



FONDAZIONE INIZIATIVE ZOOPROFILATTICHE E ZOOTECHNICHE
BRESCIA - ITALY

PROCEEDINGS OF THE 9th WORLD RABBIT CONGRESS

Verona (Italy) - June 10-13, 2008

Edited by Gerolamo Xiccato, Angela Trocino and Steven D. Lukefahr



EDITO A CURA DELLA
FONDAZIONE INIZIATIVE ZOOPROFILATTICHE
E ZOOTECHNICHE - BRESCIA

72

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- 45 - 2000 Metodi di analisi microbiologica degli alimenti
- 46 - 2000 Animali, terapia dell'anima
- 47 - 2001 Quarantacinquesimo della Fondazione Iniziative Zooprofilattiche e Zootecniche di Brescia, 1955- 2000
- 48 - 2001 Atti III Convegno Nazionale di Storia della Medicina Veterinaria
- 49 - 2001 Tipizzare le salmonelle
- 50 - 2002 Atti della giornata di studio in cardiologia veterinaria
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Associazione Scientifica
Italiana di Coniglicoltura



Università degli Studi
di Padova



WRSA
World Rabbit Science
Association



Dipartimento di
Scienze Animali
Università di Padova

PROCEEDINGS OF THE 9TH WORLD RABBIT CONGRESS

June 10-13, 2008
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Verona, Italy

Editors:
Gerolamo Xiccato, Angela Trocino, Steven D. Lukefahr

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CONTENTS

Credits	VIII
Collaborations, Patronage and Sponsors	IX
Foreword, Preface and Welcome	XIII
Paper Index	XVII
Papers of the 9 th World Rabbit Congress	1
Authors Index	475

CREDITS

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FOREWORD

The Fondazione Iniziative Zooprofilattiche e Zootecniche accepted willingly Prof. Gerolamo Xiccato's invitation to publish as a "Quaderno" of the Foundation the Proceedings of the 9th World Rabbit Congress (Verona - Italy, June 10th-13th, 2008).

The Congress will give people working in Rabbit Science the opportunity to be informed about the latest research findings in the areas of genetics, reproduction, nutrition and digestive physiology, pathology and hygiene, management and economics, welfare and ethology, product quality and processing.

The cooperation between the Foundation and the Associazione Scientifica Italiana di Coniglicoltura (ASIC), the Italian Branch of the World Rabbit Science Association, that dates from the 1990s made possible, with the publication of the Proceedings in the "Quaderni", to offer the 9th World Congress delegates a useful guide to the scientific most recent advances in rabbit science.

Dott. STEFANO CAPRETTI
The General Secretary
Fondazione Iniziative Zooprofilattiche
e Zootecniche – Brescia - Italy

PREFAZIONE

La Fondazione Iniziative Zooprofilattiche e Zootecniche ha accolto con piacere l'invito del prof. Gerolamo Xiccato a pubblicare come "Quaderno" della Fondazione gli "abstract" del 9° Congresso Mondiale di Coniglicoltura (Verona 10-13 giugno 2008).

Il Congresso offrirà ai tecnici e specialisti del settore l'opportunità di conoscere i più recenti progressi della ricerca nelle aree di genetica, riproduzione, nutrizione e fisiologia digestiva, patologia ed igiene, gestione ed economia, benessere ed etologia, qualità dei prodotti.

I rapporti di collaborazione che dagli anni '90 esistono tra la Fondazione e l'ASIC rappresentano, con l'inserimento degli "abstract" nelle collana editoriale della Fondazione, la volontà di mettere a disposizione dei congressisti una guida ai temi trattati nel 9° Convegno Mondiale di Coniglicoltura.

Dott. STEFANO CAPRETTI
Segretario Generale Fondazione
Iniziative Zooprofilattiche
e Zootecniche - Brescia

PREFACE

It is indeed a special privilege to announce the successful organization of the 9th World Rabbit Congress. Since 1976, when the 1st congress was held in Dijon, France, the World Rabbit Science Association has sponsored a congress every four years (once in Hungary, Mexico, and the USA, and twice in France, Italy, and Spain). Of course, the purpose of the congress is for rabbit scientists to assemble and to share new research information that will provide a better understanding of the biology and care of the rabbit, and to further promote the role of the rabbit as a food animal for the benefit of humans. A total of 274 scientific communications and 8 invited papers will be presented at the congress from rabbit scientists representing 34 countries.

For this congress compared to previous congresses, it is anticipated that more scientists from lesser developed countries will be in attendance due to recent efforts to form new branches in Africa, America, and in Asia. Now more than ever, the WRSA is a “world” organization of rabbit scientists. However, we should continue these efforts to further expand our borders by inviting all rabbit scientists working in every country of the world to join the WRSA and become active members.

As WRSA members, we are indebted to the efforts of all of our colleagues who assisted in working committees in the planning and organization of this congress under the superb leadership of Gerolamo Xiccato. Also, there were other colleagues who actively served as session chairs and reviewers of the numerous papers submitted; their efforts too are certainly appreciated. In addition, during the congress will be the appropriate time to acknowledge the generous monetary support of many individuals, companies, and institutions that have supported the congress. Because of the contributions of so many, it is anticipated that this will be one of the most successful congresses ever.

STEVEN D. LUKEFAHR
WRSA President

WELCOME

It is a pleasure to welcome you to the 9th World Rabbit Congress, here in Verona, Italy.

Despite kind warnings from previous convenors about the huge work of organizing the World Rabbit Congress in cooperation with World Rabbit Science Association, only a few days before the Congress could I admit that it would be harder than what I imagined. However, I could not have done it unless many persons and Institutions supported me from the beginning, sharing difficulties and successes, and I hope I do not forget to mention any one of them.

Firstly, the several Institutions who gave their patronage, collaboration and contribution to the Congress organization. A special thanks to the members and personnel of the Department of Animal Science of the University of Padova who supported me in this task.

The Italian and Foreign industries and their representatives of the rabbit production chain who made financial contributions to the Congress in a quite difficult period for the rabbit market (despite the common opinion of a low interest of private enterprises in research) is much appreciated. The increased connections between production chain and research activity will be beneficial for both.

The WRSA and its branches (France, Germany, Hungary, Italy, Spain), CIHEAM, MEKARN, the Committee “Amici del Prof. Barei”, the Department of Animal Science and the “Curso de cunicultura on-line” of the Universidad Politecnica de Valencia (Spain), the industries AIA, Lesaffre Additives and Raggio di Sole who permitted with their contribution to increase the participation of people from less developed countries.

All members of the Italian Organizing Committee who hardly worked for assuring the best management and in finding financial support for the Congress.

The WRSA Scientific Committee who continuously discussed during the four years of congress preparation about the scientific program, the topics of invited papers and round tables. Among them, a special thanks to WRSA President, Steven Lukefahr, who was always active and prompt in solving questions and support decisions.

The session referees, and all their several collaborators without names, who intensively worked at their best for the scientific success of the congress, evaluating, improving and choosing the best 274 papers to be presented out of 323 communications sent for evaluation.

The invited speakers who succeeded in preparing main papers, which include the most updated results in the fields of Genetics, Reproduction, Nutrition and digestive physiology, Pathology and hygiene, Ethology and welfare, Meat and product quality, and Management and economy, and which will guide us as rabbit scientists in our research activities for several more years.

All authors who sent their papers for presentation at the congress, all participants who will take part to the event, all persons who worked and will work during the congress for its realization.

Lastly, I would kindly thank the Fondazione Iniziative Zooprofilattiche e Zootecniche (Brescia, Italy), which agreed to publish the abstract communications and who supported the Congress organization since the original proposal.

Now, it is time to wish you good work, to enjoy the congress and your stay in Italy.

GEROLAMO XICCATO
The Convenor
of 9th World Rabbit Congress

PAPER INDEX

GENETICS

- Methods, criteria, techniques and genetic responses for rabbit selection: a review. 3
Khalil M.H., Al-Saef A.M (Saudi Arabia)
- Genetic evaluation of growth traits in a crossbreeding experiment involving line V and Baladí black rabbits in Egypt. *Abou Khadiga G., Saleh K., Nofal R., Baselga M. (Egypt)* 35
- Evaluation of the reproductive performance of a local population of rabbits in South Benin. *Akpo Y., Kpodékon T. M., Tanimomo E., Djago A.Y., Youssao A.K.I., Coudert P. (Benin)* 36
- Crossbreeding effects for carcass, tissues composition and meat quality traits in a crossing project of V-line with Saudi Gabali rabbits. *Al-Saef A.M., Khalil M.H., Al-Dobaib S.N., García M.L., Baselga M. (Saudi Arabia)* 37
- Preliminary results in a divergent selection experiment on variance of litter size in rabbits. II. Response to selection. *Argente M.J., García M.L., Muelas R., Ibáñez-Escriche N., Santacreu M.A., Blasco A. (Spain)* 38
- Effect of tissue inhibitor of metalloproteinase 1 (*timp1*) gene for embryo survival and development in a F2 rabbit cross. *Argente M.J., García M.L., Muelas R., Peiró R., Merchán M., Folch J.M., Santacreu M.A., Blasco A. (Spain)* 39
- Canalising selection on within litter variability of birth weight in rabbits: responses to selection and characteristics of the uterus of the does. *Bolet G., Garreau H., Hurtaud J., Saleil G., Esparbié J., Falières J., Theau-Clément M., Bodin L. (France)* 40
- Review on Rex Rabbit Breeding in China. *Chen Baojiang, Gu Zilin, Sun Lina, Huang Yuting, Li Sufen, Liu Yajuan, Wang Lei, Zhao Chao, Jing Cui (China)* 41
- An enterprise gross margin model to explore the influence of selection criteria for breeding programs and changes to management systems. *Eady S.J., Garreau H. (Australia)* 42
- Investigation of coat colour affecting genes in several european rabbit breeds and other leporid species. *Fontanesi L., Oulmouden A., Tazzoli M., Allain D., Deretz-Picoulet S., Robinson T.J., Pecchioli E., Cook J., Russo V. (Italy)* 43
- ZFX and ZFY gene sequences: use for molecular sexing European rabbits, European brown hares and mountain hares and perspectives for sex determination of other leporid species. *Fontanesi L., Tazzoli M., Pecchioli E., Hauffe H.C., Robinson T.J., Russo V. (Italy)* 44
- Analysis of candidate genes for meat production traits in domestic rabbit breeds. *Fontanesi L., Tazzoli M., Scotti E., Russo V. (Italy)* 45
- Strategy for developing rabbit meat production in Algeria: creation and selection of a synthetic strain. *Gacem M., Zerrouki N., Lebas F., Bolet G. (Algeria)* 46
- Effect of oviductine (*OVPGL*) gene for embryo survival and development in a F2 rabbit cross. *García M.L., Muelas R., Merchán M., Peiró R., Blasco A., Santacreu M.A., Folch J.M., Argente M.J. (Spain)* 47

Divergent selection for longevity in breeding does. <i>Garreau H., Ducrocq V., Tudela F., Saleil G., Juin H., Larzul C. (France)</i>	48
Genetic parameters of production traits and resistance to digestive disorders in a commercial rabbit population. <i>Garreau H., Eady S.J., Hurtaud J., Legarra A. (France)</i>	49
Review about rabbit breeding in China. <i>Gu Zilin, Li Sufen, Chen Baojiang, Sun Lina, Huang Yuting, Liu Yajuan, Zhao Chao, Guo Wanhua (China)</i>	50
Genetic parameters and trends of the thigh muscle volume in Pannon White rabbits. <i>Gyovai P., Nagy I., Gerencsér Zs., Metzger Sz., Radnai I., Szendrő Zs. (Hungary)</i>	51
Preliminary results in a divergent selection experiment on variance of litter size in rabbits. I. Genetic parameters. <i>Ibáñez-Escriche N., Argente M.J., García M.L., Muelas R., Santacreu M.A., Blasco A. (Spain)</i>	52
A study of environmental variance genetic control for uterine capacity in rabbits. <i>Ibáñez-Escriche N., Sorensen D., Blasco A. (Spain)</i>	53
Additive and heterotic components for post-weaning growth traits in a crossing project of V-line with Gabali rabbits in Egypt. <i>Iraqi M.M., Afifi E.A., Baselga M., Khalil M.H., García M.L. (Egypt)</i>	54
Testosterone and dihydrotestosterone production in genetically furless and furred male rabbits and effects on growth. <i>Jackson R.H., Lukefahr S.D., Stanko R.L., Flores D.O. (USA)</i>	55
RAPD markers linked to litter, lactation and growth traits in rabbits. <i>Khalil M.H., Motawei M.I., Al-Saeif A.M., Al-Sobayil K.A., El-Zarei M.F. (Saudi Arabia)</i>	56
Selection for ovulation rate in rabbits: correlated response on litter size and its components. <i>Laborda P., Mocé M.L., Climent A., Blasco A., Santacreu M.A. (Spain)</i>	57
Genetic parameters for semen traits of rabbit males: I. Production, morphology, and sperm head morphometry. <i>Lavara R., García M.L., Torres C., Vicente J.S., Baselga M. (Spain)</i>	58
Genetic parameters for semen traits of rabbit males: II. Motility. <i>Lavara R., García M.L., Torres C., Vicente J.S., Baselga M. (Spain)</i>	59
Oct-4 expression in blastocyst from two selected lines. <i>Llobat L., Vicente J.S. (Spain)</i>	60
Genetics of maternal traits in a new synthetic rabbit line under selection. <i>Mantovani R., Sartori A., Mezzadri M., Lenarduzzi M. (Italy)</i>	61
Uteroglobin levels at day 6 of gestation in two lines of rabbits divergently selected for uterine capacity. <i>Mocé M.L., Peiró R., Herrler A., Blasco A., Santacreu M.A. (Spain)</i>	62
Stability of estimated breeding values for average daily gain in Pannon White rabbits. <i>Nagy I., Farkas J., Bíró-Németh E., Radnai I., Szendrő Zs. (Hungary)</i>	63
Estimation of genetic parameters for litter size and weight traits in NZW rabbits raised in Hungary. <i>Nofal R., Hassan N., Abdel-Ghany A., Gyorgyi V. (Egypt)</i>	64
Comparison of growth performances and carcass qualities of crossbred rabbits from four sire lines in Quebec. <i>Ouyed A., Brun J.M. (Canada)</i>	65
Heterosis, direct and maternal additive effects on rabbit growth and carcass characteristics. <i>Ouyed A., Brun J.M. (Canada)</i>	66
The sex-detection in newborn rabbits by x-chromatin and PCR-SRY. <i>Parkányi V., Vašíček D., Ondruška L., Rafay J. (Slovak Republic)</i>	67

Differences in digestive efficiency between rabbit does selected for litter size at weaning and for reproductive longevity. <i>Pascual J.J., Baselga M., Blas E., Ròdenas L., Catalá A.E. (Spain)</i>	68
Relative growth of organs, tissues and retail cuts in rabbits selected for growth rate. <i>Pascual M., Pla M., Blasco A. (Spain)</i>	69
Expression of progesterone receptor related to polymorphisms in the progesterone receptor gene. <i>Peiró R., Santacreu M.A., Argente M.J., García M.L., Muelas R., Merchán M., Folch J.M., Herrler A., Blasco A. (Spain)</i>	70
Genotype x sperm dosage interaction on reproductive performance after artificial insemination. 1. Male fertility. <i>Piles M., Tusell Ll., García-Tomás M., Baselga M., García-Ispuerto I., Rafel O., Ramon J., López-Bejar M. (Spain)</i>	71
Genotype x sperm dosage interaction on reproductive performance after artificial insemination. 2. Male litter size. <i>Piles M., Tusell Ll., García-Tomás M., Baselga M., García-Ispuerto I., Rafel O., Ramon J., López-Bejar M. (Spain)</i>	72
Divergent selection for total fleece weight in the adult angora rabbit: direct response to selection on total fleece weight at first and second harvest. <i>Rafat S.A., Thébault R.G., Bonnet M., Deretz-Picoulet S., Pena-Arnaud B., De Rochambeau H., Allain D. (Iran)</i>	73
Study on relationship of Rex rabbit RAPD marker and reproductive performances. <i>Ren Keliang, Li Yanping, He Dongchang, Wu Xinsheng, Zai Ping, Liang Quanzhong, Zhangg Lijun, Zhou Shenghua, Cao Liang (China)</i>	74
Comparison of growth traits in terminal crosses of different rabbit commercial hybrids. <i>Sartori A., Lenarduzzi M., Mezzadri M., Contiero B., Mantovani R. (Italy)</i>	75
Effect of divergent selection based on CT measured hind leg muscle volume on productive and carcass traits of rabbits. <i>Szendrő Zs., Metzger Sz., Romvári R., Szabó A., Locsmándi L., Petrási Zs., Nagy I., Nagy Z., Biró-Németh E., Radnai I., Matics Zs., Horn P. (Hungary)</i>	76
The influence of weaning weight on growth of the HYPLUS broiler rabbit. <i>Vostrý L., Mach K., Jakubec V., Dokoupilová A., Majzlík I. (Czech Republic)</i>	77
Path analysis on weight, body dimension and ear type of Saibeí rabbits. <i>Wu Zhan-fu, Ma Xu-ping, Tian Shu-fei, Wu Shu-qin, Li Cun-xin, Guan Li-hui, Li Wen-hai, Wang Hai-yun (China)</i>	78

REPRODUCTION

Semen production and management of rabbit bucks. <i>Castellini C. (Italy)</i>	81
Short term effects of different diets on ovarian function and oocyte maturation of rabbit nulliparous does. <i>Arias-Álvarez M., García-García R.M., Revuelta L., Cuadrado M., Millán P., Nicodemus N., Rebollar P.G., Lorenzo P.L. (Spain)</i>	97
Pituitary gonadotropins and receptors for estrogen and GnRH in fasted does. <i>Boiti C., Galeati G., Maranesi M., Lilli L., Murgiano L., Breccia G., Dall'Aglio C., Mercati F., Gobbetti A., Zerani M. (Italy)</i>	98
The physiological dilemma of the high progesterone syndrome in rabbit does. <i>Boiti C., Guelfi G., Breccia G., Gobbetti A., Maranesi M., Zerani M. (Italy)</i>	99

Body condition score and related productive responses in rabbit does. <i>Bonanno A., Mazza F., Di Grigoli A., Alicata M.L. (Italy)</i>	100
Pheromone in rabbits: preliminary technical results on farm use in France. <i>Bouvier A.C., Jacquinet C. (France)</i>	101
Effect of a reproductive rhythm based on rabbit doe body condition on fertility and hormones. <i>Brecchia G., Cardinali R., Dal Bosco A., Boiti C., Castellini C. (Italy)</i>	102
<i>In vitro</i> viability of split rabbit embryos before and after vitrification. <i>Celestinos M., Gatica R. (Mexico)</i>	103
Manual induction of lordosis and detection of oestrus in the domestic rabbit (<i>Oryctolagus cuniculus</i>). <i>Contreras J.L., Contreras-Ferrat L.G., Canchola E., Ambriz D., Ribera J.G., Olvera J. (Mexico)</i>	104
Effect of gelatin addition to freezing extender on rabbit semen parameters and reproductive performance. <i>Cortell C., Viudes de Castro M.P. (Spain)</i>	105
Immune response to repeated rhFSH superovulation treatment in rabbit does. <i>Cortell C., Viudes de Castro M.P. (Spain)</i>	106
Motility and acrosomal integrity of frozen rabbit spermatozoa as affected by different extenders, cryoprotectants and packaging methods. <i>Daader A.H., Zeidan A.S.B. (Egypt)</i>	107
Estimation of milk production of rabbit does by cross sectional digital imaging. <i>Donkó T., Radnai I., Matics Zs., Petneházi Ö., Petrási Zs., Repa I., Szendrő Zs. (Hungary)</i>	108
Resazurin reduction test as a tool for assessment of rabbit semen quality. <i>El-Battawy K.A. (Egypt)</i>	109
Line and birth season effects on oxidative stress parameters in testis of maturing rabbits. <i>García-Tomás M., Piles M., Sánchez J., Mitjavila T. (Spain)</i>	110
Influence of environmental temperature and relative humidity on quantitative and qualitative semen traits of rabbits. <i>García-Tomás M., Tusell Ll., López-Béjar M., Ramon J., Rafel O., Piles M. (Spain)</i>	111
Effect of colour of light on the reproductive performance of rabbit does. <i>Gerencsér Zs., Matics Zs., Nagy I., Princz Z., Biró-Németh E., Radnai I., Szendrő Zs. (Hungary)</i>	112
Effect of a light stimulation on the reproductive performance of rabbit does. <i>Gerencsér Zs., Matics Zs., Nagy I., Princz Z., Orova Z., Biró-Németh E., Radnai I., Szendrő Zs. (Hungary)</i>	113
Oocyte glutathione concentration in a rabbit line selected for ovulation rate. <i>Laborda P., Santacreu M.A., García E., Mocé E., Mocé M.L. (Spain)</i>	114
Correlation between CASA and ASMA parameters in rabbit semen. <i>Lavara R., Vicente J.S., Marco-Jiménez F., Baselga M. (Spain)</i>	115
Opioid inhibition of the pulsatile luteinizing hormone release as assessed by naloxone treatment in the lactating rabbit. <i>Marongiu M.L., Gulinati A. (Italy)</i>	116
Ultrasound evaluation of ovarian follicular dynamics during early pseudopregnancy as a tool to inquire into the high progesterone (P+) syndrome of rabbit does. <i>Marongiu M.L., Gulinati A. (Italy)</i>	117
Effect of feeding regime during rearing and age at first mating on the reproductive performance of rabbit does. <i>Matics Zs., Nagy I., Biró-Németh E., Radnai I., Gerencsér Zs., Princz Z., Szendrő Zs. (Hungary)</i>	118

Influence of FSH, LH and prolactin on the components of litter size in rabbit does. <i>Muelas R., Cano P., García M.L., Esquifino A., Argente M.J. (Spain)</i>	119
Reproductive performance of rabbits fed <i>Moringa oleifera</i> as a replacement for <i>Centrosema pubescens</i> . <i>Odeyinka S.M., Oyedele O.J., Adeleke T.O., Odedire J.A. (Nigeria)</i>	120
The influence of different contact levels with male on the vaginal cytology in rabbits under the tropical humid condition. <i>Ola S.I., Oyegbade M.O. (Nigeria)</i>	121
Effect of LHRH analogue included in seminal dose on kindling rate and prolificacy of rabbits artificially inseminated. <i>Ondruška L., Parkányi V., Rafay J., Chlebec I. (Slovak Republic)</i>	122
Ovulation induction in rabbit does by intravaginal administration of the GnRH analogue [des-Gly10, D-Ala6]-LHRH ethylamide: field trial. <i>Quintela L.A., Peña A.I., Vega M.D., Gullón J., Prieto C., Barrio M., Becerra J.J., Herradón P.G. (Spain)</i>	123
Young rabbit does fed with fibrous diet during rearing: serical and productive parameters. <i>Rebollar P.G., Millán P., Schwarz B.F., Pereda N., Marco M., Lorenzo P.L., Nicodemus N. (Spain)</i>	124
Reproductive and physiological responses of rabbit does under different nutritive levels before the first parturition. <i>Rizzi C., Chiericato G.M., Dalle Zotte A. (Italy)</i>	125
Influence of doe exposure and season on reaction time and semen quality of male rabbits. <i>Rodríguez-De Lara R., Fallas-López M., Rangel-Santos R., Mariscal-Aguayo V., Martínez-Hernández P.A., García Muñiz J.G. (Mexico)</i>	126
Effect of cryopreservation on ATP content of ovulated rabbit oocytes. <i>Salveti P., Guérin P., Joly T. (France)</i>	127
Comparison of two reproductive rhythms of rabbit does. <i>Szendrő Zs., Gerencsér Zs., Matics Zs., Biró-Németh E., Nagy I. (Hungary)</i>	128
High plasmatic progesterone levels at insemination depress reproductive performance of rabbit does. <i>Theau-Clément M., Bolet G., Fortun-Lamothe L., Brecchia G., Boiti C. (France)</i>	129
Influence of photoperiod on the sexual behaviour of non-lactating rabbit does: preliminary results. <i>Theau-Clément M., Malpoux B., Lamothe E., Milcent N., Juin H., Bodin L. (France)</i>	130
Influence of a photo-stimulation on ovary and embryo recovery in nulliparous rabbit females. <i>Virág Gy., Gócza E., Hiripi L., Bősze Zs. (Hungary)</i>	131

NUTRITION AND DIGESTIVE PHYSIOLOGY

New concepts and objectives for protein-amino acid nutrition in rabbits. <i>Carabaño R., Villamide M.J., García J., Nicodemus N., Llorente A., Chamorro S., Menoyo D., García-Rebollar P., García-Ruiz A.I., de Blas J.C. (Spain)</i>	135
Mycotoxins and other contaminants in rabbit feeds. <i>Mézes M. (Hungary)</i>	157
Effect of short heat exposure, balanced feed restriction and acetic acid supplement at post weaning on growth and thermoregulation in growing rabbits during hot season. <i>Abdel-Kafy E.M., Ali W.A.H., Hoda A.S., Azoz A.A.A. (Egypt)</i>	179

Changes in oxidative profile, activity of some gastrointestinal enzymes and performance of growing rabbits during hot season due to neonatal heat exposure. <i>Abdel-Kafy E.M., Hoda A.S., Saeed A.M. (Egypt)</i>	180
Response of doe rabbits to dietary antioxidant vitamins E and C during pregnancy and lactation. <i>Abdel-Khalek A.M., Selim N.A., El-Medany Sh.A., Nada S.A. (Egypt)</i>	181
Performance evaluation of growing rabbit fed diets containing varying inclusion levels of <i>Centrosema pubescens</i> or <i>Calapogonium mucunoides</i> in the savannah zone of Nigeria. <i>Aderinola O.A., Ojebiyi O.O., Rafiu T.A., Akinlade J.A., Adepoju L.O. (Nigeria)</i>	182
Effect of dietary level and source of glutamine on intestinal health in the postweaning period. <i>Baylos M., Menoyo D., Chamorro S., Sainz A., Nicodemus N., de Blas J.C., Carabaño R. (Spain)</i>	183
<i>In vivo</i> and <i>in vitro</i> study of caecal fermentation pattern and methanogenesis in rabbits. <i>Belenguer A., Fondevila M., Balcells J., Abecia L., Lachica M., Carro M.D. (Spain)</i>	184
Effect of water restriction times of 2 and 4 hours per day on performances of growing rabbits. <i>Ben Rayana A., Ben Hamouda M., Bergaoui R. (Tunisia)</i>	185
Effects of feed restriction on the performance and carcass of growing rabbits. <i>Bergaoui R., Kammoun M., Ouerdiane K. (Tunisia)</i>	186
Effect of dietary fat quality on the performance and health of fattening rabbits. <i>Blas E., Ródenas L., Martínez E., Pascual J.J., Cervera C. (Spain)</i>	187
Effect of inulin supplementation on caecal microflora and fermentation in rabbits. <i>Bónai A., Szendrő Zs., Maertens L., Matics Zs., Fébel H., Kametler L., Tornyos G., Horn P., Kovács F., Kovács M. (Hungary)</i>	188
Effect of <i>Bacillus cereus</i> var. <i>toyoi</i> on caecal microflora and fermentation in rabbits. <i>Bónai A., Szendrő Zs., Matics Zs., Fébel H., Pósa R., Tornyos G., Horn P., Kovács F., Kovács M. (Hungary)</i>	189
Feed restriction during summer: effect on rabbit growth performance. <i>Bovera F., Di Meo C., Marono S., Vella N., Nizza A. (Italy)</i>	190
Effect of dietary supplementation of organic acids and essential oils on immune function and intestinal characteristics of experimentally infected rabbits. <i>Cardinali R., Rebollar P.G., Dal Bosco A., Cagiola M., Moscati L., Forti K., Mazzone P., Scicutella N., Rutili D., Mugnai C., Castellini C. (Italy)</i>	191
Effect of different feeding systems for young rabbit does on their development and performance until first weaning: preliminary results. <i>Cervera C., Juncos A., Martínez E., Ródenas L., Blas E., Pascual J.J. (Spain)</i>	192
Effect of dietary acidification on growth performance and caecal characteristics in rabbits. <i>Cesari V., Toschi I., Pisoni A.M., Grilli G., Cesari N. (Italy)</i>	193
Effect of dietary fat addition on growth performance, nutrient digestion and caecum fermentation in 2-3 months old meat rabbits. <i>Chen P., Li F.C. (China)</i>	194
Very low protein, aminoacid-supplied diet for heavy broiler rabbits: effects on growth, feed efficiency, carcass and meat performances. <i>Cherubini R., Barge P., Baricco G., De Poi E., Masoero G. (Italy)</i>	195

- Influence of the distribution at birth of a Heat Shock Proteins booster on growth and mortality of rabbits before and after weaning. *Colin M., Lebas F., Gutierrez G., Charrier S., Teillet B., Saliba C., Prigent A.Y. (France)* 196
- Impact of an exclusive milk vs. milk and dry feed intake till weaning on intake, growth, and on the caecal biodiversity and fibrolytic activity of the young rabbit. *Combes S., Cauquil L., Gidenne T. (France)* 197
- Assessment of loofah gourd seeds *Luffa cylindrica* (roem) on performance and some haematological indices of rabbit weaners. *Dairo F.A.S. (Nigeria)* 198
- New advances in digestive physiology of rabbits with the use of tropical forage in Cuba. *Dihigo L.E., Savón L., Sierra F., Martínez M., Hernández Y., Domínguez M., Nodas A. (Cuba)* 199
- Feed additives as they affect the fattening performance of rabbits. *Eiben Cs., Gippert T., Gódor-Surmann K., Kustos K. (Hungary)* 200
- Effect of dietary phosphorus reduction and phytase supplementation on growth of rabbits. *Eiben Cs., Gippert T., Gódor-Surmann K., Podmaniczky B., Kustos K. (Hungary)* 201
- Influence of dietary protein reduction and enzyme and/or amino acid supplementation on fattening performance of rabbits. *Eiben Cs., Gippert T., Gódor-Surmann K., Podmaniczky B., Kustos K. (Hungary)* 202
- In vitro* caecal gas production and dry matter degradability of some browse leaves in presence of enzymes from anaerobic bacterium in NZW rabbits. *El-Adawy M.M., Salem A.Z.M., Borhami B.E., Gado H.M., Khalil M.S., Abo-Zeid A. (Egypt)* 203
- Study of the effect of rosemary (*Rosmarinus officinalis*) and garlic (*Allium sativum*) essential oils on the performance of rabbit. *Erdélyi M., Matics Zs., Gerencsér Zs., Princz Z., Szendrő Zs., Mézes M. (Hungary)* 204
- In vitro* gas production of diets with inclusion of seaweed (*Lithothamnium* sp.) flour for White New Zealand rabbits. *Euler A.C.C., Ferreira W.M., Maurício R., Sousa L., Carvalho W., Teixeira E. de A., Coelho C.C.G.M., Matos C. (Brazil)* 205
- Improving the nutritive value of lupin seed for growing rabbits: α -galactosidase enzymes vs. washing. *Falcão e Cunha L., Sabino I., Castro-Solla L., Bruno-Soares A., Freire J.P. (Portugal)* 206
- Effect of feed restriction with or without free access to drinking water on performance of growing rabbits in healthy or epizootic rabbit enteropathy conditions. *Foubert C., Duperray J., Boisot P., Guyonvarch A. (France)* 207
- Effect of yeast β -glucans on rabbit performances and mortality from 35 to 63 days of age. *García-Ruiz A.I., Pérez-Bonilla A., Pérez de Ayala P., Eissen J. (Spain)* 208
- Nutritive value of carrot (whole plant), dried at low temperature, for the growing rabbit. *Goby J.P., Gidenne T. (France)* 209
- Supplementation of rabbit diet with chestnut wood extract: effect on *in vitro* gas production from three sources of carbohydrates. *Kermauner A., Lavrenčič A. (Slovenia)* 210
- Supplementation of rabbit diet with chestnut wood extract: effect on *in vitro* gas production from two sources of protein. *Kermauner A., Lavrenčič A. (Slovenia)* 211
- Live yeast stability in the digestive tract of the rabbit: relationship with digestion, growth and digestive health. *Kimsé M., Bayourthe C., Monteils V., Gidenne T. (France)* 212

Effect of different weaning age (days 21, 28 and 35) on caecal microflora and fermentation in rabbits. Kovács M., Milisits G., Szendrő Zs., Lukács H., Bónai A., Pósa R., Tornyos G., Kovács F., Horn P. (Hungary)	213
Effect of essential unsaturated fatty acids in fish oil on litters and composition of milk of rabbit does. Kowalska D. (Poland)	214
Effects of molasses incorporation in rabbit fattening diet on growth performances. Kpodékon T.M., Youssao A.K.I., Tossou C.M., Djago A.Y., Coudert P. (Benin)	215
Effect of inclusion of probiotics on micro-organisms content, health and performance of fattening rabbits: 1. Study in a commercial farm with intermediate health status. Kritas S.K., Petridou E., Fortomaris P., Tzika E., Arsenos G., Koptopoulos G. (Greece)	216
Field beans and Brewer's grains as protein source for growing rabbits in Algeria: first results on growth and carcass quality. Lounaouci-Ouyed G., Lakabi-Ioualitene D., Berchiche M., Lebas F. (Algeria)	217
Very low protein, aminoacid-supplied diet for heavy broiler rabbits: effects on nitrogen metabolism, and digital evaluation of excreta and products. Masoero G., Baricco G., Cherubini R., Barge P., Sala G., De Poi E. (Italy)	218
Joint vis-NIRS evaluation of feeds and dried feces to estimate ingestion and digestibility in growing rabbits. Masoero G., Sala G., Meineri G., Peiretti P.G. (Italy)	219
Effect of restricted feeding after weaning on the productive and carcass traits of growing rabbits. Matics Zs., Dalle Zotte A., Radnai I., Kovács M., Metzger Sz., Szendrő Zs. (Hungary)	220
Effect of adding dietary caprylic acid on the bacterial population in the rabbit caecum and stomach. McEwan N.R., Skřivanová E., Worgan H.J., Pinloche E., Newbold C.J., Marounek M. (UK)	221
Performance of growing rabbits fed <i>Lablab purpureus</i> forage with molasses mini-blocks and restricted commercial pellets. Mudunuru U., Lukefahr S.D., Nelson S.D., Flores D.O. (USA)	222
Effect of <i>Psophocarpus scandens</i> replacing para grass in the diets on feed utilization, growth rate and economic return of growing crossbred rabbits in the Mekong delta in Vietnam. Nguyen Van T., Nguyen Thi K.D. (Vietnam)	223
Effect of water spinach and sweet potato vine associated with 2 other natural plants on growth performance, carcass values and economic return of growing crossbred rabbits in the Mekong delta of Vietnam. Nguyen Van T., Nguyen Thi K.D. (Vietnam)	224
Evaluation of the nutritive potential of cassava (<i>Manihot esculenta</i> Crantz) peels-blood meal mixture on the performance characteristics of female growing rabbits in the derived savannah zone of Nigeria. Ojebiyi O.O., Farinu G.O., Babatunde G.M., Aderinola O.A. (Nigeria)	225
Use of simplified diet with cassava by-products for rabbits. Oliveira A.F.G., Scapinello C., Maria B.G., Jobim C.C., Monteiro A.C., Furuta L., Ferreira W.M. (Brazil)	226
Effects of dietary inclusion of Toyocerin® (<i>Bacillus cereus</i> var. <i>toyoi</i>) on performance, health and faecal nitrogen excretion in growing rabbits. Pascual J.J., Moya V.J., Martínez E., Calvo M.A., Adelantado C., Jiménez G., Blanch A., Castillo M. (Spain)	227

- Fiber quality to growing rabbits. *Retore M., da Silva L.P., de Toledo G.S.P., Araújo I.G., Aramburú Jr J.S., Araújo C.C.B. (Brazil)* 228
- The evolution with age of *Clostridium perfringens* concentration in soft faeces in relation with epizootic rabbit enteropathy symptoms. *Romero C., Nicodemus N., Astillero J.R., García-Ruiz A.I., de Blas J.C. (Spain)* 229
- The use of soft faeces for the prediction of the caecal contents concentration of *Clostridium perfringens* in rabbits weaned at two ages. *Romero C., Nicodemus N., García-Ruiz A.I., Astillero J.R., de Blas J.C. (Spain)* 230
- Response of growing rabbits to dietary antioxidant vitamins E and C. 1. Effect on performance. *Selim N.A., Abdel-Khalek A.M., Nada S.A., El-Medany Sh.A. (Egypt)* 231
- The use of a ginseng extract in rabbits. *Simonová M., Szabóová R., Chrastinová L., Lauková A., Haviarová M., Strompfová V., Plachá I., Faix Š., Vasilková Z., Mojto J., Rafay J. (Slovak Republic)* 232
- Combined effect of enterocin CCM4231 and sage in rabbits. *Szabóová R., Chrastinová L., Strompfová V., Simonová M., Vasilková Z., Lauková A., Čobanová K., Plachá I., Chrenková M., Mojto J., Ondruška L. (Slovak Republic)* 233
- Combined effect of bacteriocin-producing *Enterococcus faecium* CCM4231 strain and sage in rabbits. *Szabóová R., Chrastinová L., Strompfová V., Simonová M., Vasilková Z., Lauková A., Plachá I., Čobanová K., Chrenková M., Mojto J., Jurčík R. (Slovak Republic)* 234
- Productive performance of rabbits fed with diets containing ramie (*Boehmeria nivea*) hay in substitution to alfalfa (*Medicago sativa*) hay. *de Toledo G.S.P., da Silva L.P., de Quadros A.R.B., Retore M., Araújo I.G., Brum H.S., Ferreira P., Melchior R. (Brazil)* 235
- White lupin (cv. Amiga) seeds as a protein source in diet for growing rabbits: effect on growth performance, digestibility of nutrients and carcass traits. *Volek Z., Marounek M. (Czech Republic)* 236
- Effects of probiotics and nanometer implement on growth performance of Rex rabbit. *Wang Zhiheng, Gu Zilin, Huo Yanming, Chen Baojiang, Liu Yajuan, Zhao Zhujun, Zhang Guolei (China)* 237
- Effect of diet methionine rate on performances and blood protein levels of fattening rabbits. *Weissman D., Corrent E., Troislouches G., Picard E., Leroux C., Davoust C., Launay C. (France)* 238
- Digestible fibre to starch ratio and antibiotic treatment time in growing rabbits affected by epizootic rabbit enteropathy. *Xiccato G., Trocino A., Carraro L., Fragkiadakis M., Majolini D. (Italy)* 239
- Effect of mineral blocks addition on fattening rabbit performance. *Zerrouki N., Lebas F., Davoust C., Corrent E. (Algeria)* 240
- Effect of substitution rocket seed meal as a source of protein for soybean meal in diets of New Zealand White rabbits. *Zeweil H. S., Ahmed M.H., El-Adawy M.M., Zaki B. (Egypt)* 241
- Evaluation of substituting *Nigella* seed meal as a source of protein for soybean meal in diets of New Zealand White rabbits. *Zeweil H.S., Ahmed M.H., El-Adawy M.M., Zaki B. (Egypt)* 242

- Lipase activity till 35 days of age in broiler rabbits. Zita L., Fučíková A., Marounek M., Tůmová E., Skřivanová V. (Czech Republic) 243
- Effect of extract of chestnut wood inclusion (enc®) in normal and low protein aminoacid supplemented diets on heavy broiler rabbits. Zoccarato I., Gasco L., Schiavone A., Guo K., Barge P., Rotolo L., Savarino G., Masoero G. (Italy) 244

PATHOLOGY AND HYGIENE

- Viral infection of rabbits. Lavazza A., Capucci L. (Italy) 247
- Prevalence of *Staphylococcus aureus* biotypes in commercial rabbit farms. Agnoletti F., Bano L., Cocchi M., Deotto S., Ferro T., Guolo A., Drigo I., Mazzolini E. (Italy) 269
- Validation of a sampling method to detect healthy rabbit *Staphylococcus aureus* carriers. Agnoletti F., Cocchi M., Bano L., Guolo A., Bacchin C., Drigo I., Mazzolini E. (Italy) 270
- Clostridium spiroforme* drug susceptibility. Agnoletti F., Ferro T., Guolo A., Cocchi M., Drigo I., Bacchin C., Marcon B., Bano L. (Italy) 271
- Prevalence and molecular characterization of *Clostridium difficile* isolated from rabbits and detection of its main toxins. Bano L., Busani L., Cocchi M., Drigo I., Spigaglia P., Mastrantonio P., Agnoletti F. (Italy) 272
- Search for key health and welfare indicators for meat rabbit production and definition of a score method of evaluation. Cerioli M., Brivio R., Grilli G., Tittarelli C., Marasciulo V., Lavazza A. (Italy) 273
- Toxin-genotyping of *Clostridium perfringens* strains isolated from rabbits with enteric disease. Cocchi M., Drigo I., Bacchin C., Bano L., Marcon B., Agnoletti F. (Italy) 274
- Laboratory limits on dermatophyte diagnosis in rabbits with clinical lesions. Coelho A.C., Pinto M.L., Coelho A.M., Fontes M.C., Mourão J.L., Pinheiro V., Rodrigues J. (Portugal) 275
- Cooling panel type and location influence nest temperature, growth and sanitary status of rabbits during the cold season in Hungary. Colin M., Fiorenza G., Balogh K., Virág Gy. (Hungary) 276
- Infection with *Pasteurella* spp. in a New Zealand Rabbit colony. Coman C., Turcu D., Coman T., Vlase E. (Romania) 277
- PCR identification and toxin-typing of *Clostridium spiroforme* field strains. Drigo I., Bacchin C., Cocchi M., Bano L., Agnoletti F. (Italy) 278
- Establishment and application of Reverse Transcriptase-Polymerase Chain Reaction for detection of rabbit hemorrhagic disease virus. Fang Wang, Longsheng Yang, Chao-mei Li, Jiabin Xue, Weizhong Xu, Kongwang He (China) 279
- Rabbit health control by management. Finzi A., Macchioni P., Negretti P. (Italy) 280
- An outbreak of myxomatosis in Greek rabbit farms during 2007. Fragkiadaki Eir., Xylouri E., Ntafis V., Sabatakou O., Plakokefalos E., Paraskevaku E. (Greece) 281
- Rationing of drinking water supply in relationship with growth and sanitary performances of growing rabbits. Gualterio L., González-Redondo P., Negretti P., Finzi A. (Italy) 282

- Molecular characterization and phenotyping of *Pasteurella* from Swiss rabbits. Hoop R., Stahel A., Korczak B. (Switzerland) 283
- Is Epizootic Rabbit Enteropathy (ERE) a bacterial disease? Huybens N., Houeix J., Szalo M., Licois D., Mainil J., Marlier D. (Belgium) 284
- An acute outbreak of myxomatosis in two Greek rabbitries. Kritas S.K., Dovas C., Petridou E., Fortomaris P., Farsang A., Koptopoulos G. (Greece) 285
- Influence of different strains and ways of inoculation on the rabbit's response to experimental infection with *Pasteurella multocida*. Kulcsár G., Fábíán K., Barna T., Virág Gy. (Hungary) 286
- Evaluation of the protection provided by an inactivated trivalent *Pasteurella multocida* vaccine against experimental *Pasteurella* infection and pasteurellosis in commercial environment. Kulcsár G., Fábíán K., Farsang A., Virág Gy. (Hungary) 287
- Rotavirus in diarrheic rabbits: prevalence and characterization of strains in Italian farms. Lavazza A., Cerioli M., Martella V., Tittarelli C., Grilli G., Brivio R., Buonavoglia C. (Italy) 288
- Effect of predated vaccination and chinese herbal adjuvant rabbit hemorrhagic disease vaccine on immune response in young rabbits. Longsheng Yang, Jiabin Xue, Fang Wang, Yuanliang Hu, Deyun Wang, Weizhong Xu (China) 289
- In vitro* activity of robenidine hydrochloride on rabbit *Clostridium perfringens* isolates. Marien M., Vancraeynest D., De Gussem M., Baele M., Haesebrouck F. (Belgium) 290
- A method to evaluate the microbial air composition in rabbit farm. Martino P.A., Luzi F. (Italy) 291
- Bacterial infections in rabbit as companion animal: a survey of diagnostic samples in Italy. Martino P.A., Luzi F. (Italy) 292
- Possible protective effect of an autovaccine against high virulence *Staphylococcus aureus* in a rabbit skin infection model. Meulemans L., Hermans K., Lipinska U., Duchateau L., Haesebrouck F. (Belgium) 293
- Characterization of bacterial communities in caecum, hard and soft feces of rabbit using 16S rRNA genes capillary electrophoresis single-strand conformation polymorphism (CE-SSCP). Michelland R., Combes S., Cauquil L., Gidenne T., Monteils V., Fortun-Lamothe L. (France) 294
- Apramycin in the control of enteritis associated with *Escherichia coli* in rabbits. Morel Saives A., Bostvironnois C., Limet A. (France) 295
- Standardization of Nested-PCR for the detection of *Pasteurella multocida*, *Staphylococcus aureus*, Myxomatosis Virus, and Rabbit Haemorrhagic Disease Virus. Pérez de Rozas A.M., González J., Aloy N., Badiola I. (Spain) 296
- Digestive microbiota studies in rabbits by REP-PCR method. Pérez de Rozas A.M., Rosell J.M., Díaz J.V., Carabaño R., García J., González J., Aloy N., Badiola I. (Spain) 297
- Tissue depletion residues in fattening rabbits fed during 28 days with feed supplemented with 40 ppm of tiamulin hydrogen fumarate (Tiamulin® Premix 100). Pérez de Rozas A.M., Vives N., Sitjar M., García J., González J., Aloy N., Badiola I. (Spain) 298

Occurrence of <i>Campylobacter spp.</i> in Italian rabbit farms. Revez J., Rossi M., Renzi M., Zanoni R.G. (Italy)	299
Dose determination for bacitracin against <i>Clostridium perfringens</i> using a pharmacokinetic/pharmacodynamic (PK/PD) approach. Impact on consumer safety. Richez P., Richard A., Cornez B., Vancraeynest D. (France)	300
Susceptibility, resistance and antibiotic profile of bacitracin against <i>Clostridium perfringens</i> strains isolated during clinical outbreaks of Epizootic Rabbit Enteropathy. Richez P., Richard A., Cornez B., Vancraeynest D. (France)	301
Health and body condition of rabbit does on commercial farms. Rosell J.M., De La Fuente L.F. (Spain)	302
Tylosin MIC distribution from clinical isolates of <i>Clostridium perfringens</i> in France, Italy and Spain. Saggiolato M., Pradella G., Scandurra S., Bacchin C., Ferro T., Agnoletti F. (Italy)	303
Apramycin, Colistin, Neomycin and Paramomycin MIC distribution from clinical isolates of <i>Klebsiella pneumoniae</i> . Saggiolato M., Scandurra S., Pradella G., Bacchin C., Guolo A., Agnoletti F. (Italy)	304
<i>Staphylococcus aureus</i> nasal carriage in rabbits. Selva L., Viana D., Penadés J.R., Corpa J.M. (Spain)	305
Pharmacodynamics of Avilamycin towards strains of <i>Clostridium perfringens</i> isolated from Epizootic Rabbit Enteropathy. Simjee S., Heffron A.L., Agnoletti F., Pridmore A., Pradella G. (UK)	306
A note on the effect of caprylic acid on shedding of <i>Escherichia coli</i> O103 and O128 in weaned rabbits. Skřivanová E., Marounek M., Skřivanová V. (Czech Republic)	307
Genetic characterization of rabbit <i>Escherichia coli</i> strains with the use of microarray technology. Tonelli A., Badagliacca P., Bruant G., Letizia A., Di Provvido A., Harel J., Scacchia M. (Italy)	308
The anticoccidial efficacy of robenidine hydrochloride in <i>Eimeria</i> challenged rabbits. Vancraeynest D., De Gussem M., Marien M., Maertens L. (Belgium)	309
The spectrum of pathology associated with natural chronic Staphylococcal mastitis in rabbits. Viana D., Selva L., Callanan J.J., Segura P., Corpa J.M. (Spain)	310
Pheno- and genotypic characterization of <i>Pasteurella multocida</i> strains recovered from healthy and diseased rabbits. Virág Gy., Barna T., Fábíán K., Farsang A. (Hungary)	311
Mice pathogenicity of <i>Pasteurella multocida</i> strains isolated from diseased and healthy rabbits. Virág Gy., Kulcsár G., Makranszki L. (Hungary)	312
Development and application of PCR assay for detection of <i>Bordetella bronchiseptica</i> in rabbits. Wang Xin, Yun Shi-feng, Wang Fang (China)	313

ETHOLOGY AND WELFARE

Development of social and feeding behaviour in young rabbits. Coureaud G., Fortun-Lamothe L., Rödel H.G., Monclús R., Schaal B. (France)	317
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Serum chemistry and hematology values in commercial rabbits: preliminary data from industrial farms in Northern Italy. <i>Archetti I., Tittarelli C., Cerioli M., Brivio R., Grilli G., Lavazza A. (Italy)</i>	339
Raising rabbit does in platform cages. <i>Barge P., Masoero G., Chicco R. (Italy)</i>	340
Pasture availability and genotype effect in rabbits: 2. Development of gastro-intestinal tract and immune function of the vermiphorm appendix. <i>Cardinali R., Rebollar P.G., Mugnai C., Dal Bosco A., Cuadrado M., Castellini C. (Italy)</i>	341
Rabbits' preference for cages and pens with or without mirror. <i>Dalle Zotte A., Princz Z., Matics Zs., Gerencsér Zs., Metzger Sz., Szendrő Zs. (Italy)</i>	342
Effect of floor type on footpad injuries in does: a pilot study. <i>De Jong I.C., Reimert H., Rommers J.M. (The Netherlands)</i>	343
Effect of lighting program on the nursing behaviour of rabbit does. <i>Gerencsér Zs., Matics Zs., Nagy I., Princz Z., Orova Z., Biró-Németh E., Radnai I., Szendrő Zs. (Hungary)</i>	344
Guidelines for minimum standards on rabbit housing in Germany. <i>Hoy St. (Germany)</i>	345
Analysis of the behaviour of growing rabbits housed in deep litter at different stages of rearing. <i>Jekkel G., Milisits G., Nagy I., Biró-Németh E. (Hungary)</i>	346
Suitability of behavioural tests for determining the ways rabbits function in the environment and their relationship with some productive traits. <i>Kowalska D., Bielanski P., Pietras M. (Poland)</i>	347
Halal slaughter and electrical stunning in rabbits: effect on welfare and muscle characteristics. <i>López M., Carrilho M.C., Campo M.M., Lafuente R. (Spain)</i>	348
Pasture availability and genotype effects in rabbits: 1. Health and welfare. <i>Mugnai C., Dal Bosco A., Moscati L., Battistacci L., Mourvaki E., Cardinali R., Castellini C. (Italy)</i>	349
Mutual visual relationships of rabbits raised in individual cages. <i>Negretti P., Bianconi G., Finzi A. (Italy)</i>	350
Examination of feeder size for growing rabbits. <i>Orova Z., Matics Zs., Biró-Németh E., Gerencsér Zs., Nagy I., Szendrő Zs. (Hungary)</i>	351
Effect of gnawing sticks on the welfare of growing rabbits. <i>Princz Z., Nagy I., Biró-Németh E., Matics Zs., Szendrő Zs. (Hungary)</i>	352
Effect of the floor type and stocking density on the productive performance of growing rabbits. <i>Princz Z., Nagy I., Radnai I., Gerencsér Zs., Szendrő Zs. (Hungary)</i>	353
Socialization of Dutch belted rabbits for use in research: preliminary outcomes. <i>Pritt S., Wood J., Fetter B., Kline B. (USA)</i>	354
Effect of environmental conditions on productive and physiological responses in growing rabbits. <i>Rizzi C., Chiericato G.M. (Italy)</i>	355
Evaluation of rabbit welfare at stunning and slaughtering in a commercial abattoir. <i>Rota Nodari S., Lavazza A., Candotti P. (Italy)</i>	356
Temperature and cage floor enrichment affect the behavior of growing rabbits. <i>Siloto E.V., Zeferino C.P., Moura A.S.A.M.T., Fernandes S., Sartori J.R., Siqueira E.R. (Brazil)</i>	357
Effect of cage floor and stocking density on growth performance and welfare of group-housed rabbits. <i>Trocino A., Xiccato G., Majolini D., Fragkiadakis M. (Italy)</i>	358

Effects of loading method and crate position on the truck on some stress indicators in rabbits transported to the slaughterhouse. <i>Vignola G., Giammarco M., Mazzone G., Angelozzi G., Lambertini L. (Italy)</i>	359
Frequency of engagement with different materials by growing rabbits. <i>Wagner C., Weirich C., Hoy St. (Germany)</i>	360
Welfare and haematological indices of weaner rabbits as affected by stocking density. <i>Yakubu A., Adua M.M., Adamude H. (Nigeria)</i>	361
Effect of handling in pre-weaning rabbits. <i>Zucca D., Bonazza V., Heinzl E., Luzi F., Verga M. (Italy)</i>	362
Effect of environmental enrichment and group size on behaviour and production in fattening rabbits. <i>Zucca D., Heinzl E., Luzi F., Cardile H., Ricci C., Verga M. (Italy)</i>	363

MEAT QUALITY AND SAFETY

Enhancement of nutritional quality and safety in rabbit meat. <i>Hernández P. (Spain)</i>	367
NIR prediction for protein and intramuscular fat content of rabbit hind leg meat. <i>Bázár Gy., Kövér Gy., Szendrő Zs., Romvári R. (Hungary)</i>	385
Effect of limiting access to drinking water on carcass characteristics, meat quality and kidneys of rabbits. <i>Ben Rayana A., Ben Hamouda M., Kaddech A., Amara A., Bergaoui R. (Tunisia)</i>	386
Influence of preslaughter fasting on live weight loss, carcass yield and meat quality in rabbits. <i>Bianchi M., Petracci M., Venturi L., Cremonini M.A., Cavani C. (Italy)</i>	387
Use of linseed oil and antioxidant (vitamin E) in rabbit diets to improve dietetic traits of rabbit meat. <i>Bielanski P., Kowalska D. (Poland)</i>	388
Feed restriction during summer: effect on rabbit carcass traits and meat quality. <i>Bovera F., Piccolo G., D'Urso S., Nizza S., Cutrignelli M.I. (Italy)</i>	389
Microbiological quality and safety of rabbit meat in Veneto Region – Italy. <i>Comin D., Mioni R., Gallochio L., Bordin P., Maniero C. (Italy)</i>	390
Effect of feed rationing and parity order of rabbit does on growth performance and meat quality of their offspring. <i>Dalle Zotte A., Rizzi C., Chiericato G.M. (Italy)</i>	391
Effect of mother's feeding, physiological state, parity order and offspring's age on their <i>post-mortem</i> pH evolution of <i>Longissimus dorsi</i> muscle. <i>Dalle Zotte A., Rizzi C., Riovanto R. (Italy)</i>	392
Unusual spoilage in rabbit carcasses caused by <i>Janthinobacterium lividum</i> . <i>Giaccone V., Alberghini L., Biscotto A., Milandri C. (Italy)</i>	393
The effect of decreasing the omega 6/omega 3 ratio in feed on fatty acid content of rabbit meat to meet human dietary recommendations. <i>Gigaud V., Combes S. (France)</i>	394
Effect of the dietary n-3 and n-6 fatty acids on texture properties and sensory characteristics of rabbit meat. <i>Hernández P., Pla M. (Spain)</i>	395
Comparison of the slaughter characteristics of growing rabbits reared on wire net or combined (wire net/straw) floor. <i>Jekkel G., Milisits G., Biró-Németh E., Radnai I., Matics Zs., Princz Z., Gerencsér Zs. (Hungary)</i>	396

Effect of dietary supplementation with rapeseed and fish oil mixture and antioxidant on rabbit meat quality. <i>Kowalska D. (Poland)</i>	397
Commercial and microbiological qualities of frozen rabbit carcasses in Benin. <i>Kpodékon T.M., Wabi K., Seydi M., Farougou S., Djago A.Y., Akpo Y. (Benin)</i>	398
Fatty acid composition of rabbit meat when fed a linseed based diet during different periods after weaning. <i>Maertens L., Huyghebaert G., Delezie E. (Belgium)</i>	399
Effect of dietary type and level of fibre on carcass yield and its microbiological characteristics. <i>Margüenda I., Carabaño R., García-Rebollar P., Fragkiadakis M., Sevilla L., Vadillo S., Nicodemus N. (Spain)</i>	400
Results of avilamycin residues monitoring plans for the experimental use in Italy. <i>Menotta S., Fedrizzi G., Macri S., Scandurra S., Saggiorato M. (Italy)</i>	401
Effect of nutritional status of kits on carcass traits and meat quality (preliminary results). <i>Metzger Sz., Bianchi M., Cavani C., Petracci M., Gyovai M., Biró-Németh E., Radnai I., Szendrő Zs. (Hungary)</i>	402
Pasture availability and genotype effect in rabbit: 3. Performance, carcass and meat characteristics. <i>Mugnai C., Finzi A., Zamparini C., Dal Bosco A., Mourvaki E., Castellini C. (Italy)</i>	403
A critical appraisal of rabbit preslaughter conditions in a commercial production chain. <i>Petracci M., Bianchi M., Cavani C. (Italy)</i>	404
Prevalence of pathological lesions in meat rabbits at slaughtering. <i>Piccirillo A., Rampin F., Schiavon E., Poppi L., Grilli G. (Italy)</i>	405
Growth and carcass characteristics of rabbits housed in open-air or standard systems. <i>Pinheiro V., Silva S.R., Silva J.A., Outor-Monteiro D., Mourão J.L. (Portugal)</i>	406
Effect of the dietary n-3 and n-6 fatty acids on rabbit carcass and meat quality. <i>Pla M., Zomeño C., Hernández P. (Spain)</i>	407
Effect of outdoor rearing system, in floor cage, on meat quality of slow growing rabbits. <i>Prezioso G., Dalle Zotte A., D'Agata M., Russo C., Paci G. (Italy)</i>	408
Response of growing rabbits to dietary antioxidant vitamins E and C. 2. Effect on meat quality. <i>Selim N.A., Abdel-Khalek A.M., Nada S.A., El-Medany Sh.A. (Egypt)</i>	409
Estimation of rabbit hind leg muscle weight and muscularity by real time ultrasonography. <i>Silva S.R., Guedes C.M., Mourão J.L., Venâncio C., Pinheiro V. (Portugal)</i>	410
<i>In vivo</i> rabbit carcass composition and <i>Longissimus dorsi</i> muscle volume prediction by Real Time Ultrasonography. <i>Silva S.R., Mourão J.L., Guedes C.M., Pio A., Pinheiro V. (Portugal)</i>	411
Microbial contamination levels in rabbit carcasses obtained from popular markets in Toluca valley, Mexico. <i>Velázquez O.V., Alonso F.M.U., Lagunas B.S., Díaz Z.S., Gutiérrez C.A., Monroy S.H., Mendoza B.J. (Mexico)</i>	412
Effect of a high fibrous diet in the finishing period on carcass yield and meat quality of rabbits. <i>Villena P., García-Rebollar P., Rebollar P.G., Núñez N., Nicodemus N., Margüenda I., Carabaño R. (Spain)</i>	413
Colour and pH of rabbit meat and fat deposits as affected by the source and dose of dietary vitamin E supplementation. <i>Virág Gy., Eiben Cs., Tóth T., Schmidt J. (Hungary)</i>	414

- Effect of dietary vegetable oil (sunflower, linseed) and vitamin E supplementation on the fatty acid composition, oxidative stability and quality of rabbit meat. *Zsédely E., Tóth T., Eiben Cs., Virág Gy., Fábíán J., Schmidt J. (Hungary)* 415

MANAGEMENT AND ECONOMY

- A challenging role for organic rabbit production towards poverty alleviation in South East Asia. *Samkol P. (Cambodia), Lukefahr S.D. (USA)* 419
- Status and promotional strategies for rabbit production in Nigeria. *Abu O.A., Onifade A.A., Abanikanda O.T.F., Obiyan R.I. (Nigeria)* 447
- Characterization of traditional rabbit breeding system used in the South of Tunisia. *Ben Larbi M., Haddad B., Allalout S. (Tunisia)* 448
- Economic sustainability of rabbit farming innovations. *Bertazzoli A., Rivaroli S. (Italy)* 449
- Possibility of using the native breed of Popielno White rabbits for meat production. *Bielanski P., Kowalska D., Pankowski P. (Poland)* 450
- Consumers' opinion about rabbit meat consumption in Hungary. *Bodnar K., Horvath J. (Hungary)* 451
- Effect of reproductive rhythm and fresh chicory (*Cicorium intibum* L.) inclusion in the productive performance of rabbit does. *Cossu M.E., Cumini M.L., Etcheluz L., Rolla C. (Argentina)* 452
- Effects of season, generation, number of mating, parity and doe number of teat on doe and litter birth characteristics in domestic rabbit. *Fayeye T.R., Ayorinde K.L. (Nigeria)* 453
- Effects of weaning litter size and sex on postweaning bodyweight, mortality and carcass characteristics of domestic rabbit in the humid tropics. *Fayeye T.R., Ayorinde K.L. (Nigeria)* 454
- Meat production of rabbit: an alternative for the semidesert Querétano and people in extreme poverty. *Gómez Gallegos G. (Mexico)* 455
- Analysis of the efficiency and the reproductive seasonality of an alternative rabbit keeping system. *González-Redondo P., Negretti P., Finzi A. (Spain)* 456
- Effect of remating interval, weaning age and parity on rabbit doe performance under heat stress. *Guillén O., Villalobos O., García J. (Venezuela)* 457
- Enterprise organization and capital requirements in Hungary. *Horvath J., Bodnar K. (Hungary)* 458
- Commercialization of rabbit's meat in Tizi-Ouzou area, Algeria. *Kadi S.A., Djellal F., Berchiche M. (Algeria)* 459
- Survey report for present situation of technology system of China rabbit industry national survey group for technology system of rabbit industry. *Liu Hanzhong (China)* 460
- Rabbit producers organization in the state of Mexico. *Mendoza B.J., Díaz Z.S., Velázquez O.V., Alonso F.M.U. (Mexico)* 461
- Social and economic contribution of rabbit production in the state of Mexico. *Mendoza B.J., Díaz Z.S., Velázquez O.V., Alonso F.M.U., Ortega B.E. (Mexico)* 462

Influence of temperature-humidity interaction on heat and moisture production in rabbit. <i>Ogunjimi L.A.O., Oseni S.O., Lasisi F. (Nigeria)</i>	463
A proposal for the genetic improvement of rabbits for smallholder units in Nigeria. <i>Oseni S.O. (Nigeria)</i>	464
Research output in rabbit science and production emanating from Sub-Saharan Africa - Implications for future research and development efforts. <i>Oseni S.O. (Nigeria)</i>	465
Smallholder rabbit production in southwestern Nigeria: current status, emerging issues and ways forward. <i>Oseni S.O., Ajayi B.A., Komolafe S.O., Siyanbola O., Ishola M., Madamidola G. (Nigeria)</i>	466
Outdoor rearing system for fattening rabbits: effect of group size. <i>Paci G., Mozzoni C., Prezioso G., D'Agata M., Russo C. (Italy)</i>	467
Strategy on the development of small-and medium-scale rabbit farming based on farmers cooperation. A case of rabbit production in Indonesia. <i>Raharjo Y.C. (Indonesia)</i>	468
Productive performance of six genotypes of fattening rabbits fed with seven commercial feeds. <i>Suárez-López J.R., Becerril-Pérez C.M., Pro-Martínez A., Cuca-García J.M., Torres-Hernández G., Sosa-Montes E. (Mexico)</i>	469
Hungarian rabbit meat export in the aspect of the world market. <i>Szendrő Zs., Szendrő É. (Hungary)</i>	470
Circadian changes of rectal temperature and feed and water intake in adult rabbits under heat stress. <i>Villalobos O., Guillén O., García J. (Venezuela)</i>	471
Effect of cage density on performance of fattening rabbits under heat stress. <i>Villalobos O., Guillén O., García J. (Venezuela)</i>	472
A joint project to synthesize new lines of rabbits in Egypt and Saudi Arabia: emphasis for results and prospects. <i>Youssef Y.K., Iraqi M.M., El-Raffa A.M., Afifi E.A., Khalil M.H., García M.L., Baselga M. (Egypt)</i>	473
Productivity of rabbit does of a white population in Algeria. <i>Zerrouki N., Hannachi R., Lebas F., Berchiche M. (Algeria)</i>	474

GENETICS

METHODS, CRITERIA, TECHNIQUES AND GENETIC RESPONSES FOR RABBIT SELECTION: A REVIEW

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ABSTRACT

Based on an extensive review of the literature, the most common selection criteria used in selection programs for maternal lines were related to litter size at birth or at weaning, while in other cases selection programs were practiced for litter size at birth and weight at nine weeks, number of teats, traits related to the ability of the doe to lactate and nourish the progeny (e.g., weight at weaning, litter weight at weaning or total milk production), and in few cases selection for hyperprolificacy and longevity have been introduced recently. Selection for ovulation rate and uterine capacity using new reproductive techniques has been successfully performed, which can be used as an alternative to improve litter size and prenatal survival. For paternal lines, post-weaning daily gain or marketing weight are commonly selected on individual basis. New techniques, such as laparoscopy, ovariectomization, cryopreservation of embryos and semen, TOBEC (Total Body Electrical Conductivity) and X-ray scanning computerized tomography (CT), were used as tools to assist in selection programs. The application of molecular techniques in selection of rabbits so far has had a limited impact on farm animals. Major genes with large effects on litter size components have been identified. Family index or BLUP are the common procedures used to evaluate the animals genetically in selection experiments. Canalization selection model was recently used in evaluation of does and bucks in selection experiments and this model incorporated the classical genetic effects acting on the mean production level, in addition to the other genetic effects acting on the residual variance. Several synthetic maternal, paternal and multi-purpose lines were developed using different criteria and methods of selection. Selection responses were estimated commonly by regressing the estimates of the breeding values on the generation's number, or by using the control populations or the population selected divergently, or by comparing the contemporaries of two different generations using the frozen embryos of the same line. Selection responses obtained in crossbred rabbits could be periodically evaluated by estimating the crossbreeding parameters in the cross (e.g., direct and maternal additive, direct and maternal heterosis, recombination effects, etc.), or by comparing heterosis values obtained from an experiment with those of contemporary commercial farms, or by evaluating the selection responses at different stages of the programme by carrying out contemporary comparisons among purebred and crossbreds.

Studies that have compared selection responses in crossbreds with the responses in pure lines, have observed slightly higher responses in the crossbreds. Direct selection responses per generation estimated for litter size born or weaned were low or slightly moderate and ranged from 0.081 to 0.180 rabbits, while the correlated responses ranged from 0.03 to 0.18 ova for ovulation rate, and 2.0 to 3.7% for prenatal survival. Depending on modified components of litter size, selection for uterine capacity produced responses that were similar to that obtained in direct selection for litter size. Improvement in litter size caused by selection for uterine capacity was not greater than the improvement obtained from direct selection for litter size (approximately 0.1 rabbits per litter per generation). Does selected for litter size at weaning presented significant responses in feed intake (3%) and milk yield (6%). A response of 62 g per litter was recorded when selecting for litter weight at weaning, with a correlated response of 0.17 rabbits for litter size born and weaned. Estimates of direct selection responses per generation were moderate and ranged from 8.7 to 12.6 g for weaning weight, 18 to 68 g for marketing weight, 0.45 to 1.73 g/d for weight gain from weaning to marketing, and 0.05 to 0.27 g feed per g gain for feed conversion from weaning to marketing, which was associated with an increase in correlated responses in adult weight and feed consumption, but with decreasing rate in feed conversion. Selection for growth rate has little or somewhat moderate effects on carcass characteristics and meat quality when rabbits were selected at the same stage of maturity, which was associated with increases in intestinal content and decreases in dressing out percentage and fat deposits, and ultimately in pH in muscle and water holding capacity of the meat. Selection for litter weight at weaning achieved considerable responses in growth rate with maintaining high litter components and feed conversion. By selection, total fleece weight increased significantly associated with correlated improvements in live body weight and fleece qualities (bristle length and diameter, follicle ratios, compression, resilience, and fibre diameter). Selection responses estimated by different methods were in good agreement to most studies reviewed.

INTRODUCTION

Long-term selection experiments carried out in rabbits for more than 10 generations throughout the world were few compared to the major species of livestock (Baselga *et al.*, 1992; Rochambeau *et al.*, 1994, 1998; Lukefahr *et al.*, 1996; Gómez *et al.*, 2000; García and Baselga, 2002a, 2002b, 2002c; Blasco *et al.*, 2005; Khalil *et al.*, 2005). However, selection for productivity in rabbits has been performed in three directions: (1) to improve prolificacy and lactation (maternal lines), (2) to improve growth rate and carcass and meat quality traits (paternal lines) and (3) to improve total litter traits and growth traits together (multi-purpose lines). In the first case, selection was practiced mainly for litter size at birth or weaning, while in the second case the weight gain and/or carcass traits were regarded as the most important selection criteria, and the third case dealing with selection for litter size, litter weight, milk yield, and post weaning growth traits. As a result of selection, some synthetic maternal lines were developed in France (INRA2066, INRA2666 and INRA1777), in Spain (lines A,

V, PRAT, H and LP), in Saudi Arabia, (line Saudi-2), in Egypt (line APRI) and in Uruguay (line NZW and V), while the synthetic paternal lines developed were line R in Spain, Altex in USA, White Pannon in Hungary, Alexandria in Egypt, and Saudi-3 in Saudi Arabia. But, the synthetic multi-purpose lines developed were INRA1077 in France, Caldes in Spain, Botucatu in Brazil and Moshtohor in Egypt.

In selection experiments, several methodologies have been proposed to estimate selection responses. One of them was based on the estimates of the breeding values on generations and this approach depends on the genetic parameters and the model used (Estany *et al.*, 1992; Sorensen and Johanson, 1992; Garreau *et al.*, 2000; Gómez *et al.*, 2000; Moura *et al.*, 2001; Ibañez *et al.*, 2006). The other methodologies do not depend on the genetic parameters and the model itself, but are dependent on another approach through use of a control population, which could be an unselected population (Rochambeau *et al.*, 1989, 1994, 1998; Lukefahr *et al.*, 1996; Sánchez *et al.*, 2004b), or using the population selected divergently (Moura *et al.*, 1997; Gondret *et al.*, 2002; Blasco *et al.*, 2005; Mocé *et al.*, 2005; Santacreu *et al.*, 2005; Rafat *et al.*, 2007, 2008) or using the cryopreserved populations that are free of genetic drift to compare the contemporaries of two different generations (Santacreu *et al.*, 2000; Baselga and García, 2002; García and Baselga, 2002a, 2002b, 2002c; Blasco *et al.*, 2003; Piles and Blasco, 2003; Gil *et al.*, 2006).

Molecular technologies were recently used to identify the genetic diversity, gene mapping and DNA fingerprinting (Bolet *et al.*, 2000; Van Haeringen *et al.*, 2001, 2002; Korstanje *et al.*, 2003; Sacharczuk *et al.*, 2005; Chantry-Darmon *et al.*, 2006). But, the results of QTL analysis for productive and reproductive traits are not sufficiently available to be used in selection programs.

The main objectives of this article are concentrated in dealing with reviewing, generalising, and evaluating the selection experiments carried out in rabbits in some parts of the world for productive and reproductive traits in terms of: (1) methodologies used in selection; (2) criteria and techniques assisted in selection programs; (3) application of molecular techniques in selection; (4) estimation of direct and correlated selection responses; (5) programs of selection performed in breeds and those used to develop new synthetic lines.

METHODOLOGIES USED IN SELECTION

Methods applied in selection programs

Selection methods used to develop new synthetic lines of rabbits are more complicated for maternal lines than for paternal lines. This complexity is due to the fact that litter size traits are not expressed in both sexes and to the low values of heritabilities for reproductive traits (Baselga, 2004). So, it is necessary to consider as many individual and relative records as possible in the genetic evaluation of does and bucks. In addition, the generation interval for selection in maternal lines is longer than in selection of paternal lines and, consequently, it could be necessary to take into account some environmental and physiological effects in the models of evaluation (Armero *et al.*, 1995; Baselga and García, 2002; García and Baselga, 2002a, 2002b, 2002c).

The BLUP procedure was the most common procedure used in evaluation of does and bucks in selection experiments in rabbits (e.g. Estany *et al.*, 1989, 1992; García-Ximenez *et al.*, 1996; Gómez *et al.*, 1996, 2000, 2002b; Moura *et al.*, 1997; Rochambeau *et al.*, 1998; Szendrő *et al.*, 1998; Bolet and Saleil, 2002; Garreau and Rochambeau, 2003, Khalil *et al.*, 2005, 2007; El-Raffa 2007; Iraqi *et al.*, 2008; Sánchez *et al.*, 2008; Youssef *et al.*, 2008). In the last decade, a canalization procedure was also used in selection to reduce the sensitivity of selection for the environmental effects (Scheiner and Lyman, 1991; Hill, 2002). To provide an evidence for the control of environmental sensitivity, a statistical model has been proposed by San Cristobal *et al.* (1998) incorporating the classical genetic effects acting on the mean production level in addition to the other genetic effects acting on the residual variance. A multiple-trait model has a greater risk in yielding biased estimates of the genetic parameters (i.e., heritabilities, genetic correlations and selection responses) than a repeatability animal model, and, thus, all conclusions about the advantages of multiple-trait models should be evaluated with caution (Piles *et al.*, 2006).

Estimation of selection responses

Developing different reproductive techniques could facilitate the estimation of selection responses for productive traits in rabbits. Piles and Blasco (2003) suggested that response to selection for increased growth rate could be estimated in three ways: (1) comparing the selected group with the control group in a model without genetic effects, (2) comparing the selected group with the control group with a model that also included the genetic values of the animals, and (3) estimating the genetic values of all animals of the selection process then estimating the selection response as the average of the estimated genetic values in each generation. However, the most common methods used to estimate selection responses could be outlined as:

- 1) Using the control population that must be developed parallel to the selected population but without carrying out any selection (Rochambeau *et al.*, 1989, 1994, 1998; Sorensen and Johanson, 1992; Baselga, 2004; Sánchez *et al.*, 2004b) (i.e. the control population must be raised contemporaneously and under the same environment as the selected population). The control population has the advantage of providing information independent of the model used for the analysis of selected data. The main problems in using the control population in long-term experiments are: (a) the genetic drift acts on the control populations (usually for small size) and the estimate of the response to selection may be biased, and (b) the need for experimental facilities to be used in applying the selection program. Piles and Blasco (2003) stated that using frozen control populations have better advantages in optimizing the experimental facilities to reduce the genetic drift. Some bio-techniques, such as cryopreservation of embryos or semen, are used to avoid the disadvantages of maintaining the control population without selection. Cryopreserved control populations eliminate the effects of unintended selection on related traits that often occur and also decrease the effects of natural selection.
- 2) Comparing the contemporaries of two different generations by using frozen embryos of the same line (Santacreu *et al.*, 2000; García and Baselga, 2002a; Piles and Blasco, 2003; Gil *et al.*, 2006).

- 3) Using divergent selection to study the differences between two lines selected contemporarily in two directions, one direction to increase the trait and the second direction to decrease it, then contrasting both lines against the control population (Moura *et al.*, 1997; Santacreu *et al.*, 2000, 2005; Gondret *et al.*, 2002; Blasco *et al.*, 2005; Mocé *et al.*, 2005; Rafat *et al.*, 2007, 2008). In this concept, for example, Santacreu *et al.* (2005) estimated the correlated responses after 10 generations of divergent selection for litter size and its components (ovulation rate, and embryo survival and fetal survival) by contrasting both lines against a cryopreserved control population.
- 4) Using statistical methods such as mixed-model methodology and Bayesian approach that were used to estimate the genetic trends or selection responses and that are dependent on the model has been used (García and Baselga, 2002a, 2002b; Blasco *et al.*, 2005). Without the control population, Sorensen and Kennedy (1986) used mixed-model methodology to estimate the genetic response, but the results obtained by this method were highly dependent on the genetic parameters and the model used. Sorensen *et al.* (1994) provided a Bayesian way of estimating the selection response that has the advantage of taking into account the uncertainty about the variance components. Lukefahr *et al.* (1996) using mixed-model methodology estimated the responses of selection from regressing the breeding values (obtained from mixed-model analyses) or from regressing the differences between selected line and control line (from Richardson's method) on generation number. They reported that correlated responses in selected line for weaning weight and daily weight gain tended to be consistent between the two procedures. Both methods are model-dependent, and their reliabilities are dependent on the model proposed for the analysis. However, some selection experiments were analysed at the same time using two types of methods, and in most cases responses estimated by both types of approaches were in good agreement, but not always (Rochambeau *et al.*, 1998; García and Baselga, 2002a, 2002b, 2002c; Blasco *et al.*, 2003, 2005; Piles and Blasco, 2003; Tudela *et al.*, 2003; Mocé *et al.*, 2005; Santacreu *et al.*, 2005). Piles and Blasco (2003) estimated the direct selection responses in an experiment using the bio-technique of the frozen embryo transfer or using the statistical Bayesian approach; but both methods yielded similar estimates of responses.

Selection responses obtained from crossbred rabbits

In crossbreeding programmes followed by selection, it is necessary to evaluate the response of selection obtained in crossbred rabbits. Such an evaluation could be performed using one of the following approaches:

- 1) Evaluating crossbreeding parameters in the cross periodically with the aim of estimating these parameters (e.g., direct and maternal additive and heterosis, recombination effects) as performed in France, Spain, Egypt, Saudi Arabia, Brazil, etc.
- 2) Comparing heterosis estimates obtained from an experimental station with those of contemporary experiments on commercial farms as stated by Brun and Saleil (1994) who found that estimates of heterosis were remarkably similar for

total litter size and number born alive, but lower on commercial farms than at experimental station for litter size at weaning.

- 3) Evaluating the genetic selection responses at different stages of the programme by conducting the contemporary comparisons among purebred and crossbreds as performed by Tudela *et al.* (2003) in France and Costa *et al.* (2004) in Spain. In these comparisons, the maternal lines involved in the French experiment were INRA1007 (rabbits of 30th generation of selection) and INRA9077 (control line), while in the Spanish experiment the maternal lines were A and V and line A with two different generations of selection. In the French experiment, both lines were crossed with another French line and the difference in total litter size between both types of crossbred does was 1.43 rabbit, a little higher than expected from selection in INRA1077 (1.12 rabbit). In the Spanish experiment, evaluation was performed on crossbred does from mating does of V line to bucks of line A, while crossbred rabbits were the progeny from crossing crossbred does with bucks of line R. Results of this Spanish experiment could be summarized as follows: (a) all selection responses in litter size traits were in favour of crossbreds relative to purebreds since differences in total litter size, number born alive and number at weaning were 0.83, 1.16 and 0.74 rabbit, respectively (i.e., responses in crossbred does were higher than expected from the responses evaluated in the pure lines), (b) responses in crossbred progenies were lower than expected, since the response in post-weaning daily gain was 0.6 g/d, and (c) responses in feed conversion index improved.

APPLICATION OF MOLECULAR TECHNIQUES IN SELECTION

Major genes

Bosze *et al.* (2002) indicated that there was a major gene affecting litter size and that this gene gives a good evidence for a QTL mapping to be used in selection experiments. Argente *et al.* (2003a) and Santacreu *et al.* (2005) performed a complex segregation analysis for data of 10 generations of selection and they reported that there was a major gene with a large effect on implanted embryos and embryo survival and with a moderate effect on ovulation rate, foetal and prenatal survival and uterine capacity. However, this complex segregation analysis is imprecise, but these results agree with those observed by Blasco *et al.* (2005) in the first and second generation of selection. Hence, uterine capacity is highly genetically correlated with litter size as suggested by Argente *et al.* (2000), an asymmetric response in litter size should occur.

QTL analysis

Fadiel *et al.* (2003) analyzed 160 genes of rabbits depending on gene bank by providing useful information for designing more effective PCR primers in QTL analysis. Korstanje *et al.* (2003) mapped the individual rabbit chromosomes and found that the linkage group XI linked to chromosome 3 and the linkage group VI

linked to chromosome 5. They also constructed four new linkage groups assigned to chromosomes 6, 7, 12 and 19. Chantry-Darmon *et al.* (2006) built up the first genetic map for Angora and Albino rabbits using 111 markers (109 microsatellites markers and two phenotypic markers). Van Haeringen *et al.* (2001, 2002) determined 226 polymorphisms between two inbred strains of rabbits by using 15 primer combinations of AFLP markers. They reported for the first time the first genetic male map in rabbits which has a distance of 583 cM. They found four QTLs with a LOD score (log of odds) larger than 1.9 and they identified also the QTL for the hematocrit value and for three parameters responsible for cholesterol metabolism [basal serum total cholesterol level (mM), serum total cholesterol response (AUC), and relative adrenal gland weight (mg/kg body weight)].

In divergent selection in rabbits for open-field activity (OFA), Sacharczuk *et al.* (2005) reported that selection has resulted in differences in DNA fingerprinting pattern and genetic parameters of diversity, and also scanned DNA fingerprinting profiles; leading to search for minisatellite alleles potentially linked to genes determining the trait under selection. Using rabbits derived from the 8th generation of the lines selected for high (H) or low (L) levels of OFA, locomotors OFA were profiled for DNA fingerprinting and the analysis of band patterns for individual and pooled DNA fingerprints revealed that a specific band in the L line at 15 kbp was detected, while in the H line specific bands were not detected; providing evidence of a possible linkage between minisatellites and OFA in rabbits, demonstrating that studies on H and L lines may give rise to a new strategy in animal breeding and selection.

Khalil *et al.* (2008) used RAPD markers to search for the linkage between markers and quantitative traits. They used 526 rabbits in this analysis from a sire-granddaughters design in their selection program. From a total of 40 primers (10-mer) used in their study, five primers (OPA12, OPA19, OPA20, OPF09, and OPF12) were able to identify five polymorphic fragments at molecular weights of 1500, 1100, 1200, 700 and 900 bp, respectively, and only three markers of these markers (OPF12₉₀₀, OPA19₁₁₀₀, and OPF09₇₀₀) showed significant associations with phenotypic traits, which indicated the presence of linkage between the three markers for litter weights at birth, 7 and 21 days, and at weaning, litter gain at interval of 0-21 days, pre-weaning litter mortality, milk yield at lactation intervals of 0-7 and 0-21 days, and body weight at 4 and 8 weeks of age.

DIRECT AND INDIRECT SELECTION FOR DOE TRAITS

Selection criteria

The most common direct criteria used in selection programs of maternal lines were related with litter size at birth or at weaning (Estany *et al.*, 1989; Gómez *et al.*, 1996, 2002b; Rochambeau *et al.*, 1998; Capra *et al.*, 2000; El-Raffa, 2000; Baselga and García, 2002; García and Baselga, 2002a, 2002b). In some cases, selection criteria included litter size at birth and weight at nine weeks (Bolet and Saleil, 2002), number of teats (Rochambeau *et al.*, 1988), while in other cases selection programs were practiced for traits related with the ability of the doe for lactating and nourishing

the progeny, such as weight at weaning (Garreau and Rochambeau, 2003), litter weight at weaning or total milk production (Khalil *et al.*, 2002; Al-Saef *et al.*, 2008; Iraqi *et al.*, 2008; Youssef *et al.*, 2008). Fortun-Lamothe (2003) and Quevedo *et al.* (2006b) demonstrated that lactation is a priority trait for selection of the crossbred doe by taking into account competition between foetal growth and lactation, which is unfavourable for foetal growth. Selection for ovulation rate and uterine capacity has been successfully performed as indirect ways for improving prenatal survival and litter size in rabbits (Bennett and Leymaster, 1989; Ibañez *et al.*, 2004, 2006; Blasco *et al.*, 2005; Mocé *et al.*, 2005; Santacreu *et al.*, 2005). Rabbit birth weight presents a great variability within each litter (Bolet *et al.*, 1996, 2006, 2007) and reducing this heterogeneity might be useful in selection program since it induces a high mortality as a result of losses in the weakest rabbits.

Selection for hyperprolificacy in maternal lines was a successful way to improve litter size in rabbits (Cifre *et al.*, 1998a; Santacreu *et al.*, 2000). Longevity has been introduced recently in rabbit selection programs (Sánchez *et al.*, 2004a, 2008) although it is difficult to improve this trait through conventional breeding methods because of the low heritability and the time needed to obtain information. Sánchez *et al.* (2004a) concluded that both longevity and litter size are not antagonistic objectives in breeding programs because selection for one does not influence the other.

Techniques assisted in selection programs

New techniques (laparoscopy, ovariectomization, cryopreservation of embryos and semen, etc.) were used commonly as tools assisted in selection programs in rabbits. Blasco *et al.* (1994) and Bolet *et al.* (1994) reported that litter size in unilaterally ovariectomized does could be used to estimate uterine capacity in rabbits. Also, it is possible to observe the number of corpora lutea and implantation sites by laparoscopy without impairing litter size as verified by Santacreu *et al.* (1994, 1996).

Selection methods and procedures used

Rabbit litter size is mainly determined by the doe component, whereas the buck has a very small effect; therefore, it seems unnecessary to include the buck component in selection models (Piles *et al.*, 2006). In Spain, Estany *et al.* (1989) applied a family index including four sources of information (doe, dam of doe, full sisters and paternal or maternal half-sisters) to evaluate does or bucks for litter size at weaning of line A. Gómez *et al.* (1996) and Rochambeau *et al.* (1998) used a BLUP procedure in evaluation of does and bucks since this procedure is quite different from a family index in that some environmental and physiological effects are considered in the model. Response to selection would probably be the same if selecting for litter size under a repeatability animal model or using a selection index and this is because the accuracies of predicted breeding values obtained under the two models are nearly equal (Piles *et al.*, 2006).

A selection experiment for ovulation rate was practiced in the second gestation by laparoscopy in Spain where the animals were derived firstly from a synthetic line selected for litter size for 12 generations, then for uterine capacity for 11

generations, and then 5 generations in which selection was relaxed (Ibañez *et al.*, 2004). A divergent selection experiment was carried out by Garreau *et al.* (2004b) at the INRA experimental farm and a new model incorporating the genotypic value for the mean and the genotypic value for the residual variance (canalization procedure) was applied to select for homogeneity of birth weight in the litter and to estimate the correlated responses in other traits and to provide new issues relevant to validate this innovative statistical method.

Selection effect and direct responses

As presented in Table 1, genetic responses obtained from long-term selection experiments for litter size and other litter traits were found to be moderate. Some responses in litter traits were estimated exclusively by mixed-model methods (Estany *et al.*, 1989; Rochambeau *et al.*, 1994; Gómez *et al.*, 1996) where the estimates ranged from 0.05 to 0.129 rabbits born alive or weaned per litter and generation. In other cases, Rochambeau *et al.* (1998), García and Baselga (2002a, 2002b), and Tudela *et al.* (2003) reported that direct responses ranged from 0.08 to 0.14 rabbits for total number born, number born alive or weaned per litter and generation; responses estimated as genetic trends or by mixed-model were nearly similar. These responses were lower than expected and this could be attributed to: (1) additive genetic variance for litter size at weaning was low, (2) heterogeneity between parities was high (Baselga *et al.*, 1992), (3) correlations between direct and maternal effects were negative, and (4) intensity of selection was low.

Mocé *et al.* (2004, 2005) and Blasco *et al.* (2005) found that direct response to selection for uterine capacity was symmetric and they stated that selection for uterine capacity in rabbits leads to modifications in embryonic and fetal survival. The divergence rate between high and low lines in such selection experiments were 1.01 rabbits for litter size at birth, 0.88 rabbits for number born alive, 0.16 ova for ovulation rate, 0.46 embryo for implanted embryos, 0.03 embryo for embryo survival, 0.09 fetus for fetal survival, and 0.08 for prenatal survival. Garreau *et al.* (2004b) reported that selection for homogeneity of birth weight in the litter had no significant influence on other litter traits.

Table 1: Direct and correlated selection response per generation obtained for doe traits in selection experiments selected for litter size or ovulation rate or uterine capacity.

Authors	Breed or line	Methodology	Direct and/or correlated selection responses*
Selection for litter size:			
Gómez <i>et al.</i> (1996)	Line PRAT	Genetic trend using BLUP methodology	LSW = 0.09 rabbit per year
Rochambeau <i>et al.</i> (1998)	INRA1077	Genetic trend using a control population and applying BLUP methodology	LSW = 0.081 rabbit per generation
García <i>et al.</i> (2000a), (2000b)	Line V	Comparing contemporaries using cryopreservation (vitrification) population	LSW = 0.088 rabbit per generation No correlated responses were obtained for growth, feed consumption and feed efficiency traits
		<ul style="list-style-type: none"> Genetic trend using cryopreservation control population and mixed-model methodology 	Direct responses of LSW= 0.51 rabbit per litter or 0.085 rabbits per generation; with correlated responses of LSB= 0.62 rabbit per litter or 0.103 rabbit per generation; NBA= 0.57 per litter; OR = 1.08 per litter or 0.18 ova per generation; IE= 0.74 per litter; DF= 0.05 per litter; IR = - 0.14 per litter; FS = 2.18 per litter; PS = 0.34 per litter
García and Baselga (2002a), (2002b)	Line V	<ul style="list-style-type: none"> Contemporaries using cryopreservation control population and mixed-model methodology Genetic trend using mixed-model methodology 	Direct responses of 0.77 weaned rabbit per litter; with correlated responses of OR = 0.03 ova per litter; IE = - 0.15 per litter; DF = - 0.63 per litter; IR= -1.44 % per litter; FS = 5.7 % per litter; PS= 3.7 % per litter; LSB = 0.78 rabbit per litter; NBA = 0.74 rabbit per litter; Number at 63 d = 0.5 rabbit per litter LSB = 0.161 rabbit per generation; NBA = 0.175 rabbit per generation; LSW = 0.175 rabbit per generation; No at slaughter = 0.191 rabbit per generation
Selection for uterine capacity:			
Blasco <i>et al.</i> (2005)	Synthetic population derived from crossing NZW and Californian	Genetic trend in divergent selection using Bayesian method	Divergence rate = 1.5 rabbits per generation
Mocé <i>et al.</i> (2005)		Genetic trend in divergent selection using Bayesian method	Per litter: LSB = - 0.008 rabbits; NBA = 0.20 rabbits; OR = - 0.009 ova; IE = - 0.60; ES = - 0.05; FS = 0.08; PS = 0.02
Santacreu <i>et al.</i> (2005)		Genetic trend in divergent selection using Bayesian method	Per litter: LSB = 0.47 rabbits; NBA = 0.15 rabbits; OR = - 0.32 ova; IE = - 0.28; ES = 0.04; FS = 0.04; PS = 0.05
Selection for ovulation rate:			
Ibañez <i>et al.</i> (2006)	Line V	Genetic trend in phenotypic selection using Bayesian method in analysis	Per litter: OR = 1.8 ova; IE = 1.44; LS = 0.49 rabbits; Prenatal survival = - 0.009; ES = 0.05; FS = - 0.09

*LSB: litter size at birth; NBA: number born alive; LSW: litter size at weaning; OR: ovulation rate; IE: implanted embryos; DF: number of dead foetus; ES: embryo survival; IR: implantation rate; FS: foetus survival; PS: prenatal survival.

Selection effect and correlated responses

Rochambeau *et al.* (1988) found that number of teats increased indirectly in a population selected for litter size at weaning respective to the control population. As shown in Table 1, selection for ovulation rate was associated with a correlated increase in litter size compared to direct selection for litter size as reported by Santacreu *et al.* (2005) in Spain, while selection for uterine capacity was associated with indirect response in number of teats as reported by Mocé *et al.* (2000) in France.

Selection for litter size in rabbits showed that the magnitudes of correlated responses in the components of litter size were varied from one experiment to another (Quevedo *et al.*, 2006a, 2006b). García and Baselga (2002a, 2002b) showed that selection for litter size was associated with an increase in ovulation rate (0.18 more ova for ovulation rate per generation) with non-significant changes for prenatal survival. However, differences for embryo survival may be due to differences in fertilization rate, embryo viability, or other factors related to the oviduct or/and uterine physiology of the doe.

Ibañez *et al.* (2004) reported that selection response for ovulation rate was 0.97 ova, while the correlated responses for implanted embryos and litter size were found to be 0.79 embryo and 0.32 rabbit, respectively. After 10 generations of divergent selection, the correlated responses obtained by Santacreu *et al.* (2005) for litter size and its components (ovulation rate, embryo survival, and fetal survival) were asymmetric, divergence rate between high and low lines was 2.35 rabbits, mainly because of higher correlated response in the low line (1.88 rabbits).

As shown in Table 1, selection for uterine capacity for several generations has been performed successfully in rabbits, and it produced a response that was similar to that found in experiments in which direct selection for litter size was practiced (Argente *et al.*, 1997; Blasco *et al.*, 2000, 2005; Santacreu *et al.*, 2005). The observed increase in litter size caused by selection for uterine capacity was not greater than the improvement obtained from direct selection for litter size (approximately 0.1 rabbits per litter per generation), while the correlated response in number born alive was asymmetric and less than that for litter size (Rochambeau *et al.*, 1998; García and Baselga, 2002a, 2002b). Argente *et al.* (2000) found a large genetic correlation between litter size and uterine capacity, which supports the non-asymmetric response in uterine capacity as detected by Blasco *et al.* (2005). The correlated selection divergence rate for uterine capacity between high and low lines reported by Santacreu *et al.* (2005) were 2.35 rabbits in litter size at birth (reflecting the role of a major gene affecting uterine capacity and litter size as stated as Bosze *et al.*, 2002 and Argente *et al.*, 2003a), associated with 1.84 rabbits for number born alive, 0.43 ova for ovulation rate, 1.79 embryo for implanted embryos, 0.10 embryo for embryo survival, 0.13 fetus for fetal survival, and 0.19 for prenatal survival, while the respective selection difference between high and control line were 0.47, 0.15, -0.32, -0.28, 0.0, 0.04, and 0.05.

Quevedo *et al.* (2005, 2006b) found that does selected for litter size at weaning presented significantly higher feed intake (3%) and milk yield (6%) during the first 21 days of lactation, while the responses at late stages of lactation were not significant. Selection effects on weaning weight (28 days) reported recently by Quevedo *et al.* (2006) agrees with the results obtained by García and Baselga (2002c), who found

that the effects of selection for litter size at weaning on weaning weight corrected for litter size at birth were limited. For data not corrected for litter size, the individual weaning weight was decreased by selection (Costa *et al.*, 2004). In contrast, Khalil *et al.* (2004) reported that selection responses for litter weight at weaning was 62 g per litter; associated with a correlated response of about 0.17 rabbit per litter per generation for litter size born and weaned.

Selection programs in synthetic maternal lines developed

Summaries for maternal lines developed through selection are presented in Table 2. In France, a maternal line of INRA2066 was directly selected for litter size at birth and this line was used to produce the most common parental female of INRA1077 x INRA2066 (Rochambeau, 1998; Bolet and Saleil, 2002; Garreau *et al.*, 2004a). A new selection experiment was started in 2003 to develop the line INRA1777 through selection for litter size at birth, together with individual weaning weight (Garreau and Rochambeau, 2003); longevity was added later as a new criterion (Garreau *et al.*, 2001, 2004a).

In Spain, long-term selection experiments were carried out to produce synthetic lines to be used on commercial farms. Details concerning these new lines were presented by Baselga (2004). In 1976, the Spanish line A was developed from NZW rabbits and selection was practiced using a family index including litter size at weaning of the doe, dam, full-sisters and half-sisters. The Spanish line V was founded in 1981 as a synthetic line and this line was selected for litter size at weaning using a BLUP procedure under an animal-repeatability model (Estany *et al.*, 1989). In 1992, the PRAT line was developed in Barcelona from a closed population (with 178 crossbred animals). In this line, selection was practiced for litter size at weaning using a BLUP procedure under an animal-repeatability model (Gómez *et al.*, 1996). The foundation of line H was based on the detection of hyperprolific does and this line is selected for litter size at birth. Recently, a new maternal line in Spain named LP was established following a scheme similar to that applied in selection for hyperprolificacy taking hyperlongevity and litter size as selection criteria. Details concerning constitution and evaluation of such a long-lived productive line of rabbits were presented by Sánchez *et al.* (2008).

In Saudi Arabia, Saudi 2 was synthesized from crossing Saudi Gabali with V-line rabbits (75% from V line and 25% from Saudi Gabali) and selected for litter weight at weaning and individual weight at 84 d. Details concerning the development of this new line were presented by Khalil *et al.* (2002, 2005) and Al-Saef *et al.* (2008).

In Egypt, Baladi Red bucks (B) were mated with V line does to produce a new synthetic line named APRI with genetic structure of $((\frac{1}{2}B\frac{1}{2}V)^2)^2$. This line was selected for litter weight at weaning. In Uruguay, two lines were developed through selection for litter size at weaning, where the first line named Uruguay NZW and the second line named Uruguay V-line (Capra *et al.*, 2000).

Table 2: Selection programs in synthetic maternal lines developed in some parts of the world.

Synthetic line and authors	Founder breeds	Selection criteria	Selection methodology	Number (interval) of generations	Selection response per generation+
French selection experiments:					
INRA2066, Bolet and Saleil (2002)	Californian, Giant Himalayan	Birth litter size	BLUP procedure	More than 34 generation	LSB= 0.12 rabbit per litter; LSW= 0.07 rabbit per litter; LWW= 34 g per litter; WW= -4.4 g per rabbit
INRA2666, Bolet and Saleil (2002)	INRA2066 and V-Line	Litter size	BLUP procedure		
INRA1777, Garreau and Rochambeau (2003)	INRA1077	Birth litter size + weaning weight + longevity	BLUP procedure	More than five generations	
Spanish selection experiments:					
Line A, Estany <i>et al.</i> (1989)	NZW	Weaning litter size	Family index including litter size at weaning of doe, dam, full-sisters and half-sisters	More than 33 generation (9 months)	LSW= 0.1 rabbit
Line V, Estany <i>et al.</i> (1989)	Four specialized maternal lines	Weaning litter size	BLUP procedure under an animal-repeatability model	More than 30 generation (9 months)	LSW= 0.03 rabbit
Line PRAT, Gómez <i>et al.</i> (1996), (2002b)	A closed population with crossbred animals	Weaning litter size	BLUP procedure under an animal-repeatability model		
Line H, García-Ximenez <i>et al.</i> (1996)	Hyper-prolific V line does	Birth litter size	BLUP with applying embryo cryopreservation technique	More than 11 generation (9 months)	
Line LP, Sánchez <i>et al.</i> (2008)	Line H	Hyper-longevity + Birth litter size	BLUP procedure		
Selection experiments in developing countries:					
Saudi-2, Saudi Arabia, Khalil <i>et al.</i> (2005)	V line and Saudi Gabali	Weaning litter weight + 84-d weight	BLUP procedure under an animal-repeatability model	More than 10 generations (9 months)	LSB= 0.18 rabbit per litter; LSW= 0.16 rabbit per litter; LWW= 62 g per litter; WW= 8.6 g per rabbit
APRI, Egypt, Youssef <i>et al.</i> (2008)	V line, Baladi Red	Weaning litter weight	BLUP procedure under an animal-repeatability model	More than 5 generations	
Uruguay NZW, Capra <i>et al.</i> (2000)	NZW	Weaning litter size	BLUP procedure	More than 5 generations	
Uruguay V, Capra <i>et al.</i> (2000)	V line	Weaning litter size	BLUP procedure	More than 5 generations	

+LSB: litter size at birth; NBA: Number born alive; LSW: litter size at weaning; LWW: litter weight at weaning; WW: weaning weight.

DIRECT AND INDIRECT SELECTION FOR GROWTH, CARCASS AND MEAT QUALITY TRAITS

Selection criteria

Selection for rapid growth rate has been largely introduced to develop sire lines to modify the whole pattern of growth, feed efficiency, and tissue composition, thus affecting carcass and meat quality traits. Current programs of rabbit selection normally include terminal sires produced from selection schemes commonly practiced for post-weaning daily gain (Rochambeau *et al.*, 1989; Estany *et al.*, 1992; Hernández *et al.*, 1997, 2004; Moura *et al.*, 1997; Piles *et al.*, 2000, 2004; Gómez *et al.*, 2002a; Sánchez *et al.*, 2004b; El-Raffa, 2007) or for body weight at the market age (Lukefahr *et al.*, 1996; Gondret *et al.*, 2002; Khalil *et al.*, 2002, 2005; Larzul *et al.*, 2005; Al-Saef *et al.*, 2008). In practice, criterion of post-weaning growth is effective in selection programs because it is very easy to record and it has a negative and favourable genetic correlation with feed conversion index, and therefore this trait is very important for an efficient rabbit production (Moura *et al.*, 1997; Piles *et al.*, 2004).

The average daily gain is the preferred trait for selection during post-weaning period, suggesting that individual selection could be used successfully to improve this trait because this trait is less affected by common litter effects than the individual weights at specific ages and it has moderate or high heritabilities that vary in magnitude from 0.13 to 0.48 (Rochambeau *et al.*, 1989; Estany *et al.*, 1992; Ferraz and Eler, 1996; Krogmeier *et al.*, 1994; Lukefahr *et al.*, 1996; McNitt and Lukefahr, 1996; Moura *et al.*, 1997; García and Baselga, 2002c; Piles *et al.*, 2004). Moura *et al.* (1997) stated that selection based on an index including both growth rate and feed conversion ratio would be more efficient for improving feed efficiency than selecting solely for growth rate. Although feed conversion ratio is the most important trait in meat rabbit production (Armero and Blasco, 1992; Piles *et al.*, 2004), this criterion was not considered commonly in selection programs. Baselga (2004) reported that feed conversion index is not used directly in selection because it is expensive to record and would need electronic devices to enable recording of individual feed intake. Since feeding costs represent about 70% of the rabbit meat production costs, feed conversion (g feed per g gain) could be an economic trait in direct selection (Moura *et al.*, 1997; Larzul and Rochambeau, 2005).

Techniques assisted in selection programs

Applying the technique of TOBEC (Total Body Electrical Conductivity), Milisits and Levai (2002) demonstrated that selection for TOBEC value was associated with indirect improvement in carcass composition, observing a difference of 22.6% between animals selected for low and high fat content. A technique of X-ray computerized tomography (CT) was utilized to assess *in vivo* body composition in selection of rabbits (Nagy *et al.*, 2006) and this technique of selection was used successfully in sheep in UK, Australia and New Zealand (Simm, 1987; Jones *et al.*, 2002, 2004). Using such technique in rabbit research are summarised by Romvári *et al.* (1996) and the results of using CT as an aid of selection in Pannon White rabbits were reported by Szendrő *et al.* (1996) and Nagy *et al.* (2006) taking into account

the genetic correlations between average cross-sectional area of *m. Longissimus dorsi* (measured by CT) and a carcass trait (Szendrő *et al.*, 1992). However, high scanning costs in evaluating the animals by CT would be a limiting factor to use such technique in selection program.

Szendrő *et al.* (1996, 2004) selected indirectly for daily gain and carcass quality in Pannon White breed by measuring the average surface of *m. Longissimus dorsi* (L value) at 10.5 weeks of age by using a computerized tomography technique and applying BLUP for L-value to select growing rabbits. A two-step procedure of selection was used where the first step was for daily weight gain between 5 and 10 weeks of age, and the next one was for the L-value obtained from CT scanning. Results demonstrated that rabbits with higher L-values in *m. Longissimus dorsi* were associated with higher meat weight of the hind legs (Szendrő *et al.*, 1992). The effectiveness of selection for carcass traits was confirmed by Metzger *et al.* (2004) and Szendrő *et al.* (2004) using CT technique to compare between different genotypes (Pannon White, Hyplus, and their crosses). In this experiment, BLUP for L-values measured for the scanned animals showed that selection based on CT measurement could be effective to improve carcass traits in rabbits.

Selection methods

In general, individual mass selection was used as the common methodology to select for growth traits in paternal lines of rabbits and this is because this method is the simplest procedure to be applied for heritable traits expressed in both sexes. By this way, time, labour and resources can be reduced and the generation interval could be shortened to about 6 mo (Baselga, 2004). Since average daily gain in weight and feed conversion are moderately to highly heritable, having high and negative genetic correlations between them, applying mass selection for average daily gain was associated with favorable correlated selection responses (Feki *et al.*, 1996; Moura *et al.*, 1997). Lukefahr *et al.* (1996) concluded that individual selection was effective for increasing marketing weight following five generations of selection.

A two-way selection experiment for improving average daily gain and feed conversion was performed by Moura *et al.* (1997) who reported that this program could be performed at least in the first generations of selection without difficulties in measuring feed consumption. Another divergent selection experiment was performed by Larzul and Rochambeau (2005) in order to estimate the consequences of selection for feed efficiency (30-65 d) on growth, feed efficiency, and carcass composition (fatness of the males was estimated using the technique of TOBEC at 65 days of age). Piles *et al.* (2004) concluded that growth rate and feed conversion ratio should be used in an index in order to improve the selection response for feed conversion ratio, which is the most important trait in rabbit production (i.e., feed conversion ratio would be improved by more than 77% if an index including growth rate and feed conversion ratio was used compared to indirect selection using growth rate).

Progeny testing was practiced to select directly for dressing out percentage (Nagy *et al.*, 2006), although the costs of this method are high and it lengthens the generation interval. This method was practiced previously by Varewyck *et al.* (1986) and Szendrő *et al.* (1988).

Selection effect and direct responses

Selection experiments for growth rate in rabbits reporting successful responses in most experiments (Table 3).

Table 3: Direct and/or correlated selection response per generation obtained for growth and carcass traits in selection experiments.

Authors	Breed or line	Selection criteria	Methodology	Direct and/ or correlated selection responses*
Rochambeau <i>et al.</i> (1989)	INRA1077	Growth rate (4-11 wks)	Genetic trend for phenotypic selection using mixed-model	ADG= 0.83 g/d per year
Lukefahr <i>et al.</i> (1996)	F ₂ population of ½ Flemish Giant, ¼ Californian, ¼ Champagne	70-day weight (market weight)	Genetic trend for phenotypic selection using mixed-model	Per generation: W70= 29.4 g; WW = 9.5 g; ADG (28-70 d) = 0.47 g/d; CY = -0.24%; PL = -0.04%; MBR = 0.1
Garreau <i>et al.</i> (2000)	White Pannon	Growth rate (6-10 wks)	Annual genetic trends using BLUP in multiple-trait model.	ADG = 0.64 g/d per year; W10 = 18.5 g per year.
García and Baselga (2002c)	Line V	Litter size at weaning	Contemporaries using control cryopreserved population and mixed-model methodology	Per generation: WW = 1.4 g/d; ADG (28-63d) = 0.11 g/d; W63 = 1.5 g/d; FC = -3.3 g/d feed; FCI = 0.01; For all traits, the responses were less than 0.3% of the population mean.
Piles and Blasco (2003)	Synthetic line selected for growth rate	Growth rate (4-9 wks)	Phenotype selection using control population and Bayesian inferences	Growth rate = 7% relative to the population mean before selection
Hernández <i>et al.</i> (2004)	Line R	Growth rate (9-13 wks)	Contemporaries using control population (differences between selected and control groups)	Per generation: SW = 118 g; CCW = 53 g; DP = - 0.22%; PL = 0.23%; KP = 0.04%; LHP = 0.02%; RCW = 46 g; FP = - 0.3%; LP = 0.6%; MBR = 0.42
Sánchez <i>et al.</i> (2004b)	Line R	Daily gain (28-63 d)	Contemporaries using cryopreserved population (as control) and an animal model	Per generation: ADG= 0.18 g/d; WW= 8.7 g; W63= 94.6 g; FC= 0.05 g feed per g gain

*WW: weaning weight; W10: weight at 10 weeks; W63: weight at 63 d; SW: slaughter weight; W70: marketing weight at 70-d; ADG: average daily gain; CY: carcass yield percentages (hot carcass weight/ preslaughter weight times 100); PL: percent of liver; LP: loin percent; MBR: meat to bone ratio; FC: feed consumption; FCI: feed conversion index; CCW: chilled carcass weight; DP: dressing percent; KP: kidney percent; LHP: set of organs percent (thymus + trachea + esophagus + lungs +heart); RCW: reference carcass weight; FP: dissectible fat percent.

In this concept, direct selection responses for average daily gain or for body weight at market time were verified (Mgheni and Christensen, 1985; Lukefahr *et al.*, 1996; McNitt and Lukefahr, 1996; Moura *et al.*, 1997; Blasco *et al.*, 2003; Piles and Blasco, 2003; Nagy *et al.*, 2006), while in some cases the responses were less than expected (Rochambeau *et al.*, 1989; Estany *et al.*, 1992; Gondret *et al.*, 2002; Sánchez *et al.*, 2004b). Such contradictions in results of selection responses may be due to the overlapping of generations, especially when a control population was not used in the same generation of the selected population, or may be due to the appearance of a disease such as enterocolitis.

The rate of genetic progress in marketing weight per generation (29.4 g or 1.3% per generation) obtained by Lukefahr *et al.* (1996) was similar to the annual genetic improvement often reported for this trait in other livestock species (i.e., producers willing to select for increased 70-day body weight may have genetic improvement in weaning weights and in average daily gains and also in lean-to-bone ratio). Using a control line or mixed-model methodology, direct responses obtained for growth rate from weaning to marketing age were moderate and ranged from 0.45 to 1.73 grams per day per generation for daily weight gain, while the responses for weight at market age ranged between 18 and 68 g per generation (Rochambeau *et al.*, 1989, 1994; Estany *et al.*, 1992; Lukefahr *et al.*, 1996; Moura *et al.*, 1997; Szendrő *et al.*, 1998; Garreau *et al.*, 2000; Khalil *et al.*, 2002, 2005; Hernández *et al.*, 2004; Sánchez *et al.*, 2004b). At commercial slaughter age (9 wk), Blasco *et al.* (2003) and Piles and Blasco (2003) found that selected animals had a higher growth rate of 7% relative to the population mean before selection and the slaughter weight was also higher in the selected group. However, weaning weight remained practically the same where the two methods used in estimating the selection response (control population vs. Bayesian inference) yielded similar results, thus validating the model used for the analysis. Gondret *et al.* (2002) and Larzul *et al.* (2005) found that body weights have been increased by selection, while carcass and muscle traits did not differ significantly between highly selected animals and the animals of a cryopreserved control population.

Selection effect and correlated responses

The estimates of correlated selection responses for growth, feed conversion and carcass traits available in literature are limited and the only available estimates are presented in Table 3. However, selecting at a fixed slaughter weight was associated with increases in feed consumption and decreases in feed conversion (Feki *et al.*, 1996); intestinal content increased, and dressing percentage decreased (Gómez *et al.*, 1998; Pla *et al.*, 1998), fat deposits reduced, ultimately pH in muscle and water holding capacity of the meat diminished (Piles *et al.*, 2000; Gondret *et al.*, 2003).

With regards to correlated selection responses for feed efficiency, resulting from direct selection for growth rate, some investigators (Torres *et al.*, 1992; Feki *et al.*, 1996; Moura *et al.*, 1997; Larzul and Rochambeau, 2005) found that selection for growth rate was associated with an improvement in feed efficiency. Heritability values for growth rate and feed conversion ratio were moderate or high and the correlations between both traits were also moderate or high (-0.82 as cited by Moura

et al., 1997; from -0.4 to -0.49 as cited by Piles *et al.*, 2004). In two elliptical selection experiments, Piles *et al.* (2004) indicated that selection for growth rate was expected to yield a similar correlated response for feed conversion ratio in sire Caldes and R lines of rabbits. In contrast, the effects of selection for growth rate on post-weaning feed intake were not significant (Costa *et al.*, 2004; Piles *et al.*, 2004; Sánchez *et al.*, 2004b).

Few experiments have assessed the consequences of selection for growth rate on carcass and meat quality traits in rabbits. In this respect, Lukefahr *et al.* (1996) reported that selection for marketing weight was associated with favorable correlated responses in daily weight gain and lean to bone ratio. However, the correlated responses were not significantly different from zero for growth traits, but the correlated responses for carcass traits were all positive and significant (Table 3). Comparing Richardson's procedure with mixed-model procedure, similar correlated responses of 0.1 and 0.21 units per generation of selection were observed in the selected line for lean to bone ratio. Hernández *et al.* (2004) indicated that selection for growth rate has little effect on carcass characteristics when rabbits were measured at the same stage of maturity because there was no increase in fat content of the carcass and there was an improvement in the meat-to-bone ratio, while meat quality at the same stage of maturity was affected a little by selection, only producing a decrease in water holding capacity (Table 3).

Piles *et al.* (2000, 2006), Gondret *et al.* (2002), Ramirez *et al.* (2004) and Gil *et al.* (2006) reported that genetic selection for growth rate did not affect carcass traits, muscle traits, protein degradation of *longissimus* muscle, and meat texture properties. In contrast, Piles *et al.* (2004) reported that selection for growth rate was associated with decreases in feed conversion rate, but may have also been associated with decreases in carcass and meat quality. By comparing two lines selected for live body weight at 63 days of age, and using a cryopreserved control population raised contemporaneously with selected rabbits of 5th generation, Larzul *et al.* (2005) attained considerable correlated selection responses in growth rate, carcass, and muscle traits. Rochambeau *et al.* (1994) and Quevedo *et al.* (2005) reported that selection for increased litter size resulted in a decrease in individual weight at weaning, although total weight of the litter at weaning increased. Modifications in the selection objective for maternal lines were attempted by Rochambeau (1998) by including the weight at 63 days in addition to litter size to increase simultaneously litter size and individual weight.

As presented in Table 3, when the comparisons were done at a constant litter size at birth, the correlated responses in growth traits obtained from selection for litter size at weaning did not show any significant responses for weaning weight, weight at market time, post-weaning daily gain, daily feed intake and feed conversion index (Baselga and García, 2002; García and Baselga, 2002c).

Selection programs in synthetic paternal lines developed

The paternal lines developed for use on small and large commercial scales are presented in Table 4. In Hungary, White Pannon rabbits were created since 1991 by crossing New Zealand White rabbits with Californian and then selection was

practiced for growth rate between 6-10 weeks of age (Szendrő *et al.*, 1998). In Spain, line R was individually selected for daily gain between 28-63 days and the main objective of selection was directed to improve feed efficiency; this line was developed for use on commercial farms (Estany *et al.*, 1992). In USA, Lukefahr *et al.* (1996) described the development of a large terminal-sire breed, known as the ALTEX, selected for 70-day market weight, which has a breed foundation of ¼ Californian, ¼ Champagne d' Argent, and ½ Flemish Giant. In Saudi Arabia, Saudi-3 was established from ¼ V line and ¾ Saudi Gabali and selected for litter weight at weaning and individual weight at 84 d. The details concerning the development of this new line were presented by Khalil *et al.* (2002, 2005) and Al-Saef *et al.* (2008). In Egypt, a synthetic paternal line of Alexandria originated at Alexandria University from crossing V line with the Baladi Black, and selection was practiced for daily weight gain during 28-63 days of age (El-Raffa, 2007).

Table 4: Selection programs for synthetic paternal line development.

Synthetic line and country of work	Authors	Founder breeds	Selection criteria	Selection methodology	Number (interval) of generations	Selection responses per generation*
Line R, Spain	Estany <i>et al.</i> (1992)	Californian, specialized paternal line	Growth rate (28-63 d)	Individual selection using BLUP	12 generation, (6 mo)	ADG= 0.5 g/d
Altex, USA	Lukefahr <i>et al.</i> (1996)	Californian, Champagne d' Argent, Flemish Giant	70-day market weight	Individual selection using mixed-model	More than eight generations	W70= 29.4 g; WW = 9.5 g; ADG (28-70 d) = 0.47 g/d
White Pannon, Hungary	Szendrő <i>et al.</i> (1998)	NZW, Californian	Growth rate (6-10 wks)	Individual selection using BLUP	Since 1992	ADG= 0.6 g/d per year
Alexandria, Egypt	El-Raffa (2007)	Line V, Baladi Black	Daily body weight gain (28-63d)	Individual selection using BLUP	More than five generations, (10 mo)	W12= 38 g; ADG= 0.6 g/d; LSB= 0.14 rabbits per litter; LSW= 0.12 rabbits per litter; LWLW= 35 g per litter
Saudi-3, Saudi Arabia	Khalil <i>et al.</i> (2002), (2005)	Line V and Saudi Gabali	Weaning litter weight + 84-d weight	Individual selection using BLUP	More than eight generations (9 mo)	W12= 38 g; ADG= 0.6 g/d; LSB= 0.14 rabbits per litter; LSW= 0.12 rabbits per litter; LWLW= 35 g per litter

*ADG: average daily gain; WW: weaning weight; W70: weight at 10 weeks; W12: weight at 12 weeks; LSB: litter size at birth; LSW: litter size at weaning; LWLW: litter weight at weaning.

DIRECT AND INDIRECT SELECTION FOR TOTAL OBJECTIVES

Criteria and methods of selection

Ramon *et al.* (1992) and Utrillas *et al.* (1992) proposed demographic and phenotypic analyses in a synthetic population of rabbits selected for total objectives of litter weight at 60 days through overlapping generations. However, multi-purpose lines were developed as a total objective through simultaneous selection for litter size and growth traits. This selection strategy was successfully developed in Spain, France, Brazil, and Egypt. In Spain, a two-stage selection program was practiced using two criteria (litter size at weaning and individual daily weight gain) and applying the method of independent culling level of selection (Gómez *et al.*, 2000a), while the French program of selection was practiced for litter size at birth and individual weight at 63 days (Rochambeau, 1998; Garreau and Rochambeau, 2003). In Brazil, a selection index including litter size at weaning, individual weaning weight, weaning litter weight and individual weight at 70 days of age was used (Moura *et al.*, 2001), while in Egypt, selection was practiced for litter weight at weaning and individual weight at 56 days (Youssef *et al.*, 2008).

Multi-purpose synthetic lines developed

Table 5 shows multi-purpose synthetic lines developed in some parts of the world. In France, a multi-purpose line of INRA1077 was developed by selecting for litter size at birth and for individual weight at 63 days to produce the most common parental females of INRA1077x INRA2066 (Bolet and Saleil, 2002; Garreau and Rochambeau, 2003). From 1983 to 1992, the Caldes line in Spain was formed by selecting for litter weight at weaning in the first stage, while in the second stage the individuals were chosen for post-weaning growth. Since 1992, animals of this line are selected for growth rate between 32 and 60 days. Details concerning the foundation breeds and selection methods used in developing the Caldes line were described by Rafel *et al.* (1988) and Utrillas *et al.* (1992). In Brazil, a multi-purpose selection program was initiated in 1992 to develop a multi-purpose line using a selection index, including litter size and weight at weaning and post-weaning growth traits and this line was named the Botucatu (Moura *et al.*, 2001). In Egypt, a multi-purpose selection program was started in March 2003 to produce a synthetic line (named Moshtohor), resulting from crossing Sinai Gabali with V-line and selection was practiced for litter weight at weaning and live weight at 56 days (Iraqi *et al.*, 2007, 2008).

Table 5: Selection programs for the development of multi-purpose synthetic lines.

Synthetic line and country of work	Authors	Founder breeds	Selection criteria	Selection methodology	Direct response per generation*
INRA1077, France	Bolet and Saleil (2002)	NZW, Bouscat	Weaning litter size then birth litter size + 63 d weight	Individual selection applying BLUP	LSB= 0.11 rabbits per litter; LSW= 0.08 rabbits per litter; LWW= 47 g; WW= -3.4 g
Botucatu, Brazil	Moura <i>et al.</i> (2001)	Norfolk English line	Weaning litter size and weight + growth rate (28-70 d)	Selection index	
Caldes, Spain	Gómez <i>et al.</i> (2002a)	Six lines in Caldes de Montbui, Barcelona	Litter weight at 56-d weight + growth rate (32-60 d)	Two-stage selection applying BLUP	LWW= 30.7 g; ADG= 1.1 g/d
Giant de España, Spain	López and Sierra (2002)	Flemish Giant, Lebrele Español	Weaning litter size + growth rate during fattening	Independent culling levels selection	
Moshtohor, Egypt	Iraqi <i>et al.</i> (2008)	Sinai Gabali, line V	Litter weight+ 56-d weight	Two-stage selection using BLUP	

*LSB: litter size at birth; LSW: litter size at weaning; LWW: litter weight at weaning; WW: weaning weight; ADG: average daily gain.

Direct and correlated selection responses

Estimates of direct and correlated selection responses obtained from selection experiments are presented in Table 6. Lines selected based on different objectives showed that selection for growth rate have a better feed efficiency than selection for reproductive traits (Torres *et al.*, 1992; Feki *et al.*, 1996).

In Spain, selection responses obtained from selecting for litter size at weaning and daily weight gain in the Caldes line (Gómez *et al.*, 2000) are summarized in Table 6 that indicates: (1) direct response for litter size at weaning was 0.03 rabbits per litter, while indirect responses for litter weight at weaning, and individual weight at weaning were 30.7 g per litter and 11 g per rabbit per year, respectively; (2) direct response for daily gain was around 1.06 g per day, with an indirect response of 38 g per rabbit per year for individual weight at 60 days; (3) ability of the doe to raise her litter was also improved because genetic response for litter size at weaning was higher than the response for litter size at birth; (4) selection for litter weight at 60 days achieved positive responses for growth rate, while maintaining high reproductive performances; (5) selection for a total objective has achieved responses for growth traits without impairment of litter size at weaning.

Table 6: Direct and/or correlated selection response per generation obtained from multi-purpose selection experiments.

Authors	Breed or line	Selection criteria	Methodology	Direct and/or correlated selection responses per generation*
Moura <i>et al.</i> (1997)	Composite population	Daily gain + feed conversion (28-70 d)	Genetic trend in divergent selection program using BLUP	ADG = 1.23 g/d; FCI = -0.20 g feed per g gain
Gómez <i>et al.</i> (2000)	Caldes line	LWW + ADG (32-60 d)	Annual genetic trends using BLUP	LSB = 0.0 rabbits per litter; LSW = 0.03 rabbits per litter; LWW=30.7 g per litter; ADG = 1.06 g/d; WW = 11 g per rabbit; W60 = 38 g per rabbit
Moura <i>et al.</i> (2001)	Botucato	LSW, WW, LWW, 70-d weight	Genetic trend in multiple-trait selection using mixed-model methodology	NBA = 0.034 rabbits per litter; LSW = 0.039 rabbits per litter; LWW = 35.2 g per litter; WW= 6.74 g per rabbit; W70 = 17.2 g per rabbit

*LSB: litter size at birth; NBA: number born alive; LSW: litter size at weaning; LWW: litter weight at weaning; WW: weaning weight; W60: weight at 60 days; W70: weight at 70 days; ADG: average daily gain; FCI: feed conversion index.

Salaün *et al.* (2001) in France stated that annual genetic gain for selecting litter weight at weaning was 342 g, which was equal to 1.7% of the phenotypic mean or 3.2% of the phenotypic standard deviation. In Brazil, Moura *et al.* (2001) reported that the annual genetic gain for litter size at weaning, weaning litter weight and 70-d market weight in multi-purpose selection program were 0.04 rabbits per litter, 35.2 g per litter, and 17.2 g per rabbit, respectively.

DIRECT AND INDIRECT SELECTION FOR WOOL TRAITS

In the literature, results dealing with selection experiments for wool traits in Angora rabbits are very limited (Rafat *et al.*, 2007).

Selection criteria and methods

Selection for total fleece weight was successfully performed in French Angora rabbits (Rochambeau *et al.*, 2000; Allain *et al.*, 2004; Rafat *et al.*, 2007, 2008). However, it is unclear whether or not high fleece weight is associated with an increase in other fleece characteristics (length, diameter, compression and secondary to primary follicle ratio) in Angora rabbits. Nonetheless, selection for total fleece weight is a simple and easy criterion to measure, and it is very efficient to improve weight of 1st class quality wool, which is an important economic trait in French Angora wool production.

Direct and correlated selection responses

In order to estimate direct and correlated selection responses for wool production and other wool quality traits, an 8-year divergent selection experiment was carried out on French Angora rabbits based on selection for total fleece weight (Rafat *et al.*, 2007, 2008). Results obtained in this experiment indicated that: (1) response in total fleece weight was substantial with an annual divergence rate between the high and low lines in mean breeding values to be 80.95 g or 3.04 genetic standard deviations; (2) correlated responses in other fleece quality traits were significant with divergence rates of 2.96, 2.78 and 1.21 genetic standard deviations for weight of 1st class quality wool (W1), weight of 2nd class quality wool (W2), and wool homogeneity (the ratio of W1 to total fleece weight), respectively; (3) live body weight increased significantly by selection for total fleece weight; (4) a positive divergence rate of 0.92 genetic standard deviations between the two selection lines was observed for bristle length, while negative divergence rates of 1.00, 1.31, 0.38 and 0.50 genetic standard deviations were observed for compression, resilience, bristle diameter and fiber diameter, respectively.

Allain *et al.* (2004) reported that direct selection for fleece weight in long Angora rabbits was associated by positive divergence rates of 3.0, 0.7 and 0.9 genetic standard deviations for fleece weight, fleece homogeneity and bristle length, respectively, while negative divergence rates of 0.9, 0.9, 1.1 and 0.4 genetic standard deviations were observed for lock structure, compression, resilience and live body weight, respectively. No other results on correlated selection responses for total fleece weight or for fleece characteristics in Angora rabbits have been published. Similar to the results of Angora rabbits, Bai *et al.* (2006) suggested that selection for cashmere weight in goats was very effective, which has led to slow genetic progress in fibre length due to its negative genetic correlation with cashmere weight. In another study, Redden *et al.* (2005) concluded that selection for increased cashmere weight results in a reduction in quality and value of the fleece.

CONCLUSIONS AND FUTURE PROSPECTS

- 1) In developed countries, specialized maternal or paternal lines were mostly developed for use on commercial farms, while in developing countries the synthesis of multi-purpose lines are necessary for use in national breeding programmes for the rabbit industry.
- 2) Spanish V-line rabbits genetically selected for more than 35 generations have been introduced in various countries (as live animals or as frozen embryos) using recent bio-techniques and applying selection and/or crossbreeding with local lines. This line has been widely distributed in some countries of the world, such as France, Egypt, Saudi Arabia, Turkey, and Uruguay.
- 3) Direct selection has had little or moderate effects on litter size, prenatal litter components, and meat quality traits, while it has had major effects on post weaning growth and carcass traits, feed conversion, and fleece weight in Angora rabbits. Direct selection for feed efficiency is less efficient than selection for growth rate for improving feed conversion ratio.

- 4) Selection for increasing total fleece weight has resulted in beneficial effects on other fleece quality traits and on live body weights in Angora rabbits. Detecting QTL and major genes for hair follicle production are considered to be a valuable tool for selection programs of selection in Angora rabbits (Allain et al., 2004).
- 5) New techniques, such as laparoscopy, ovariectomization, cryopreservation of embryos and semen, TOBEC (Total Body Electrical Conductivity), and X-ray scanning computerized tomography (CT), have been introduced successfully as tools to assist in selection programs. Selection based on the X-ray computerized tomography measurement could be effective to improve carcass traits in rabbits.
- 6) Results of available molecular techniques are not presently of sufficient accuracy to be used in selection programs in rabbits; although the Spanish and French teams have verified the hypothesis that there are major genes affecting components of litter size, uterine capacity and ovulation rate. A genetic map with microsatellite markers distributed every 10 to 20 cM along the rabbit genome is available as stated by Chantry-Darmon *et al.* (2004). This breakthrough will help to efficiently construct linkage maps, based on molecular markers for use in selection programs. Simultaneously, the corresponding cytogenetic maps were established in order to provide the chromosomal position of all the genetic markers.
- 7) To date, marker-assisted selection (MAS) is not generally used in current rabbit selection programs, and the recent molecular technologies were used only to identify genetic diversity, gene mapping and DNA fingerprinting in different breeds of rabbits.
- 8) As to future prospects, localizing loci of genes of economic interest are necessary to identify the candidate animals in selection programs and to elucidate the molecular nature of the few already verified major genes.

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GENETIC EVALUATION OF GROWTH TRAITS IN A CROSSBREEDING EXPERIMENT INVOLVING LINE V AND BALADI BLACK RABBITS IN EGYPT

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Five genetic groups of rabbits were produced in a crossbreeding experiment involving the Spanish synthetic maternal line V, Egyptian Baladi Black (BB), their first reciprocal crosses (F1) and the second generation (F2) by *inter se* mating of the F1 to evaluate their body weight (BW) and daily weight gain (DG) from weaning (4 weeks) to marketing (12 weeks). A total of 2622 rabbits coming from 24 sires and 114 dams (892 purebred and 1730 crossbred progeny) were controlled to evaluate these genotypes. The study was conducted through two successive years during the first four parities of 114 dams. Statistical model included the fixed effects of animal genotype, year-season, parity order and sex along with the random effect of environmental common litter effects and additive genetic values.

Crossbreeding genetic parameters (direct and maternal additive effects, direct and maternal heterosis) were estimated. Highly significant differences ($P < 0.001$) were detected for all fixed effects on all traits. Estimates of crossbreeding genetic parameters showed a superiority of line V fryers compared with other genotypes, and Baladi Black had the poorest performance. Estimates of direct heterosis (7 to 11.8%) and maternal heterosis (-0.4 to 7.4%) mainly showed significant positive effects for crossing on growth traits. Estimates of direct and maternal genetic effects showed a positive pattern in favor of line V. The growth traits were significantly affected by direct genetic effects. The maternal effects were less important. These results suggest that applying crossing between line V and Baladi Black rabbits could be an effective breeding method for producing fryers superior in growth traits in Egypt.

EVALUATION OF THE REPRODUCTIVE PERFORMANCE OF A LOCAL POPULATION OF RABBITS IN SOUTH BENIN

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The aim of this study was to make an initial evaluation of the does (n=147) which will be introduced in a selection program in CECURI. The reproductive parameters of 400 litters were recorded and analyzed. The fertility rate of the herd was 65% and the interval between kindling varied from 63 to 44 days according to the parity. The mean weight of the litters at weaning was higher in the second than in the first parities (1612 g vs. 2313 g; $P < 0.05$). The mean individual mean weight at 28 days was 396 ± 132 g. The rate of weaning was 94, 90 and 86%, respectively for the first, the second and the third kindling. The mean litter size was 5.7 born alive. It was significantly lower in the first litter (5.1) than in the following parities. The stillbirth rate was 2.5, 2.3 and 2.2%, respectively for the first, the second and the third kindling of the does.

CROSSBREEDING EFFECTS FOR CARCASS, TISSUES COMPOSITION AND MEAT QUALITY TRAITS IN A CROSSING PROJECT OF V-LINE WITH SAUDI GABALI RABBITS

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A five-year crossbreeding project involving Spanish maternal line called V-line (V) and Saudi Gabali (S) rabbits was carried out to produce 14 genetic groups of V, S, $\frac{1}{2}V\frac{1}{2}S$, $\frac{1}{2}S\frac{1}{2}V$, $\frac{3}{4}V\frac{1}{4}S$, $\frac{3}{4}S\frac{1}{4}V$, $(\frac{1}{2}V\frac{1}{2}S)^2$, $(\frac{1}{2}S\frac{1}{2}V)^2$, $(\frac{3}{4}V\frac{1}{4}S)^2$, $(\frac{3}{4}S\frac{1}{4}V)^2$, $((\frac{3}{4}V\frac{1}{4}S)^2)^2$, $((\frac{3}{4}S\frac{1}{4}V)^2)^2$, Saudi 2 (synthetic maternal line), and Saudi 3 (synthetic paternal line). A total number of 2770 rabbits produced by 91 sires and 402 dams were used to evaluate carcass components, tissue composition and meat quality traits. A generalized least square procedure was used to estimate additive and heterotic effects (direct, maternal, and grand-maternal). The estimates of direct additive effects were significant and in favour of V line rabbits for the majority of traits studied, ranging from 3.8 to 9.0% for slaughter and edible carcass components, 3.4 to 10% for non-edible traits, -3.1 to 9.8% for tissues compositions, and -14.9 to 2.5% for meat quality traits. Maternal additive effects were significantly in favour of V line by 1.66% for meat ether extract (11.1% relative to the average of the V line and Gabali as purebreds). Grand-maternal additive effects were not significant in most traits studied except dry matter and ash contents in meat since the effect of the V line was higher than the effect of the Gabali by 0.5% and 1.39%, respectively (0.7% and 15.4% of the respective averages of the pure breeds). Heterosis estimated for non-edible traits were mostly positive and only significant for head weight (direct and grand-maternal heterosis), fur weight (grand-maternal heterosis), lung weight (maternal and grand-maternal heterosis) and viscera weight (maternal and grand-maternal heterosis); the estimates were small relative to the average of purebreds reaching 6.4% as maximum value. Estimates of direct, maternal and grand-maternal heterosis for meat weight were found to be consistent and positive (3.9, 4.5 and 5.0%, respectively) associated with significant direct heterosis for fat weight (12.2%), maternal heterosis for meat bone ratio (4.5%), and maternal and grand-maternal heterosis for dry matter in meat. The estimates of direct heterosis for protein content in meat were significantly positive (1.4%), but the estimates for grand-maternal heterosis were significantly negative (-2.1%). For fat content in meat, the estimates of direct (-8.3%) and maternal heterosis (-11.9%) were significant, while for ash content the estimates for maternal (23.7%) and grand-maternal heterosis (30.1%) were significantly positive.

**PRELIMINARY RESULTS IN A DIVERGENT SELECTION
EXPERIMENT ON VARIANCE OF LITTER SIZE IN RABBITS.
II. RESPONSE TO SELECTION**

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A divergent selection experiment on phenotypic variance of litter size was carried out in rabbits. Selection was based on phenotypic variance of litter size for each doe after correcting litter size for the fixed effects of year-season and lactation status (PVC). Selection pressure on does was approximately 30% in each line. Males were chosen within sire families in order to avoid the increase of inbreeding. The total number of records for litter size and PVC were 1929 and 534, respectively. Results of the first generation of selection were analyzed using Bayesian methods. The High line showed a higher PVC than the Low line in the first generation of selection. The difference between lines (D) was 0.73 (P(D>0)=96%). This difference in PVC was associated with an increase in the phenotypic variance of litter size (D=1.49, P(D>0)=100%) and a decrease in litter size in the High line (D=-0.38 kits, P(D>0)=93%). The first preliminary results of this study suggest that the variance of litter size seems to be under genetic control.

EFFECT OF TISSUE INHIBITOR OF METALLOPROTEINASE 1 (TIMP1) GENE FOR EMBRYO SURVIVAL AND DEVELOPMENT IN A F₂ RABBIT CROSS

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The effect on early embryo survival and development of a diallelic 1423A>G SNP found in the promoter region of the tissue inhibitor of metalloproteinase 1 gene (*TIMP1*) was studied in an F₂ rabbit population. The F₂ population comes from High (H) and Low (L) lines selected divergently for uterine capacity during 10 generations. A total of 171 and 159 intact does were slaughtered at 48 h and 72 h of gestation respectively to determine whether *TIMP1* gene was associated with ovulation rate, fertilization rate, early embryo survival and embryonic stage of development. We did not find differences between homozygote genotypes in ovulation rate and fertilization rate at 48 and 72 h of gestation. At 48 h of gestation, AA genotype showed a similar early embryo survival and embryonic stage of development to GG genotype. However AA genotype, the genotype most frequent in the line selected to increase uterine capacity, had 0.88 embryos (P(D>0)=90%) more at 72 h of gestation than GG genotype and also had a higher embryonic stage of development, showing a lower percentage of early morulae (D_m=-16.33%, P(D<0)=92%), a higher percentage of compacted morulae (D_m=19.85%, P(D>0)=92%), and similar percentage of blastocysts (D=3.52%) at this stage of gestation. A more advanced embryonic stage of development seems to be associated with a higher embryo survival at 72 h of gestation.

CANALISING SELECTION ON WITHIN LITTER VARIABILITY OF BIRTH WEIGHT IN RABBITS: RESPONSES TO SELECTION AND CHARACTERISTICS OF THE UTERUS OF THE DOES

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A divergent selection experiment on within litter homogeneity of birth weight in rabbits was carried out at INRA. The two lines have been created by selecting breeding does and bucks from the female strain AGP22 bred at the Grimaud Frères Sélection Company. This involved a new model incorporating a genotypic value for the mean and a genotypic value for the environmental variance. There was a favourable selection response with a significant difference in within-litter standard deviation of birth weight between the lines selected for increasing (HOM) or decreasing (HET) the homogeneity. In generation 7, the difference between lines reached 1.6 g, i.e. 19% of the mean of this standard deviation. The standard deviation of weaning weight diverged in generation 7 by 9 g, i.e. 12% of the mean standard deviation. There was a favourable correlated response for the young survival from birth to weaning and litter size at weaning, and no effect of selection for variability on individual weight of the young at birth. At the end of the 3rd and the 6th generation, after the weaning of their last litter, females were sacrificed to collect the uterine horns and measure their initial length and their length after elongation with a weight of 50 g and then 70 g. The length and the elongation in the homogeneous line were significantly higher, whatever the weight added. It is the first time that a positive response to a canalising selection, acting on variability and not on the mean of a trait, was observed in domestic mammals. Given this significant result, the breeding company associated with this work implemented this method of selection in one of its selected strains. At INRA, this experiment continues for better understanding the physiological mechanisms and looking for the genes implied in this response.

REVIEW ON REX RABBIT BREEDING IN CHINA

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This article outlines the concepts of the Rex Rabbit variety and strain and points out the present situation of the Rex Rabbit strain in China. Six main problems of Rex Rabbit breeding in China are examined, e.g. paying more attention to weight, growth, the length of hair, the back hair, phenotypic trait and adulthood but ignoring wholesome coordination, hair quality, other aspects, other parts, hereditary basis and other growth stage; three kinds of simple selective breeding methods of Rex rabbit are proposed in this paper, e.g. synthesis, comprehensive selective index and simple selective breeding table method.

AN ENTERPRISE GROSS MARGIN MODEL TO EXPLORE THE INFLUENCE OF SELECTION CRITERIA FOR BREEDING PROGRAMS AND CHANGES TO MANAGEMENT SYSTEMS

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The profit model developed was used to investigate and provide insight into two scenarios – the profitability of restricted feeding in grower rabbits and the relative importance of some selection traits for rabbit breeding. Using published parameters for production levels and cost inputs, the model calculated outputs that were consistent with survey values for the best 25% of French rabbit breeders. The model demonstrated that restricted feeding of growers, which was introduced in France to help combat epizootic rabbit enterocolitis (ERE), may be a profitable undertaking regardless of the presence of ERE, due to improvement of feed conversion ratio. Under the assumptions used, the relative importance of traits in the breeding objective was highest for production traits, reproduction being the highest then growth, with fitness traits, such as resistance to ERE and longevity, contributing much less to profit. However, relative economic values need to be viewed in the light of associated changes in other traits, and traits such as longevity may indeed have a higher value if used as a *de facto* trait to select for poor fertility and disease resistance in breeding does.

INVESTIGATION OF COAT COLOUR AFFECTING GENES IN SEVERAL EUROPEAN RABBIT BREEDS AND OTHER LEPORID SPECIES

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Pigmentation in mammals is mainly determined by the distribution of pheomelanin and eumelanin pigments which produce red/yellow and dark phenotypes, respectively. The relative amount of eumelanin and pheomelanin in the melanocytes is controlled primarily by two loci, the *Extension* and *Agouti* loci. *Extension* locus encodes the melanocortin 1 receptor (MC1R). *MC1R* mutations have been identified to alter coat colour and pigment synthesis in several mammals. Analysing almost the complete coding region of the *Oryctolagus cuniculus* *MC1R* gene, we recently identified two mutations associated with red (recessive allele *e* of the *Extension* locus) or black (*E^D* or *E^S*, dominant black or steel, weaker version of *E^D*) coat colours in different European rabbit breeds. Here we completed the sequence of the 953 bp coding region of the *MC1R* gene in *O. cuniculus* excluding the presence of additional common disrupting or functional mutations. *Agouti* locus encodes for the agouti signalling protein (ASIP). In European rabbit, classical studies have suggested the presence of three alleles at the *Agouti* locus: *A* (wild type allele), *a'* (black and tan) and *a* (non-agouti). We sequenced the *O. cuniculus* *ASIP* exon 2 region and identified three mutations. Two were synonymous substitutions and one was an insertion of 1 bp. This insertion causes a frameshift of the translation suggesting that this mutation might be the molecular basis of the recessive black non-agouti allele at the *Agouti* locus (*a* allele). Genotyping this mutation in a larger number of animals confirmed the fixation of the insertion in all animals of breeds with black/dark coat colour. In addition, *MC1R* gene and *ASIP* exon 2 were sequenced in other Leporid species obtaining useful information to study these two coat colour genes from an evolutionary point of view.

ZFX AND ZFY GENE SEQUENCES: USE FOR MOLECULAR SEXING EUROPEAN RABBITS, EUROPEAN BROWN HARES AND MOUNTAIN HARES AND PERSPECTIVES FOR SEX DETERMINATION OF OTHER LEPORID SPECIES

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We have developed a new molecular sexing method for three leporid species (*Oryctolagus cuniculus*, *Lepus europaeus* and *Lepus timidus*) based on the analysis by PCR-RFLP of point mutations that differentiate the *ZFX* and *ZFY* gene sequences. Polymorphic positions on the X and Y chromosomes of male samples were recognizable as double peaks in the sequencing chromatograms. Comparison of these sequences with those obtained from the females of *O. cuniculus*, *L. europaeus* and *L. timidus* allowed us to deduce the sequence of the Y fragment for these species. Analysis of the obtained sequences revealed an *AluI* restriction site in the female but not in the male sequences and a *BglIII* restriction site in the male but not in the female sequences. A *HinfI* restriction site was present only in male *O. cuniculus* sequences but not in the female fragment. Digestion of the amplified fragments obtained in all collected samples for these three species produced the expected fragments for all analysed samples (70 European rabbits, 37 European brown hares and 24 mountain hares), for which sex was recorded during their collection proving a high accuracy of the methods. The method described here enables the identification or confirmation of gender of tissue samples from three species of *Leporidae* using restriction enzymes that cut male or female fragments. The specificity of this method means that the probability of incorrect sex identification due to polymorphisms within species that might involve the two gender specific restriction sites is low enough to be ignored. Then, we obtained sequence information for the *ZFX/ZFY* loci for other 6 leporid species (*Bunolagus monticularis*, *Lepus americanus*, *Pentalagus furnessi*, *Romerolagus diazi*, *Sylvilagus floridanus* and *Sylvilagus nuttallii*) including a few threatened taxa. Several polymorphic sites can be identified between sexes and among species. However, as few samples were available for these species further investigation should be carried out to develop and evaluate appropriate sexing protocols.

ANALYSIS OF CANDIDATE GENES FOR MEAT PRODUCTION TRAITS IN DOMESTIC RABBIT BREEDS

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A candidate gene approach has been already successfully applied to identify several DNA markers associated with production traits in livestock. The principle is based on the fact that variability within genes coding for protein products involved in key physiological mechanisms and metabolic pathways directly or indirectly involved in determining an economic trait (e.g. feed efficiency, muscle mass accretion, reproduction efficiency, disease resistance, etc.) might probably explain a fraction of the genetic variability for the production trait itself. Growth hormone (*GH*) and myostatin (*MSTN*) genes play important roles in animal growth, development and muscle mass accretion. For their functions, these two genes can be considered candidate genes for meat production traits. Here we resequenced parts of these two genes in four rabbit breeds (Belgian Hare, Burgundy Fawn, Checkered Giant and Giant Grey) in order to identify DNA markers useful for association studies with economic traits. On the whole, resequencing of the *GH* and *MSTN* genes generated sequence information for 9988 bp. No mutation was detected in the sequenced regions of the *GH* gene suggesting the absence of common polymorphisms in this rabbit gene. Resequencing of the rabbit *MSTN* gene identified only a single nucleotide polymorphism (C>T) in intron 2. A PCR-RFLP protocol was designed to investigate this mutation in a larger number of rabbits (15 Checkered Giant, 9 Giant Grey, 6 Dwarf, 4 Burgundy Fawn, 3 Giant White, 3 Lop, 2 Belgian Hare, 1 New Zealand White). Allele frequencies across breeds were 0.51 for allele *C* and 0.49 for allele *T*. Considering the breeds for which at least 5 animals were analysed, allele *C* frequency was 0.56 in Checkered Giant, 0.60 in Burgundy Fawn and Giant Grey and 0.83 in Dwarf. For its allele distribution the identified polymorphism seems an useful gene marker for association studies with production traits in rabbits.

STRATEGY FOR DEVELOPING RABBIT MEAT PRODUCTION IN ALGERIA: CREATION AND SELECTION OF A SYNTHETIC STRAIN

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There is in Algeria a local population well adapted to the climatic conditions, whose prolificacy and adult weight are too low. In the frame of a co-operation between INRA and ITELV, a new synthetic strain has been formed from the insemination of females of this local population by fresh semen of males from the INRA2666 strain. The main interest of the crossbreeding between breeds or strains is to profit from their complementarity and the effect of heterosis. The two possible strategies are, on one hand, the crossbreeding at each generation between the selected pure stocks to produce F1 parental females and, on the other hand, the constitution of a synthetic line whose nucleus is submitted to selection. The first solution has the advantage of exploiting at each generation the entirety of the effect of heterosis, but it requires a complex scheme based on the maintenance and selection of the pure stocks and the multiplication and diffusion of the crossbred females. This solution seemed too complex to set up in Algeria, where the structures were not enough developed to accompany this process. This is why the creation of a synthetic line was chosen, making possible to ensure the independence of the farmers. Indeed, while not excluding the terminal crossbreeding with a male, this solution lets to the farmers the faculty to adapt their strategy of renewal of their herd to their possibilities: they can renew their stock themselves without loss of its genetic level, they can also buy males or the two sexes to the nucleus, permanently or punctually to profit from the genetic progress carried out. After 4 generations of matings, this synthetic strain can be considered as stable. This analysis is not a fair comparison of the generations, because of the year-season effects; however, compared to the average characteristics of the local population, the F4 females have a prolificacy higher by approximately 1.8 young rabbits born alive, a strongly decreased stillbirth rate, they weigh approximately 500 g more. They do not seem to be more sensitive to the summer conditions than the local population. However, their litter size at weaning and the weight of the young rabbits at slaughter are yet too low. These two traits will be the main objective of selection of the nucleus.

EFFECT OF OVIDUCTINE (*OVPGL*) GENE FOR EMBRYO SURVIVAL AND DEVELOPMENT IN A F2 RABBIT CROSS

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The aim of this study was to analyze the association between the effect of 1413 C>G SNP and the microsatellite located in the promoter region of the *OVPGL* gene and the embryo survival and development at 48 and 72 h of gestation in an F2 population. This population was obtained by crossing two lines divergently selected for high and low uterine capacity. The GG genotype for 1413 C>G SNP and the allele 455 for the microsatellite were more frequent in the low line. A total of 172 and 159 female rabbits were slaughtered at 48 and 72 h of gestation. The ovulation rate, the fertilization rate, early embryo survival and embryonic stage of development were recorded at 48 and 72 h of gestation. At 72 h of gestation, the GG genotype had higher early embryo survival ($P(D<0)=86\%$; $D_m=-0.56$ embryos; $Pr=73\%$) and percentage of early morulae ($P(D<0)=86\%$; $D_m=-10.32\%$; $Pr=60\%$) than the CC genotype. With respect to the microsatellite located in the promoter region, the -/- genotype had higher ovulation rate than the 455/455 genotype. At 48 h of gestation, we found similar fertilization rate in both homozygote genotypes ($Ps=66\%$) but the -/- genotype had higher early embryo survival and percentage of early morulae than the 455/455 genotype ($Pr=85\%$ and 78%). At 72 h of gestation, the -/- genotype had higher fertilization rate, early embryo survival and percentage of early morulae and lower percentage of blastocysts than the 455/455 genotype. The conclusions of this study were that the GG genotype of the SNP and the 455 allele of the microsatellite located in the promoter region of the *OVPGL* gene were associated with a better embryo survival and development at the first stages of gestation.

DIVERGENT SELECTION FOR LONGEVITY IN BREEDING DOES

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A divergent selection for functional longevity, based on genetic merit estimated through survival analysis techniques, was carried out in the INRA 1077 rabbit line. The experiment was carried out to estimate the efficiency of such a selection and to analyse the consequences on other reproduction traits. Given the herd management, length of productive life was measured as the number of artificial inseminations of rabbit does without any culling for infertility. A total of 48 males were progeny tested based on the longevity of ten daughters (5 in the INRA SAGA farm and 5 in the INRA EASM farm). Based on their estimated genetic merit, 5 “high longevity” and 5 “low longevity” males were selected and produced a new generation (5 sons/sire). These 48 males were similarly progeny tested to estimate the direct and correlated responses to selection. A significant difference in longevity (+0.92 AI i.e. 39 days) was observed between the two lines. The differences of survival between the 2 lines in the SAGA farm were mainly due to culling (26% in the low line vs. 14% in the high line) whereas the mortality was similar in the 2 lines. In the EASM farm, mortality and culling were both higher in the low line than in the high line (33% vs. 15% and 19% vs. 7%, respectively). Except for the total number born which was higher in the low line, there was no difference between the two lines for reproduction traits recorded for each kindling. Nevertheless, because of the difference in the number of litters between the 2 lines, the sum of young rabbits born alive per doe and the sum of young rabbits weaned per doe were higher in the high line (+5 kits).

GENETIC PARAMETERS OF PRODUCTION TRAITS AND RESISTANCE TO DIGESTIVE DISORDERS IN A COMMERCIAL RABBIT POPULATION

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A Bayesian analysis was performed in order to estimate the genetic parameters of a binary trait based on the observed signs of enteropathy, 63-day body weight, carcass yield and perirenal fat percentage in a commercial paternal line. There were 53,222 rabbits inspected for signs of disease and 2,646 slaughtered rabbits for carcass traits from 1999 to July 2007. The incidence of digestive disorder was 8% of the population and 66% of these rabbits died prior to 63-day weighing. Estimated heritabilities were equal to 0.08, 0.36, 0.24 and 0.64 for the disease trait, 63-day body weight, carcass yield and perirenal fat percentage, respectively. The genetic correlations between the disease trait, on one hand, and the 63-d body weight and the carcass yield, on the other hand, were negative (-0.19 and -0.34, respectively) and so favorable. The genetic correlation between the disease score and the perirenal fat percentage was close to zero (-0.07). Therefore, it would be possible to reduce the incidence of enteropathy by including this binary trait in a breeding program, if its relative economic value is high enough to warrant its inclusion.

REVIEW ABOUT RABBIT BREEDING IN CHINA

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This article reviews the main achievement of domestic rabbit breeding in China and summarizes the main experience and some insufficiencies of domestic rabbit breeding. Amongst the achievements are the introduction of quality breeds that have economic implications both in meat and fur production and the advancement in breeding technology and selections. The article discusses the role and importance of government support in promoting and regulating the rabbit industry in China.

GENETIC PARAMETERS AND TRENDS OF THE THIGH MUSCLE VOLUME IN PANNON WHITE RABBITS

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Genetic parameters and genetic trends for average daily weight gain between the age of 5 and 10 weeks (ADG) and thigh muscle volume (TMV) were analyzed for 19751 and 2602 Pannon White rabbits, respectively, reared in 3093 litters and born between 2003 and 2007. Rabbits with higher daily weight gain than the average of their kindling batch were chosen (based on individual ADG performances), thereafter CT (computerised tomography) scanning was performed on these rabbits measuring thigh muscle volume. Estimated heritabilities were moderate for average daily gain (0.27 ± 0.02) and moderately low for thigh muscle volume (0.21 ± 0.03). Litter effects were low for both traits (0.14 ± 0.01 and 0.14 ± 0.02 , respectively). Genetic correlation coefficient estimate between average daily gain and thigh muscle volume was low (0.14 ± 0.08). Applying BLUP the estimated selection response was 0.0879 g/year-month (i.e. 1.05 g/year) for average daily weight gain; 0.3341 cm³/year-month (i.e. 4.01 g/year) for thigh muscle volume.

**PRELIMINARY RESULTS IN A DIVERGENT SELECTION
EXPERIMENT ON VARIANCE OF LITTER SIZE IN RABBITS.
I. GENETIC PARAMETERS**

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Data from a single generation divergent selection experiment for the phenotypic variance of litter size in rabbits were analysed to estimate genetic parameters on the environmental variance of litter size using a model which postulates that environmental variance is partly under genetic control. Selection was based on the phenotypic variance of litter size for each doe after correcting litter size for the fixed effects of year-season and lactation status. Selection pressure on does was approximately 30% in each line. Males were chosen within sire families in order to avoid the increase in inbreeding. The total number of records for litter size was 1929. The posterior mean of additive variance at the variance level was 0.12 and the highest posterior interval at 95% did not include zero. The estimated genetic correlation between the additive genetic effects on the mean and those on the variance was -0.74 with a posterior interval far away from zero. The first preliminary results of this study support the model postulating that environmental variation for litter size is partly under genetic control, and it would suggest that selection for environmental variance can be successful on both the mean and variance of litter size.

A STUDY OF ENVIRONMENTAL VARIANCE GENETIC CONTROL FOR UTERINE CAPACITY IN RABBITS

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Data from ten generations divergent selection experiment for uterine capacity in rabbits were analysed to estimate the genetic parameters using a model which postulates that environmental variance is partly under genetic control. The posterior mean of additive variance at variance level was 0.12 and the highest posterior interval at 95% did not include zero. The estimated correlation between the additive genetic effects on the mean and those on the variance was -0.74 with a posterior interval far away from zero. A study of model fit/model comparison was also carried out using three different approaches: 1) a version of model checking, based on the regression of the average sampling variance of records within individuals on mean phenotypic values; 2) the deviance information criterion, an index that encapsulates the fit of a model and its complexity; 3) cross validation based on CPOs. The three approaches provided statistical support for the genetically structured heterogeneous variance model.

ADDITIVE AND HETEROTIC COMPONENTS FOR POST-WEANING GROWTH TRAITS IN A CROSSING PROJECT OF V-LINE WITH GABALI RABBITS IN EGYPT

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This study was carried out within a four years project that aimed to develop a synthetic line (M) between the Sinai Gabali breed (G) and the V-line (V). Data of 6278 young rabbits sired by 242 sires and dam reared by 540 dams coming from six genetic groups of rabbits (G and V purebreds and their crosses, F1, F2, F3 and M line) were used. Body weight at weaning (BW4), 8 (BW8) and 12 (BW12) weeks of age, and daily gain during 4-8 (DG4-8) and 8-12 (DG8-12) weeks were recorded. Using mixed model methodology, estimable functions of genetic type effects were computed and, based on them and the matrix of their variance-covariance errors, the crossbreeding parameters were estimated. It was discussed which parameters could be accurately estimated concluding that no matter six genetic groups were involved, only the difference between G and V for the direct additive effects (D_{G-V}), the individual heterosis (H^I) and the maternal heterosis (H^M) should be considered. The differences between G breed and V line were significant for almost all traits in favor of G breed. Means of F1 rabbits for most traits were significantly higher than the two purebreds. The trend for the average of F2, F3 and M lines was to be intermediate between the purebreds and the F1, excepting BW4 and BW8. Positive values of D_{G-V} , the majority of them significant, were obtained on all post-weaning growth traits, confirming the superiority of G over V for growth traits. Percentages of these estimates to the means of the two purebred parents were 5.2, 6.6, 5.3, 11.1 and 21.3% for BW4, BW8, BW12, DG4-8 and DG8-12, respectively, showing an increasing trend as the trait is recorded later. Estimates of H^I were always positive and significant for several of the studied traits. Percentages of H^I were 6.9, 3.6, 5.4, 9.7 and 6.1% for BW4, BW8, BW12 (significant), DG4-8 (significant) and DG8-12 (significant), respectively. Percentages of H^M for the same traits are 7.9, 4.8, -0.0, -8.0 and 2.6%, significant for BW4 and DG4-8. The estimates of the direct additive effects, the values and sign of the average of H^I and H^M estimated in this experiment, and the complementarity between G (better in growth) and the V line (better in prolificacy), all of them are indicators of the interest of the cross between Gabali and V lines and of their synthetic, the Moshtohor line.

TESTOSTERONE AND DIHYDROTESTOSTERONE PRODUCTION IN GENETICALLY FURLESS AND FURRED MALE RABBITS AND EFFECTS ON GROWTH

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Rabbits are highly prone to heat stress in arid and tropical environments where a tremendous potential exists in lesser developed countries for small-scale meat rabbit projects to alleviate hunger and poverty. One solution may be the breeding of rare, genetically furless rabbits, which is inherited as a simple autosomal recessive gene. To elucidate the physiological mechanism of furless gene expression, our hypothesis reflected the model of androgenic alopecia in humans (i.e., male patterned baldness) involving elevated levels of testosterone (T) and dihydrotestosterone (DHT), which could possibly account for the growth advantage in furless male rabbits as reported ifrom previous trials. The primary aim of this investigation was to determine if a difference exists between furred and furless male rabbits for mean serum T and DHT concentration, and secondly if a relationship exists between T and DHT with growth performance. Data were collected in summers over a 3-year period (2005 to 2007). Matings were made between a homozygous furless parent and a heterozygous furred parent, which produced 35 litters. Ninety-six weaned female and male fryers (range in initial age of 42 to 56 d with mean body weight of 809 g) were randomly assigned to growing cages containing two or three either furless or furred fryers from different litters. Following a 42-d growing period, blood was drawn from males (n=40) to evaluate serum T and DHT levels. Models consisted of the fixed effect of treatment (furred vs. furless) and random effects of batch (i.e., a contemporary group effect of litters born within 1 wk), litter nested within batch, and residual error. Data showed a large range of T values from 23 to 7,534 pg/ml (SD of 1,106 and CV of 102%), as well as for DHT with values ranging from 23 to 1,308 pg/ml (SD of 217 and CV of 94.9%). Results from ANOVA revealed that furred compared to furless male rabbits had similar least-squares means for T of $1,154 \pm 364$ and $1,020 \pm 364$ pg/ml ($P=0.7560$) and likewise for DHT of 235 ± 57 and 221 ± 57 pg/ml ($P=0.8563$), respectively. Furless male rabbits tended ($P=0.1022$) to have more rapid ADG than furred male rabbits (23.9 vs. 21.3 g/d) and have numerically ($P=0.1409$) heavier final body weights (1,913 vs. 1,782 g). The residual correlation between T and DHT was small and negative ($r=-0.30$; $P>0.05$). The residual correlation between T and ADG was -0.19 ($P>0.05$) and between DHT and ADG was -0.45 ($P<0.05$). Our experimental results do not support the androgenic alopecia model to account for major growth differences between furred and furless male rabbits in our population.

RAPD MARKERS LINKED TO LITTER, LACTATION AND GROWTH TRAITS IN RABBITS

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A five-years crossing scheme involving the Spanish V-line (V) and Saudi Gabali (S) rabbits was practiced during five generations to produce new synthetic lines: Saudi 2 with structure of $((\frac{3}{4}V\frac{1}{4}S)^2)$ to be used as maternal line, and Saudi 3 with structure of $((\frac{3}{4}S\frac{1}{4}V)^2)$ to be used as paternal line. To perform the genetic analysis, DNA from four sires of the parental generation (V-line and Saudi Gabali purebreds) were used to determine which genetic markers (from 40 RAPD markers used) can be used to differentiate between individuals. To reach high accuracy of homogeneity within families, 526 grand-daughter progenies from the 5th generation (Saudi2, with the structure of $((\frac{3}{4}V\frac{1}{4}S)^2)$) were used to perform the genetic and statistical analyses. Traits under investigation included litter size at birth (LSB) and weaning (LSW), litter weight at birth (LWB), 7 days (LW7), 21 days (LW21) and weaning (LWW), litter gain at days interval of 0-21 (LG021) and 0-28 (LG028), pre-weaning litter mortality (PLM), milk yield at lactation intervals of 0-7 days (MY07), 0-21 days (MY021), 7-21 days (MY721), 21-28 days (MY2128), total 0-28 days (TMY), and body weight at 4 and 8 weeks of age. Application of the RAPD (Random Amplified Polymorphic DNA) technique with 40 primers revealed five polymorphisms between the strains. In single-marker analysis, RAPD marker of OPF09₇₀₀ explained variation ranged from 10 to 14.7% for litter weight (LWB, LW7, LW21 and LWW) and gains (LG021), PLM and milk yield (MY07, MY21). RAPD marker of OPA19₁₁₀₀ explained variations ranging from 10 to 14% for LW7, LW21, LWW, LG021 and PLM, while OPF12₉₀₀ marker explained 14.7 and 16.8% of the variation for body weight at 4 and 8 weeks of age, respectively.

SELECTION FOR OVULATION RATE IN RABBITS: CORRELATED RESPONSE ON LITTER SIZE AND ITS COMPONENTS

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The aim of this work was to evaluate the response to six generations of selection for ovulation rate. The line was derived from a line selected for litter size during 12 generations and then for uterine capacity for 10 generations. Selection was relaxed during 6 generations and then animals were selected for ovulation rate during 6 generations. Selection was based on phenotypic value for ovulation rate and pressure of selection for does was close to 30%. To avoid increase of inbreeding, males were selected within sire families. Line size was approximately 20 males and 80 females per generation. Does were mated for the first time at 18-20 weeks of age and 11-12 days after each parturition thereafter. Laparoscopies were performed on all does at day 12 of their second gestation. Traits recorded in second parity were: ovulation rate (OR), estimated as number of corpora lutea; number of implanted embryos (IE) estimated as number of implantation sites; litter size (LS) estimated as total number of born rabbits, and prenatal survival (PS=LS/OR). All the traits were recorded only in the second parity with the exception of LS which was recorded over four parities. Data from 524 laparoscopies and 1874 parities were analyzed using Bayesian methods. Heritabilities of OR, IE, LS and PS were 0.27, 0.18, 0.06 and 0.07. Selection increased OR in 1.5 oocytes but correlated response on LS was lower (0.4 kits). Correlated response on IE and PS were 1.25 and -0.04, respectively.

GENETIC PARAMETERS FOR SEMEN TRAITS OF RABBIT MALES: I. PRODUCTION, MORPHOLOGY, AND SPERM HEAD MORPHOMETRY

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(Co)variance genetic components of buck semen characteristics and growth traits were estimated using the REML procedure applied to bivariate animal models. The estimations were based on data from 1456 ejaculates from 283 bucks belonging to one of the paternal lines more commonly used in Spanish farms. The ejaculates were collected from 2005 to 2007 at two different insemination stations. The traits studied were: ejaculate volume (V, ml), concentration (Cn, 10⁶/ml), total number of spermatozoa per ejaculate (Prod, 10⁶), percentage of spermatozoa with normal apical rigde (NAR, %), percentage of sperm morphological abnormalities (ANOR, %), length of head spermatozoa (L, μ m), width of sperm head (W, μ m), area of sperm head (A, μ m²), perimeter of sperm head (P, μ m) and 4 derived head shape parameters: ellipticity FUN1 [L/W], rugosity FUN2 [4 π A/P²], elongation FUN3 [(L/W)/(L-W)], regularity FUN4 [π LW/4A].

Equal model equations for all semen traits included the artificial insemination station, the period, the year-season and the order of ejaculate as fixed effects; the animal, the permanent environmental and non-additive genetic effect of the male over all its ejaculates, and the residual as random effects. The model of daily weight gain, from 28 days to 63 days of age, included litter size at birth as covariate, parity order and year-season as fixed effects and the animal, the common litter and the residual as random effects. The following heritabilities were estimated: ejaculate volume 0.091, concentration 0.053, total number of spermatozoa per ejaculated 0.097, percentage of spermatozoa with normal apical rigde 0.247, percentage of sperm morphological abnormalities 0.643, length of sperm head 0.309, width of sperm head 0.341, area of sperm head 0.279, perimeter of sperm head 0.105, FUN1 0.508, FUN2 0.518, FUN3 0.220 and FUN4 0.067. The significant genetic correlations with daily gain in the fattening period were: concentration -0.695, total number of spermatozoa per ejaculated -0.360, percentage of spermatozoa with normal apical rigde -0.350, percentage of sperm morphological abnormalities 0.334, width of sperm head -0.617, area of sperm head -0.600, perimeter of sperm head -0.646, FUN1 0.367, FUN2 0.374.

GENETIC PARAMETERS FOR SEMEN TRAITS OF RABBIT MALES: II. MOTILITY

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The objective of this study was to estimate the genetic parameters of semen motility in a rabbit line selected for increased growth rate in the fattening period. Bucks (n=283) from line R, selected during 25 generations, located in two different insemination stations were used. The animals were weighed at weaning (28 days of age) and at the age of slaughter (63 days) and daily gain (DG, g/d) was computed. The estimations were based on data from 1172 ejaculates. The reproductive rhythm of males were two ejaculates per week, the training period started at five months of age, the semen samples were taken one week after finishing the training period and three months later this period. Sperm motility parameters, assessed using a computer-assisted sperm analysis (CASA) system were: total motile sperm (MOT, %), average path velocity (VAP, $\mu\text{m/s}$), curvilinear velocity (VCL, $\mu\text{m/s}$), straight-line velocity (VSL, $\mu\text{m/s}$), linearity index (LIN, %), amplitude of lateral head displacement (ALH, μm), wobble (WOB, %), straightness (STR, %) and dance (DNC, $\mu\text{m}^2/\text{s}$).

Estimates of heritability, permanent effect and genetic correlation between growth rate and motility characteristic were obtained from the solutions of a bivariate animal model. The parameters of sperm motility studied with CASA methodology had low estimated heritabilities and, in general, they were higher than the estimated permanent effects. Estimates of the heritability were 0.156 ± 0.041 for MOT, 0.171 ± 0.062 for VAP and 0.189 ± 0.062 for LIN, 0.119 ± 0.046 for STR, and 0.186 ± 0.064 for WOB. The majority of traits had a negative and moderate genetic correlation with daily gain, and this correlation was -0.927 for percentage of total motile sperm.

OCT-4 EXPRESSION IN BLASTOCYST FROM TWO SELECTED LINES

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Pre-implantation development is characterized by distinct biological steps including first cleavage division, activation of the embryonic genome, compaction, and blastocyst formation. These changes need the expression well orchestrated of genes both of the maternal and/or embryonic genome. Usually prenatal survival seems to be mainly determined by the female, whereas the embryo-fetus plays a secondary role. In this preliminary study we focused on a major developmental gene expressed mainly by the embryo.

Oct-4 expression patterns, transcript of POU5F1 gene, have been studied in several mammalian species, but are not known in rabbit. In other species, this transcript was highly expressed in early stages. It is related with undifferentiated stages by up-regulation of other genes as IFN, Sox-2 or Ets-2. The Oct-4 is related with cellular differentiation in mammalian embryos. In fact, Oct-4 is expressed in embryonic cell but the expression is not equal in different stage of development. The objective of this study was to examine the Oct-4 expression in pre-implantatory blastocyst from two rabbit lines selected for litter size. Embryos were recovered at the fifth and sixth day post-mating from 15 females of two selected lines by litter size at weaning. Total RNA was extracted and retrotranscribed individually from 117 blastocysts. The quantitative PCR for the Oct-4 expression was realized and the β -actin was used for housekeeping gene. The relative expression ratio of Oct-4 gene was calculated with the $\Delta\Delta C_t$ method normalized to β -actin gene (ACTB). The results showed that the Oct-4 expression is not related with ovulation rate and selected line, but the relationship between Oct-4 expression and age of embryos is statistically significant (the means of Oct-4 relative expression were 89.16 and 303.06 for days 5 and 6 respectively, $P < 0.05$). The characterization of profile secretion of this transcript can be a valuable tool to accurate assessment of rabbit embryo viability in genetic and biotechnological studies as *in vitro* production or cryopreservation of embryos.

GENETICS OF MATERNAL TRAITS IN A NEW SYNTHETIC RABBIT LINE UNDER SELECTION

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The study aimed to estimate genetic parameters in a synthetic rabbit line selected for maternal traits and compare different animal model methods, i.e. single (ST) vs. multiple trait (MT) approach to be used to calculate estimated breeding values (EBVs). Data on 4462 kitting belonging to 1151 does were used and classified for different generation (G, 3 levels), month of kitting (M, 12 levels), GxM interaction, parity order (9 levels) and age class within parity (18 levels). Random permanent environment and additive genetic effects were included in Animal Model accounting for a total of 1297 animals. ST and MT approach were used alternatively for 3 traits measured: 1) total number of born, 2) number of born alive and 3) mortality rate up to weaning. ST and MT approaches were compared by using Percentage of Square Biases (PSB), Means Absolute Deviation (MAD), Coefficient of determination (R^2) and correlations between actual values and estimated breeding values (EBVs). Correlation coefficients were calculated between rankings for EBVs obtained with ST or MT method for each variable. The parameters used to compare the models indicated that, despite small differences in term of PSB, MAD or R^2 , the ST animal model could be preferred. However, a general poor R^2 for all methods and variables considered was observed, suggesting the presence of undetermined environmental factors that could influence the overall variability of each trait studied. Moreover, for the mortality rate variable a very huge amount of biases was detected, probably because of the large asymmetry of distribution for this variable. Heritability estimates were similar in both ST and MT approach: low for total number of born (0.075-0.076 for ST and MT, respectively) and number of born alive kits (0.048-0.053 for ST and MT, respectively), and basically zero for the mortality rate up to weaning (0.0004-0.0007 for ST and MT, respectively). Genetic correlations produced by the MT method were generally high (0.61 to 0.97) and in an expected range for the considered variables. The correlation between rankings of animal based on ST or MT EBVs confirmed a considerable similarity between the two methods as regard the EBVs for total number of born or number of born alive kits (correlation between 0.93 and 0.98), but inconsistent for the mortality rate, suggesting the need to avoid such a variable in selection for maternal traits. The easiest ST animal model for total number of born or number of born alive could therefore be used to predict breeding values for selecting maternal line of rabbits.

UTEROGLOBIN LEVELS AT DAY 6 OF GESTATION IN TWO LINES OF RABBITS DIVERGENTLY SELECTED FOR UTERINE CAPACITY

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The aim of this work was to study the expression of uteroglobin in uterine fluid from two lines of rabbits divergently selected for uterine capacity during ten generations. In addition, the relation between embryo survival and uteroglobin expression and between progesterone levels in uterine flushings and uteroglobin expression were studied. Females from the 12th generation were used. Nineteen females from High line and fifteen females from Low line were slaughtered at day 6 of gestation. The genital tracts were removed and uterine flushings were obtained to measure uteroglobin expression and progesterone concentration. Number of recovered embryos (RE), ovulation rate (OR) and embryo survival (ES=RE/OR) were counted. The uteroglobin expression and progesterone concentration in uterine fluid was 2.44 ± 1.29 and 15.48 ± 5.88 ng/ml, respectively. Progesterone concentration and uteroglobin expression in uterine fluid were similar in both lines. Uteroglobin was slightly correlated with levels of progesterone in uterine fluid ($r=0.29$; $P<0.05$). However, uteroglobin expression was not correlated to the number of embryos ($r=-0.21$; $P>0.05$) or to embryo survival ($r=-0.13$; $P>0.05$). Thus, differences in embryo survival between lines do not seem to be caused by differences in uteroglobin expression at day 6 of gestation.

STABILITY OF ESTIMATED BREEDING VALUES FOR AVERAGE DAILY GAIN IN PANNON WHITE RABBITS

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Stability of estimated breeding values for average daily gain (ADG) between the age of 5 and 10 weeks was analyzed for 50,869 Pannon White rabbits, reared in 7,911 litters and born between 1999 and 2007. The data set was divided into successive 5-year long periods (1999-2003, 2000-2004, 2001-2005, 2002-2006 and 2003-2007), then after selecting the appropriate part of the pedigree for these sub-datasets genetic parameters and breeding values were estimated for ADG using REML and BLUP methods. Estimated heritabilities for the successive 5-year long periods were moderate and stable (0.24 ± 0.01 , 0.28 ± 0.02 , 0.25 ± 0.02 , 0.25 ± 0.02 and 0.26 ± 0.02). Magnitudes of random litter effects were low and stable (0.17 ± 0.01 , 0.16 ± 0.01 , 0.16 ± 0.01 , 0.16 ± 0.01 and 0.15 ± 0.01). After breeding value estimation the most recent sub-dataset (2003-2007) was merged pair wise with each sub-dataset (2002-2006, 2001-2005, 2000-2004 and 1999-2003) using inner join. Thus, in the merged datasets only those records of most recent sub-dataset (2003-2007) were included that matched the other datasets records (i.e. records of rabbits born in 2003-2006, 2003-2005, 2003-2004 and 2003, respectively). In these merged datasets, each rabbit had two breeding values for ADG based on two different 5-year long dataset. The breeding values based on the most recent dataset were regressed on the breeding values based on other 5-year long datasets. With the successive years the coefficients of determination decreased (0.976 , 0.963 , 0.929 and 0.848). However, the coefficients of determination were moderately high, even when the proportion of the common rabbits in the merged datasets was low. Using a rolling base dataset, therefore, did not result the instability of breeding values estimated for ADG.

ESTIMATION OF GENETIC PARAMETERS FOR LITTER SIZE AND WEIGHT TRAITS IN NZW RABBITS RAISED IN HUNGARY

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The genetic parameters of litter size and weight traits were estimated in the New Zealand White (NZW) breed raised in a rabbit farm that belongs to the Institute for Small Animal Research, Godollo, Hungary. Using Multi-Trait Derivative-Free Restricted Maximum Likelihood (DF-REML) procedure applied to a multiple trait animal model, five traits were analyzed: litter size at birth (LSB), litter size at weaning (LSW) at 6 weeks, litter size at marketing (LSM) at 10 weeks, litter weight at weaning (LWW) and litter weight at marketing (LWM). The genetic parameters were then used to estimate genetic trends between 1992 and 1997, using the BLUP methodology. The data consisted of 3956 litters from 525 dams and 212 sires. Heritability of LSB, LSW, LSM, LWW and LWM were: 0.03, 0.03, 0.03, 0.09, and 0.07 respectively. LSW was strongly correlated with LWW and LSM, as well as LWW with LSM while the correlations between LSB and LSW or LWW were positive but low. The number of sires having positive transmitting ability (TA) records reached less than 50% at all traits while the number of dams having positive TA records reached <50% at all traits studied. The ranks of sire TA were generally low 46.2, 45.2, 44.3, 45.7 and 46.6 while 56.1, 60.3, 61.1, 62.1 and 62.8 considerable high of dams for LSB, LSW, LSM, LWW and LWM traits of NZW rabbits.

COMPARISON OF GROWTH PERFORMANCES AND CARCASS QUALITIES OF CROSSBRED RABBITS FROM FOUR SIRE LINES IN QUEBEC

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A total of 146 male and female rabbits coming from four sire lines, Californian (CA), American Chinchilla (CH), Géant Blanc du Bouscat (GB) and New-Zealand white (NZ) mated to New-Zealand white dams were used for this study. The objective was to evaluate the combining ability of these sire lines selected for 63-day body weight by measuring growth and carcass traits of their offspring. Kits used for this experiment were weaned at 5 weeks of age. At weaning, three young rabbits were randomly taken from each litter. Rabbits were identified, weighed individually and placed in individual cages for the fattening period. Rabbits were fed *ad libitum* and good quality drinking water was available continuously. Rabbits were individually weighed and slaughtered after 18 h fasting from feeds only. The commercial carcass weight including liver, kidneys and perirenal fat was taken after 2 hours chilling at 4°C. After dissection, fore part, intermediate part and hind part of carcass were measured. Dressing out percentage was also calculated as chilled carcass weight x 100/live weight. One of the hind leg was used to evaluate meat/bone ratio. Statistical analyses were performed using the procedure GLM of SAS. Results of this study shows highly significant differences ($P < 0.0001$) for individual live weight at 35 d, average daily gain, commercial carcass weight, intermediate part yield and hind part yield ($P < 0.001$) between the four sire lines compared. Rabbits coming from GB line had the best growth traits and commercial carcass weight. However, concerning carcass traits, CH sire line significantly increased intermediate and hind part yield of their progeny whereas GB sire line decreased these traits (30 vs. 29% and 36 vs. 35% for intermediate and hind part yield respectively). Rabbits coming from the 2nd litter were significantly heavier at weaning and had the heaviest commercial carcasses. There was no significant effect of parity on carcass traits. Higher growth traits and commercial carcass weight were obtained with lower litter size at birth. For carcass qualities, there was no significant difference between rabbits coming from different litter size except for hind part yield.

HETEROSIS, DIRECT AND MATERNAL ADDITIVE EFFECTS ON RABBIT GROWTH AND CARCASS CHARACTERISTICS

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A total of 142 male and female rabbits of two breeds, Californian (CA) and New-Zealand White (NZ), and their reciprocal crosses were used. This study aimed to estimate heterosis, direct and maternal additive effects as well as some non genetic effects on rabbit growth and carcass characteristics in order to identify the best crossbreeding plan to use for rabbit meat production under Quebec conditions. Kits used for this experiment were weaned at 5 weeks of age. Each rabbit was identified and weighed individually at weaning and at 63 days of age. During the fattening period, rabbits were placed in individual cages. Rabbits were slaughtered after 18 h fasting from feeds only. The commercial carcass including liver, kidneys and perirenal fat was weighed after 2 hours chilling at 4°C. After dissection, fore part, intermediate part and hind part of carcass were measured. Dressing out percentage was calculated as chilled carcass weight x 100/live weight. One of the hind legs was used to evaluate meat/bone ratio. Statistical analyses were performed using the procedure GLM of SAS. Results showed significant differences between breed types for individual live weight at 35 d, average daily gain, live weight at 63 d, fore part, intermediate part and hind part yields. Overall, for growth performances (ADG and live weight at 63 d) and hind part yield, breed types from NZ dams had better performances than those from CA dams. Concerning the intermediate part yield (and carcass yield, but non significantly) a different classification was observed, with better performances of CA sired breed types. There were no significant effects of breed type on commercial carcass weight, commercial carcass yield and meat/bone ratio. The lower the litter size at birth, the heavier the individual weight at weaning, at 63 d and the commercial carcass weight were. Rabbits coming from the 2nd parity litters were significantly heavier at weaning and had the highest commercial carcass weight. Crossbreeding parameters were calculated from linear contrasts between breed types means. Breed NZ had positive direct effects on growth rate and 63-d body weight but negative ones on carcass yield and the proportion of intermediate part of the carcass. Maternal and heterosis effects were generally non significant.

THE SEX-DETECTION IN NEWBORN RABBITS BY X-CHROMATIN AND PCR-SRY

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The aim of the present study was gonosomal screening of newborn rabbits. The neutrophil leucocyte peripheral blood X-chromatins and *SRY* sequences were analysed in 16 newborn rabbits, native to two litters of female lines P91 and M91. The neutrophile X-chromatin was detected in 11 newborn rabbits, while X-chromatin was not detected in 5 young. *SRY*-PCR fragments (242 bp) were analysed in 5 newborn rabbits, while PCR fragments were missing in 11 young. All young were evaluated according to outer genitals at 3 weeks of age. By these 3 techniques were identically detected 5 males and 11 females. The appearance of the female X-chromatin frequency was within the range 6.66%-20%. Ranking of the neutrophile X-chromatin is exact, quick and relatively cheap detection system of the sex in newborn rabbits selected for tissue cultures. The authors emphasize the relevance of the cytological analysis (occurrence X chromatin in female neutrophils) as an additional screening for all *SRY* negative samples. This technology provides partly selective tool for categorization of the females and males in further reproductive process. Both sexes of newborn rabbits can be used for control of production of special enzymes under genetic control of the gonosomes.

DIFFERENCES IN DIGESTIVE EFFICIENCY BETWEEN RABBIT DOES SELECTED FOR LITTER SIZE AT WEANING AND FOR REPRODUCTIVE LONGEVITY

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The main aim of the present work was to assess how selection for reproductive longevity criteria affects the digestive utilisation of reproductive rabbit does in lactation, compared with another common selection criterion, litter size at weaning. A second objective was to analyse and propose correction of the method to improve reliability in the determination of apparent digestibility coefficients of rabbit does in lactation. Fourteen does were used from line V, selected for litter size at weaning, and 13 does from a “longevous” productive line (LP). After standardising the litter size at birth to 10 kits, on day 6 of lactation the does were housed in metabolic cages. In order to determine the apparent digestibility coefficients, after an adaptation period of 7 days, the faeces were collected individually during 4 days (13 to 16 of lactation). To analyse the effect of the intake regularity and/or milk yield of the doe on the reliability of the determination of the apparent digestibility coefficient of dry matter digestibility (dDM), the correlation between the standard deviation of ingestion and milk production was analysed with the individual residues obtained in the dDM determination by a full-cross validation procedure. The standard deviation of the milk production, both in the previous period and during the experimental phase, was not correlated with reliability in the determination of dDM. Nevertheless, a clear and significant correlation was observed between the variability of consumption during the 4 days prior to the experimental phase and this reliability ($r=+0.57$; $P<0.01$). The LP does consumed 22 g DM/d more than the V does ($P>0.10$) and had lower apparent digestibility coefficients for the dry matter, organic matter and gross energy compared with the V does (2.3, 2.5 and 2.1%, respectively; $P<0.05$). In conclusion, it could be hypothesized that the selection for litter size at weaning in reproductive rabbit does may have selected animals more efficient in the use of the nutritional resources.

RELATIVE GROWTH OF ORGANS, TISSUES AND RETAIL CUTS IN RABBITS SELECTED FOR GROWTH RATE

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The effect of selection for growth rate on the organs, parts and tissues of rabbit was studied in line R, a line selected for growth rate in the Polytechnic University of Valencia. Rabbits belonged to two groups. The group C was formed with the offspring of embryos recovered and vitrified when the line was in the 7th generation of selection. The group S was formed with rabbits belonging to the 18th generation of the line. Both groups were reared contemporary and under the same conditions. A total of 313 rabbits were slaughtered at 4, 9, 13, 20 and 40 weeks old (approximately 15 rabbits per group, sex and age). The weight of the different organs, parts and tissues of the carcass were related to the body weight by using the Huxley's allometric coefficient (k). Their degrees of maturity were related to the degree of maturity of the animal by the Butterfield's allometric coefficient (q). Values obtained for k and q coefficients led to similar patterns of growth in most of the components studied. Full gastrointestinal tract ($k=0.75$, $q=1.92$) and organs such as liver ($k=0.70$, $q=3.18$), kidneys ($k=0.60$, $q=2.28$) and thoracic viscera ($k=0.86$, $q=1.33$) were early maturing whereas the chilled ($k=1.08$, $q=0.89$) and reference carcass ($k=1.16$; $q=0.77$) were late maturing. The retail cuts of the reference carcass were isometric (forelegs, k and q not different from 1) or late maturing (breast and ribs, $k=1.13$ and $q=0.74$; loin, $k=1.24$ and $q=0.74$; hind legs, $k=1.14$ and $q=0.84$; abdominal walls, $k=1.30$ and $q=0.67$). Dissectible fat of the carcass ($k=1.45$; $q=0.20$) and meat of the hind leg ($k=1.24$; $q=0.73$) were late maturing and bone had an early development ($k=0.74$; $q=1.48$). Lumbar circumference ($k=1.26$; $q=0.62$) was later maturing than the carcass length ($k=1.06$; $q=0.91$). No effect of selection for growth rate on k , and q values of any of the components studied was found.

EXPRESSION OF PROGESTERONE RECEPTOR RELATED TO POLYMORPHISMS IN THE PROGESTERONE RECEPTOR GENE

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The effect of the 2464G>A SNP found in the promoter region of the progesterone receptor gene on progesterone receptor (PR) expression was evaluated by Western blot analysis. This SNP was associated to two lines divergently selected for uterine capacity. The GG genotype was the genotype more frequent in the line selected to increase uterine capacity. Two isoforms were obtained: the PR-B, previously described in rabbits, and the PR-A isoform, not described in rabbits before. The GG genotype showed lower PR-B and PR-A expression than the AA genotype in the oviduct. Higher both PR expressions were obtained at day 3 of gestation in the oviduct with respect to day 2. Similar PR-A expression was observed in the ampulla and isthmus, meanwhile a higher PR-B expression was found in the isthmus.

GENOTYPE X SPERM DOSAGE INTERACTION ON REPRODUCTIVE PERFORMANCE AFTER ARTIFICIAL INSEMINATION.

1. MALE FERTILITY

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Failures in fertilization or embryogenesis have been shown to be in part of semen origin. Fertilization rate depends on the quality and the number of spermatozoa of the dose of artificial insemination. Thus, individual variation in male fertility could be better observed under conditions of low sperm concentration of the AI dose. The aim of this research was to estimate genetic parameters of male fertility after AI with low and high sperm dosage, considered as different traits. The interaction genotype x sperm dosage was estimated to know whether there is individual variation for the effect of sperm dosage on fertility. A total of 6655 AI was performed, involving 2527 crossbred females that were inseminated with homospermic semen doses coming from 250 bucks of the Caldes line. Fertility (defined as success/failure to conception) after AI with doses of 10×10^6 (F_{10}) or 40×10^6 spermatozoa/ml (F_{40}) was considered as a binary trait. Data were analyzed under a bivariate threshold model. The model for the underlying variables, corresponding to F_{10} and F_{40} , included the systematic effects of: the physiological status of the female, day of insemination-operator, and buck age-building, and the male additive genetic effects, the male non additive genetic plus permanent environmental effects, the female genetic plus permanent environmental effects, the environmental permanent effects of male and day of IA, and a random residual effect. The mean of the marginal posterior distribution for F_{10} minus F_{40} was estimated to be -0.13 (s.d.: 0.02), which indicates a clear effect of the sperm dosage on fertility. However, 40×10^6 spermatozoa/ml seems to be not high enough to compensate deficiencies in sperm characteristics precluding sperm access to the ovum and fertilization when homospermic doses are used. It is because F_{40} was still lower than fertility after natural mating (NM). Heritabilities seem to be similar for F_{10} and F_{40} and both of them could be higher than heritability of male fertility after NM. The importance of the genotype x sperm dosage interaction was almost negligible (<12% of the mean of the additive variance), since additive variances were similar for both traits and their genetic correlation was close to 1.

GENOTYPE X SPERM DOSAGE INTERACTION ON REPRODUCTIVE PERFORMANCE AFTER ARTIFICIAL INSEMINATION. 2. MALE LITTER SIZE

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Failures in fertilization or embryogenesis have been shown to be in part of semen origin. When artificial insemination (AI) is practised in prolific species, under conditions of low sperm concentration of the AI dose, differences among males in number of kits born per litter (TB) could be better observed than when natural mating (NM) is practised, due to the effect of the individual variation of semen characteristics with an effect on this trait that can be compensated increasing the number of spermatozoa. This research aimed to estimate genetic parameters of male on TB after AI with low (10×10^6 spermatozoa/ml, TB₁₀) and high (40×10^6 spermatozoa/ml, TB₄₀) sperm dosage, considered as different traits, and also the interaction between the genotype and the sperm dosage, to know whether there is individual genetic variation on the effect of sperm dosage on TB. The number of records was 1650 and 1856 for TB₁₀ and TB₄₀, respectively, corresponding to 1129 females and 202 males for TB₁₀ and 1188 females and 209 males for TB₄₀. The pedigree, referred to the males, included 733 individuals. The mean of the estimated marginal posterior distribution (EMPD) for TB₁₀ minus TB₄₀ was -1.25 kits (s.d.: 0.22), which supposed an increase of 17.3% in TB over the mean of the EMPD for TB₁₀. Heritability of male TB after AI is higher than the corresponding value after NM. The additive genetic variances for TB₁₀ and TB₄₀ were no significantly different and the genetic correlation between both traits can be considered as high. Consequently, the estimated value of the genotype x sperm dosage interaction was almost negligible (mode (mean) <1.5%, <16% of the average of the means of the EMPD for the additive variances) and therefore, no individual genetic variation was observed for the effect of sperm dosage on the total number of kits born. Estimates of genetic parameters suggest that most of the genes affecting TB₁₀ are also affecting TB₄₀ and thus, within the range of sperm dosage studied, selection to improve male TB after AI could be performed at any sperm dosage, and could have a higher response to selection than selection for male TB after NM.

DIVERGENT SELECTION FOR TOTAL FLEECE WEIGHT IN THE ADULT ANGORA RABBIT: DIRECT RESPONSE TO SELECTION ON TOTAL FLEECE WEIGHT AT FIRST AND SECOND HARVEST

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In order to explore genetic variability of wool production and other quantitative traits, an 8-cohort divergent selection experiment for total fleece weight (TFW) was carried out in French Angora rabbits. Studies were made on the wool production of a total of 669 female rabbits born between 1994 and 2001 and having produced wool from first to 12th harvests. The aim of the selection experiment was to obtain two divergent lines (low and high) on TFW. From preliminary analysis, the dataset was separated into three subsets according to the harvest number: one for each of the first two harvests and one for the third to the 12th harvests. In this paper, wool production data of the first and second harvests were analysed separately. Response to selection for total fleece weight at 3-12 harvest (TFW3-12) on this trait at first and second harvest was the aim of this paper. The second objective was to study the possibility of using the first or second harvest for estimation of breeding value and selection of total fleece weight in French Angora rabbit. Data were preliminary analysed for non genetic factors by SAS GLM procedure. Then genetic parameters and breeding value estimates were carried out using a BLUP animal model using with ASReml. A linear mixed model for a bivariate analysis of total fleece weight at first or second harvest and TFW3-12 was used. Heritability estimates of total fleece weight at first and second harvest, 0.36 and 0.38 respectively, were similar to that observed at later harvests (0.35). Genetic correlation between TFW3-12 and fleece weight at first harvest was close to zero indicating that wool production at first harvest is a different trait from that of following harvests. Genetic correlation estimates observed at second harvest was high, 0.76, and response to selection at second harvest was similar to that observed for TFW3-12. These observations confirm that total fleece weight at first harvest is a different trait from TFW3-12. In French Angora rabbits the high genetic correlation between TFW3-12 and total fleece weight at second harvest proposes the possibility of selection at this harvest for TFW3-12.

STUDY ON RELATIONSHIP OF REX RABBIT RAPD MARKER AND REPRODUCTIVE PERFORMANCES

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RAPD (Random Amplified Polymorphic DNA) marker was applied to study the relationship between some reproductive performances in Rex Rabbit. In the study 15 random primers were selected to PCR for genomes DNA and to detect the amplification product using agarose gel electrophoresis. The study showed some relationship between four primers (OPA1, OPA7, OPA14 and OPA15) with productive performance of Rex Rabbit. From them nine, six, eight and eight bands were obtained respectively. Two groups with or without and N^o.2 band from OPA1 showed significant (P<0.05) and highly significant (P<0.01) differences in the birth weight and the birth litter size. As for the two groups with or without N^o.4 band from OPA7 showed distinguished (P<0.05) or significant distinguished (P<0.01) differences in the litter size, living litter size, birth weight and birth litter size. The two groups with or without N^o.6 band from OPA14 showed significant differences (P<0.05) in the birth weight and birth litter size. The two groups with or without N^o.2 band from OPA15 showed distinguished significant differences (P<0.01) in litter size, living litter size and birth litter size.

COMPARISON OF GROWTH TRAITS IN TERMINAL CROSSES OF DIFFERENT RABBIT COMMERCIAL HYBRIDS

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An experimental trial was carried out on three commercial rabbit farms (A, B and C) by evaluating the growth traits of terminal crosses obtained from both bucks of different genetic level of a new Italian synthetic line and two different commercial hybrids. A total no. 230 does (82, 80 and 68 in A, B and C farms, respectively) were artificially inseminated simultaneously on the same season (i.e., autumn) using semen from bucks of a new Italian synthetic line, pooled by genetic level (excellent E, medium M, and fair F) depending on different mean genetic index of bucks for average daily gain (ADG) up to 81 d. In addition, in A farm, other 15 and 14 does were inseminated with semen of two types of common commercial hybrids (H₁ and H₂), and 19 does were inseminated with mixed semen from bucks of E and M genetic levels (i.e. normal commercial semen sold, N). After weaning, a standard performance trial on young rabbits was carried out and weights were registered individually at 32, 60 and 81 days. Data were used to compare the growth traits within the new Italian hybrid line (WH) and among the Italian hybrid line and the other commercial hybrids (AH). In the WH comparison, weight at 60 d (W60) and the ADG were greatly influenced in rabbits born from bucks of different genetic level, with animals obtained from E bucks that resulted 51 g heavier at 60 d and gained on average 3.2 g/d faster than rabbit born from bucks of M and F genetic level. The ADG was also significantly influenced by farm management: 41.2 vs. 39.2 vs. 37.6 g/d for A, B and C farms respectively (P<0.01). In the AH comparison different hybrids showed no different W60 in fattened rabbits. ADG resulted significantly higher in H₂ hybrids than in H₁ (42.8 vs. 39.6 g/d, respectively), while N semen (i.e. the mixture of semen from E and M genetic levels) produced rabbit that had an intermediate growth rate compared with the other two commercial hybrids (41.2 g/d). However, the rabbits obtained from buck of E genetic level were significantly heavier at 60 d, showing an ADG equivalent to that exhibited by more selected commercial hybrid as the H₂ (41.4 g/d).

EFFECT OF DIVERGENT SELECTION BASED ON CT MEASURED HIND LEG MUSCLE VOLUME ON PRODUCTIVE AND CARCASS TRAITS OF RABBITS

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Rabbits were divergently selected for muscle content on hind legs, as measured by computerized tomography (CT). Progenies of does and bucks in the first and second generations were compared: M=progenies of minus selected parents of the first generation (n=71), P=progenies of plus selected parents of the first generation (n=64), MM=progenies of minus selected parents of the second generation (n=25), PP=progenies of the plus selected parents of the second generation (n=57). Forty 10-week old rabbits in the M and P groups and 24 animals in the MM and PP groups were scanned by CT and their muscle volume of their legs was measured. All these rabbits were slaughtered and the carcasses were dissected. In the progenies of the first generation the production was similar, but in the progenies of the second generation the feed intake (128 vs. 138 g/d, $P<0.001$) and feed conversion ratio (2.81 vs. 3.01 g/g, $P<0.001$) were better in PP than in MM groups; body weight and weight gain were similar. Significant differences were found in the progenies of the first generation: weight of skin, kidneys and periscapular fat was higher in M group, while the weight of hind part of carcass was higher in P group. Proportion of fat deposits and that of mid part to reference carcass were higher in M rabbits, while the proportion of hind part was higher in the P group. More pronounced differences were found in the progenies of the second generation: the weight of perirenal (29.4 vs. 23.8 g, $P<0.05$) and periscapular fat (6.05 vs. 13.0 g, $P<0.001$) was lower, while the weight of the hind part (473 vs. 439 g, $P<0.05$) and hind legs (355 vs. 326 g, $P<0.05$) was higher in PP rabbits. The proportion of gastrointestinal tract to body weight was lower in PP group (16.7 vs. 18.1%, $P<0.05$). Significantly lower proportion of perirenal and periscapular fat and fore part of carcass (1.90 vs. 2.40%, $P<0.01$; 0.49 vs. 1.07 g, $P<0.001$; 29.4 vs. 30.1%, $P<0.05$, respectively), while higher proportion of hind part and hind legs to reference carcass (38.2 vs. 36.3%, 28.7 vs. 26.9%, $P<0.001$, respectively) were measured in PP group compared to MM rabbits. Results of the experiment give some evidence for effectiveness of CT based selection. If the selection objective is to increase the volume of hind leg muscles, not only the carcass traits but also the feed intake and feed conversion ratio will improve.

THE INFLUENCE OF WEANING WEIGHT ON GROWTH OF THE HYPLUS BROILER RABBIT

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The final crossbreds of the broiler rabbit HYPLUS (product of the company Grimaud Frères) were fattened from 42 to 84 days of age. The following traits were weekly recorded: body weight, average daily weight gain, average daily consumption of feed and feed conversion ratio with regard to the effect of the genotype (σ^7 PS59 \times ϕ PS19; σ^7 PS119 \times ϕ PS19), replication, interaction genotype \times replication and weight at 42 days of age (group 1: weight lower than 1300 g and group 2: weight greater than 1300 g). The highest difference in body weight between both genotypes was found at the age of 70 days, when the difference was 5.6%. During the whole fattening period the genotype (59 \times 19) showed lower feed conversion ratio ($P < 0.001$) and higher average daily gain ($P < 0.05$). The effect of replication was not-significant in most of the traits. The first replication showed a significantly higher value in the average daily gains than the second replication ($P < 0.05$). The interaction genotype \times replication was significant in the body weight at 42, 56, 63 and 70 days of age and in the feed consumption from 49 to 56 days. Rabbits of group 1 weighed 2655 g at the end of fattening period and rabbits of group 2 weighed 2892 g respectively. No growth compensation during the fattening period was observed in group 1. The body weight at 42 days of age had a significant influence on body weight at the end of fattening.

PATH ANALYSIS ON WEIGHT, BODY DIMENSION AND EAR TYPE OF SAIBEI RABBITS

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Weight, body dimension and ear type were studied on 105 six-month Saibei rabbits by path analysis. The results showed that the correlation coefficients between six-month weight on one hand and body length, chest girth, ear length, ear width on another hand were respectively 0.730, 0.736, 0.465 and 0.354. Path coefficients for them were 0.526, 0.535, 0.264, -0.148. The summation of the determination coefficients was 0.849, and the error coefficient was 1-0.849 by analyzing the determination coefficients. The best multiple linear regression equation for Saibei rabbits was established.

REPRODUCTION

SEMEN PRODUCTION AND MANAGEMENT OF RABBIT BUCKS

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ABSTRACT

The aim of this review was to summarise recent knowledge of the physiology of semen production and management of rabbit bucks. As in other mammals, the spermatogenesis is affected by many environmental factors (temperature, light, age of animals, feeding strategies, health status) as well as by semen handling (dilution, temperature, and condition of storage). Optimal conditions of rearing rabbit bucks can improve the quality of semen permitting to produce additional doses with higher and more stable fertilizing ability. In addition to the use of rabbit as a component of the food chain, rabbit bucks or spermatozoa could be useful as *in vivo* or *in vitro* toxicological models for some chemical compounds. A peculiar aspect of rabbit semen is the large presence of granules produced by prostate gland which, after ejaculation, make contact with spermatozoa. These granules seem implicated in the synchronization between ovulation time and the acrosome reaction. Since in rabbit does the ovulation occurs after several hours from mating, it is highly hypothesisable that during this lag-phase seminal particles contribute to delayed premature capacitation and acrosome reaction and maximize the likelihood that an ovulated egg would meet spermatozoa in the best functional state.

INTRODUCTION

In commercial rabbit farms, Artificial Insemination (AI) is widely employed and this diffusion has contributed to the increase in knowledge of spermatozoa metabolism and management of rabbit bucks. Many factors affect seminal traits (Boiti *et al*, 2005) and thus it is crucial to define suitable protocols to improve spermatozoa characteristics (Brun *et al.*, 2002 a, b). Hence, it is possible to produce more doses of semen with higher “expected” fertility and with less variability.

Semen is a mixture of spermatozoa, produced by testicles, and seminal plasma secreted at different sites by accessories glands and by the epididymus, which are combined at the time of ejaculation. Seminal plasma also contains other particles of different size which affect the spermatozoa behaviour during the transit along the female reproductive tract (see Figure 1).

Semen evaluation must provide information on the fertilizing ability of spermatozoa. The most relevant parameters correlated with the fertility rate are the number of spermatozoa inseminated and their motility, although the use of a single attribute is

not sufficiently accurate to predict the fertilizing ability of the semen (Love *et al.*, 1998; Colenbrander *et al.*, 2003; Lavara *et al.*, 2005). Additional semen traits or composite indexes better predict the fertilizing capacity of spermatozoa. Quinteiro *et al.* (2007), developed a composite index using a multivariate regression approach by entering several parameters of rabbit semen (motility, sperm abnormalities and altered acrosomes), which better predicts the fertilizing ability and the prolificacy of semen samples.

The low correlation between individual semen traits and fertility can also be explained by other reasons such as the use of sperm/AI in excess that masks the effect of several semen quality traits on fertility (Castellini and Lattaioli, 1999; Tardif *et al.*, 1999).

However, variation in the seminal characteristics is known to be affected by many factors (genetic strain, feeding, health status, rearing condition, season, age and collection frequency), thus contributing to the large variability in semen traits (Alvariño, 2000). Furthermore, the complexity of semen evaluation is such that substantial variability among laboratories can be introduced in the evaluation of sperm parameters (sperm counts, motility and morphology; WHO, 1999).

The purpose of this paper was to analyse the main physiological aspects of rabbit semen production and the effect of the management of bucks.

ROLE OF SEMINAL GRANULES IN THE PHYSIOLOGY OF SPERM AFTER EJACULATION

Recent scientific evidence has shown that the particulate fraction of seminal plasma plays an important role in reproductive physiology of several mammal species. These particles are secreted by different accessory glands of the male reproductive apparatus and thus their biochemical composition and function vary from species to species.

Seminal particles have different dimensions: large granules about the sperm head dimension, abundant in rabbit semen (Zaniboni *et al.*, 2004), and small particles described in several other mammalian species (Ronquist *et al.*, 1978; Breitbart and Rubinstein, 1982; Garwal and Vanha Pertulla, 1987; Fornes *et al.*, 1991; El-Hajj *et al.*, 2004).

The electron microscopic view of these particles (Figure 1) shows a prevalent round shape and the presence of clubbed cytoplasmic protrusions with small detaching vesicles. According to Metz *et al.* (1968), rabbit semen granules are not homogeneous and are composed by different populations of vesicles. They show different sizes (0.5-6 μm diameter) and are generally surrounded by a bilaminar membrane containing a scarcely organized electron dense material.

It has been postulated that these particles modulate the capacitation process and acrosome reaction (AR) of spermatozoa (Davis, 1974), kinetics of sperm (Stegmayr and Ronquist 1982; Fabiani *et al.*, 1995), immune-response of female tracts (Kelly *et al.*, 1991; Skibinski *et al.*, 1992; Miodrag *et al.*, 1995; Johansson *et al.*, 2004;), as well as the transit of spermatozoa in the female tract.

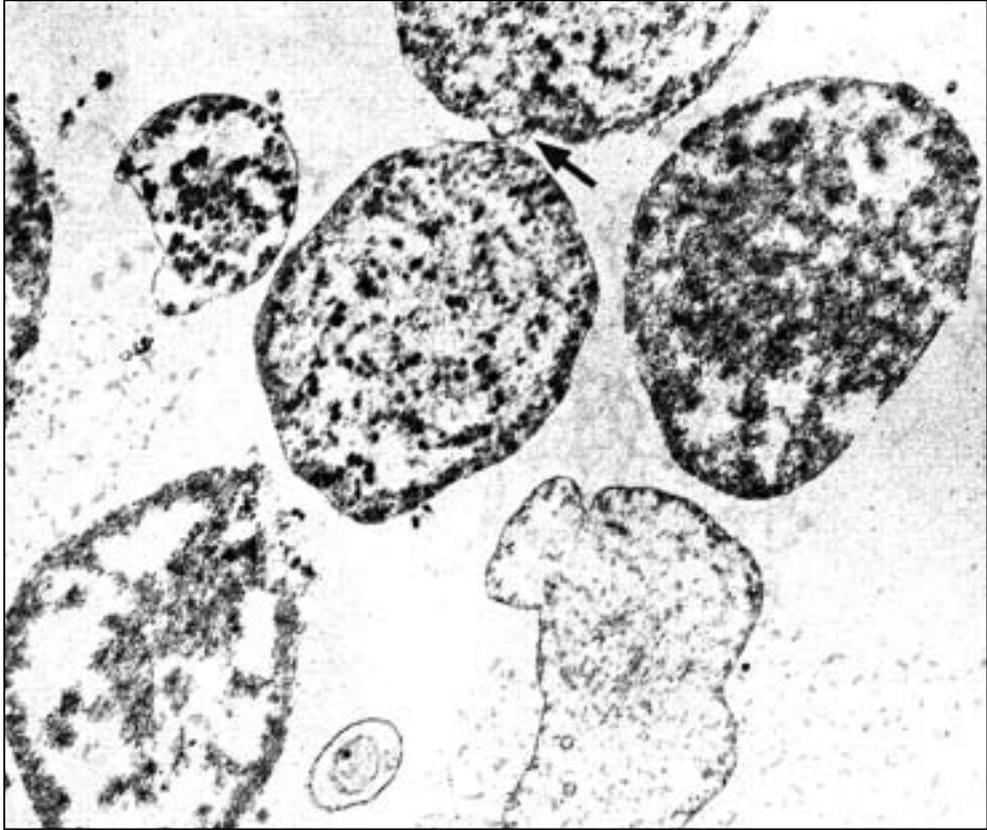


Figure 1: Transmission Electron Microscopy of rabbit seminal granules (original magnification $\times 11,500$); arrow: small detaching particles.

These particles, largely found in rabbit semen ($450 \times 10^6/\text{ml}$; Castellini *et al.*, 2006b), seem to be mainly involved in the control of capacitation and the acrosome reaction (Table 1). In fact, the presence of seminal granules significantly reduces the response of spermatozoa to *in vitro* inducers of the acrosome reaction and as a result the level of capacitated spermatozoa is almost equal to zero. On the contrary, when granules are eliminated by Percoll[®] centrifugation the decapacitative effect is virtually removed.

Table 1: Effect of seminal granules on spontaneous or induced acrosome reaction and capacitated sperm of rabbit (modified from Cardinali *et al.*, 2008).

	Spontaneous acrosome reaction (%)	Induced acrosome reaction (%)	Capacitated (%)
Raw semen	12.5 ^a	34.5 ^a	22.0 ^b
Percoll ® selected sperms	28.5 ^b	59.9 ^b	31.4 ^c
Percoll ® selected sperms + granules	26.2 ^b	28.5 ^a	2.3 ^a
Pooled Standard Error	5.9	4.2	1.9

^{a,c} on the same column: $P \leq 0.05$

The decapacitative effect would be ascribed to the release of decapacitating factors by such particles as showed by the lipid composition and the occurrence of ecto-enzymes. Indeed, seminal particles are characterized by large amounts of cholesterol and sphingomyelin (respectively 310 µg/10⁹ and 10.20 % of fatty acids - Castellini *et al.*, 2006b) and, contrary to most of biological membranes, spermatozoa included, have a molar ratio of phospholipid: cholesterol of approximately 1:2 (Arvidson *et al.*, 1989; Arienti *et al.*, 1997). It is well-known that cholesterol and phospholipids modulate membrane fluidity (Darin-Bennett and White, 1977). In spermatozoa, cholesterol is regarded as the main inhibitor of AR since cholesterol release is required for the activation of a trans-membrane signal transduction leading to sperm capacitation.

Granules and seminal plasma probably act as donors of sterols in order to protect spermatozoa against environmental shock and premature acrosome reaction. According to Cross and Mahasreshti (1997), the inhibitory activity of seminal plasma on AR is mainly contained in such granules and cholesterol is the major inhibitor.

In addition, it should be considered that seminal granules are very rich in tocopherol (38.7 mmol/l more than 50% of semen tocopherol – Mourvaki *et al.*, 2008). The antioxidant activity of tocopherol (Saez *et al.*, 1998), lowering the free radicals in the semen (de Lamirande *et al.*, 1997), could contribute to reducing the responsiveness of spermatozoa to exogenous stimuli.

Nicander *et al.* (1974) demonstrated that these particles are secreted by prostate gland, mainly in the first lobe, called pro-prostate (Figure 2), and their role as modulators of capacitation and AR along the female tract is particularly sound in rabbit. The ovulation reaction in the rabbit female is not spontaneous but is induced by coitus (Jones *et al.*, 1976). Ovulation occurs about 10-16 hours after mating and during this lag-phase rabbit spermatozoa must avoid premature capacitation and AR and seminal particles contribute in delaying this process.

Giojalas *et al.* (2004), comparing human and rabbit spermatozoa, suggest that the timing and duration of the capacitation is programmed according to the egg availability in the oviduct: long in periodic ovulators and short in induced ovulators, such as rabbits. Indeed, Brackett *et al.* (1982) reported that *in vitro* capacitation of raw rabbit semen is long and difficult, whereas Percoll ®-selected spermatozoa (without granules) show a faster rate of capacitation. This circumstance maximizes the possibilities that an ovulated egg would meet spermatozoa in the best functional state.

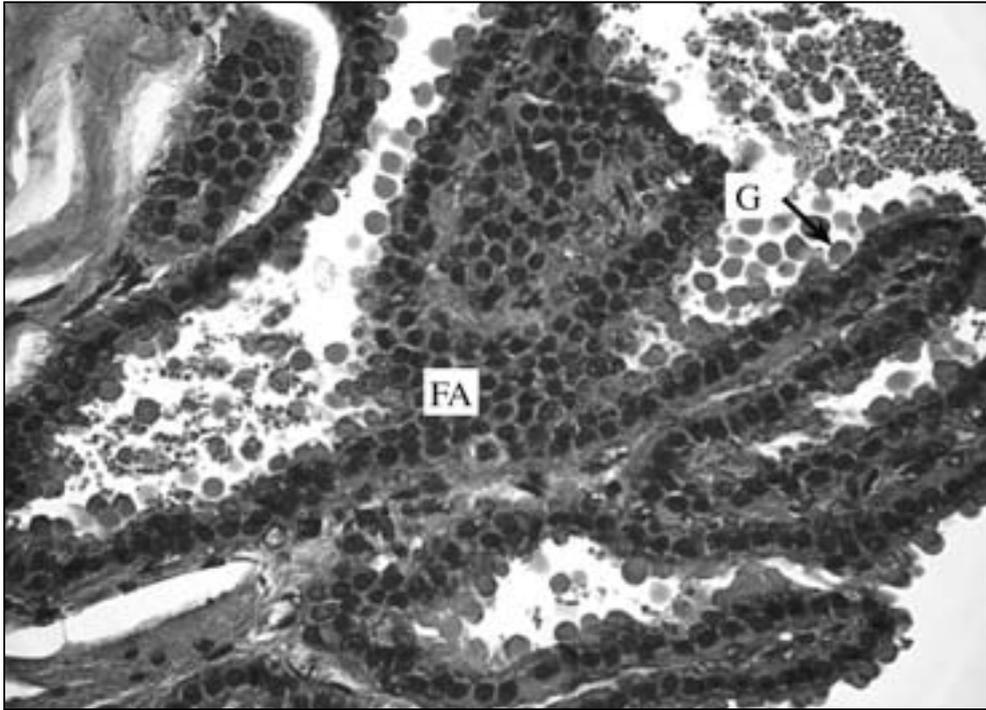


Figure 2: Histology of rabbit pro-prostate (original magnification x 200). Fibrovascular axes (FA) and seminal granules (G) still attached to the secretory cells and free in the gland lumen (from Cardinali *et al.*, 2008).

According to this hypothesis involving seminal plasma, the acrosome is stabilized by two groups of factors: some of them are of epididymus origin (Davis, 1973; Reynolds *et al.*, 1989; Fraser *et al.*, 1990), and are probably effective during spermatozoan maturation, whereas other factors (i.e. seminal particles) may have a crucial role in regulating AR during the passage in the female apparatus and are secreted by other glands of male reproductive apparatus.

FACTORS INFLUENCING SEMEN PRODUCTION

Individual and genetic

The variability of semen characteristics in male rabbits is generally high (Moce *et al.*, 2005); however, the sperm traits of some genetic strains exposed to strict protocols of rearing (light, temperature, feed) and collection frequencies has shown lower variability within and between bucks (Theau-Clement *et al.*, 2003).

Differences between genetic types of bucks have also been found for semen characteristics and fertility. Viudes *et al.* (2004), and Brun *et al.* (2002a, 2004) observed differences in semen characteristics for males from different genetic lines and from crossbred and purebred males. Crossbred sires tended to express a moderate

advantage for various semen traits, but when semen from these bucks was used for AI, a negative heterotic effect was observed. Therefore, the use of crossbred males may not provide a major advantage with respect to the use of purebred males from sire lines. These differences could be explained by differences in maternal genetic effects and the existence of heterosis for this trait (Garcia *et al.*, 2006).

Frequency of collection

Collection frequency has an important effect on semen characteristics: two ejaculates collected once a week (with an interval of at least 15 min) allows for the best semen production (Bencheikh, 1995, Moce *et al.*, 2000) both in terms of quality and quantity. Conversely, too long collection frequency (every 14 d) exerts a depressive effect on sperm output probably for the scarce sexual stimulus followed by androgen reduction (Castellini *et al.*, 2006a).

Collection frequency affects not only sperm production but also the concentration of seminal granules: daily collection, in comparison to a collection every week, reduced the spermatozoa and granule concentration even if granules showed a more stable and higher production (Castellini *et al.*, 2006a).

Light

Light length affects the hypothalamus-pituitary axis and consequently hormonal release and spermatozoa production. A daily constant 16L:8D light program increases sperm production (qualitative and quantitative aspects) compared with a shorter light length (8L:16D, Theau-Clément *et al.*, 1994).

By contrast, light intensity did not significantly affect semen characteristics (Besenfelder *et al.*, 2004).

Age

Sexual maturity occurs approximately at 5 months (depending on the strain) and semen quality generally decreases in older rabbit bucks. Recently, some authors showed that the sperm chromatin structure of the semen of rabbits between 5 and 28 months of age significantly changed. Changes in chromatin structure suggested a relatively high stability of sperm chromatin in the rabbit. The lowest percentage of sperm with damaged chromatin (1.7 -2.4%) was observed between 6 and 16 months of age. Decreased sperm chromatin stability was found in ejaculates taken from male rabbits less than 5 months and more than 20 months of age (Gogol *et al.*, 2002).

Spermatozoa of aged animals show less stable membranes and they seem more vulnerable to dietary deficiency of polyunsaturated fatty acids (PUFA; Castellini *et al.*, 2003a).

Health status

It is widely known that inflammation of the male reproductive apparatus (O'Bryan *et al.*, 2000) worsens various testicle functions and seminal characteristics by affecting biosynthesis of pro-inflammatory eicosanoids (prostaglandins and leukotriens) and cytokines (Knapp, 1990).

A high concentration of leukocytes during spermatogenesis or after ejaculation

caused by inflammation/infection can deeply reduce the integrity of acrosome by increasing free radical production. Bucks' health has to be regularly controlled mainly in aged animals.

Feeding protocols

Regarding the quantity of feed that should be administered to rabbit bucks, Luzi *et al.* (1996) showed that a restricted dietary protocol reduces *libido* and some seminal traits. However, the most important factor is not the amount of diet furnished but its chemical characteristics.

Specific recommendations for rabbit bucks are not available (de Blas and Wiseman, 1998), and only some specific requirements have been established.

Crude protein

Diets with more than 15% of crude protein are recommended to assure suitable sperm production (Nizza *et al.*, 2000).

Fat

More critical than the total amount of fat is a balanced fatty acid composition (Wesley, 1998). In mammalian spermatozoa, a very high amount of lipids are PUFA of n-3 and n-6 series (Apel-Paz *et al.*, 2003), which are associated with the membrane fluidity and its competence. Animal species are not able to synthesize essential PUFA (C18:2n-6 or C18:3n-3) and the diet has to provide adequate amounts of these fatty acids in the form of precursors (C18) or elongated ($C \geq 20$) fatty acids.

Our previous research has shown that dietary addition of PUFA n-3 modified several traits of rabbit spermatozoa (Table 2: Castellini *et al.*, 2003b; Castellini *et al.*, 2004). Relevant modifications regarding the motility and the kinetic traits of sperm cells (curvilinear velocity and lateral head displacement) is probably due to higher membrane elasticity in spermatozoa of n-3 supplemented group. The best results occurred when 2% fish or 20% flaxseed were added to the diets (Castellini *et al.*, 2005).

Table 2: Effect of dietary supplementation with n-3 fatty acids on semen traits of rabbits (modified from Castellini *et al.*, 2005).

Treatment		Control	Fish 2%	Flaxseed 5%	Flaxseed 20%
Concentration	n.x10 ⁶ ml	353 ^a	410 ^b	389 ^{ab}	493 ^b
Motile cells	%	69.9 ^a	76.6 ^b	72.9 ^{ab}	79.0 ^b
Curvilinear velocity	µm/sec	108 ^a	123 ^b	115 ^a	130 ^b
Linearity	%	48.5 ^b	42.6 ^a	47.1 ^b	43.5 ^a
Lateral head displacement	µm	3.62 ^a	4.09 ^b	3.87 ^{ab}	4.34 ^b
Spontaneous acrosome reaction	%	13.5	13.5	15.6	14.0
Induced acrosome reaction	%	31.0	36.6	35.4	37.7
Capacitated sperm	%	17.5 ^a	23.1 ^b	19.8 ^{ab}	23.7 ^b

^{a,c} on the same row: $P \leq 0.05$

On the contrary, high levels of cholesterol in the diet alter the metabolism of Sertoli cells (Yamamoto *et al.*, 1999) and the normal process of spermatogenesis (Mann and Lutwak-Mann, 1981).

Antioxidant protection

The high unsaturation levels of spermatozoa membrane render these cells very susceptible to peroxidation (de Lamirande *et al.*, 1997), which degrades membrane structure, sperm metabolism and DNA integrity (Jones *et al.*, 1979).

The more common way to increase the antioxidant stability of semen is to fortify diets with antioxidant molecules. Alpha-tocopherol (α -T) is retained as one of the most powerful antioxidants and high dietary levels of α -T, mainly if associated with vitamin C (Castellini *et al.*, 2000a, c; Castellini *et al.*, 2001b; Castellini *et al.*, 2003b), is effective in limiting the oxidative damage of semen.

However, the bioavailability of α -T in blood and in semen is non-linear: rabbit bucks fed 200 mg/kg α -T acetate showed a proportional increase after only 1 week of administration (Castellini *et al.*, 2002; Castellini *et al.*, 2006c). Studies using deuterated α -T demonstrated that newly administered α -T rapidly displaced ‘old’ α -T in plasma rather than proportionately increasing the total plasma α -T level (Traber *et al.*, 2001). It should be noted that α -T in rabbits has a lower bioavailability than in humans (Galli *et al.*, 2001) probably because of the low fat, high fiber of rabbit diet which limits vitamin E absorption (Iuliano *et al.*, 2001).

To decide the amount of α -T to be used as well as the way in which it should be administered, a suitable tool was the analysis of the major α -T catabolite (Carboxyethoxychroman: CEHC) in rabbit urine. Rabbit bucks supplemented with a mono-dose of α -T acetate (800 and 1600 mg/animal) after 24 hours showed large differences in CEHC concentration according to the dose administered (Figure 3) but at the same plasma level of α -T. After 72 hours the CEHC level in the urine was the same without any increase in the α -T plasma level.

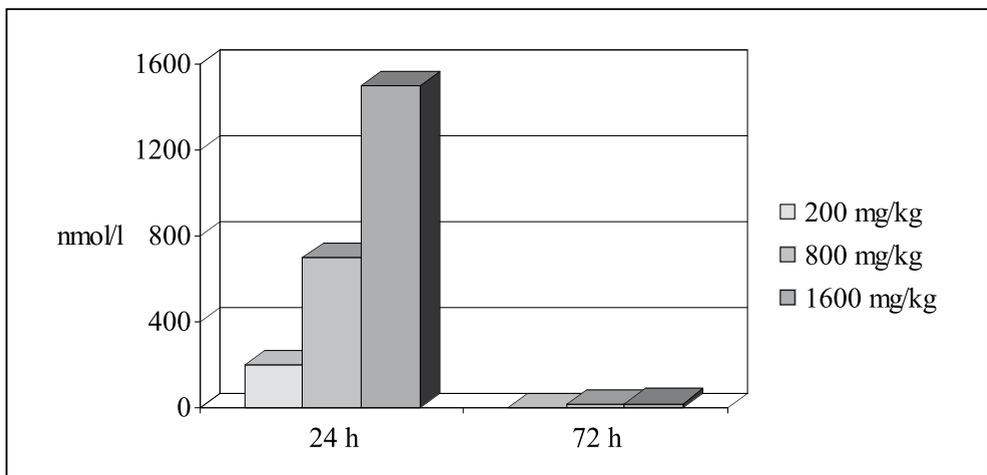


Figure 3: Carboxyethoxychroman (CEHC) levels in urine of rabbit bucks 24–72 h after α -T administration.

Considered together, these findings may be used to define the amount of α -T to be used as well as the way in which it should be administered. Doses that exceed the capability of saturable enzymes and plasma transporters have no effect on the α -T bioavailability because α -T in excess is eliminated. Therefore, continuous supplementation not exceeding the plasma α -T threshold (absence of CEHC) is more efficient than the use of single mega-dose (Castellini *et al.*, 2007a).

In semen, The trend of α -T is even more complex than in plasma. As in other species, the standard level of α -T in rabbit semen is relatively low (0.2—0.9 mmol/l; Marin-Guzman *et al.*, 1997). To increase this level, a long lag-phase of α -T accumulation occurs according to the time needed for reaching the site of action and then further time is needed for spermatozoa to differentiate and mature (about 45–50 days). When dietary supplementation is extended beyond that period, the α -T concentration in testis and spermatozoa increases, reaching a steady state after about 3 months (Castellini *et al.*, 2006c). Although the increase of α -T in semen is not particularly high, its oxidative stability is greatly enhanced because antioxidant efficiency of α -T mainly depends on its incorporation into the spermatozoa membranes. This was also confirmed by the fact that the antioxidant protection of semen is not greatly reduced after dilution (Castellini *et al.*, 2000a).

Even if α -T is the main natural form of Vitamin E existing in the body, eight other molecules are included in the definition of vitamin E, α -, β -, γ - and δ -tocopherol whose amount and role are largely unknown. The higher level of α -T compared to other homologues was probably due to the high concentration of this compound in the standard diet (90%). Such high amounts of α -T compromise the access of the other isoforms to saturable lipoproteins, binding proteins and enzymes that implicated in their transport, distribution and metabolism, and this may explain the low amounts of these compounds in semen.

According to Mourvaki *et al.* (2008), α -T, β -T, γ -T and δ -T are not homogeneously distributed within rabbit semen fractions (spermatozoa, seminal plasma and granules). Alpha-T is the main lipid antioxidant in all fractions, while γ -T, β -T and δ -T are more abundant in germ cells and seminal plasma, respectively. Differences in the chemical structure might account for the differential distribution of the minor tocopherol isoforms within fractions. More polar tocopherols (δ -T) have a higher affinity for aqueous seminal plasma, while less polar tocopherols (α -T > β -T > γ -T ordered by non-polarity) have a higher affinity for the lipid-rich cell membrane and seminal granules.

This antioxidant protection is essential after semen storage or when diets have a relevant amount of PUFA (Figure 4). When diets are fortified with different sources (fish and flaxseed) and levels (2-20%) of PUFA, the antioxidant stability of semen decreases. In addition, these dietary protocols determine a higher susceptibility of spermatozoa to capacitation and the acrosome reaction (Castellini *et al.*, 2001a; 2005).

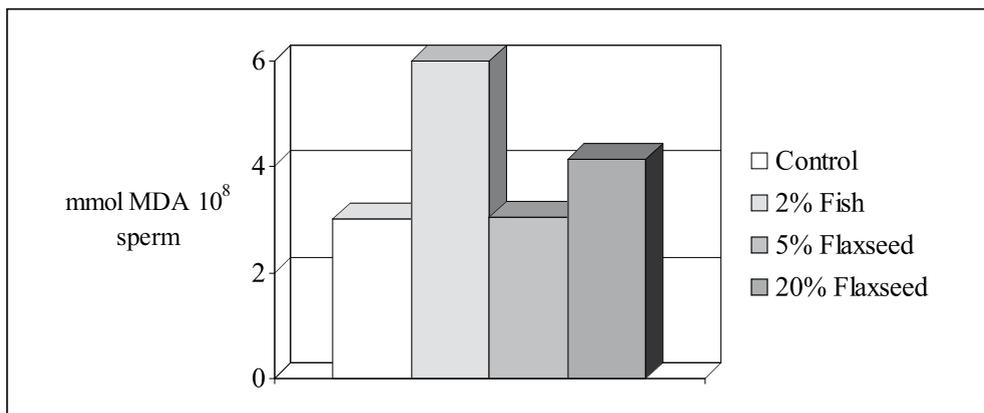


Figure 4: Effect of dietary supplementation with n-3 fatty acids on antioxidant stability of rabbit semen.

SEMEN COLLECTION AND HANDLING

Some authors reported that a previous stimulation of the buck increases sperm concentration. For this aim, a doe can be put on the top of the cage for few minutes. The type of artificial vagina influences the adaptation of buck to the collection. An artificial vagina with a wider collection hole increases the number of bucks adapted to the collection (Dal Bosco *et al.*, 1996). At each collection time, a sterile artificial vagina should be used. Indeed, considerable bacterial contaminants come from the environment, and it is important to hygienically collect the semen sample (Mercier and Rideaud, 1990).

As a general rule, during semen handling, any shock of sample (temperature, chemical, oxygen) may be reduced to avoid reduced fertility (Boiti *et al.*, 2005).

Within 5 min from collection, semen should be diluted (1/2-1/5) with a buffer medium at the same temperature to avoid heat or cold shocks. Generally, all the semen handling has a negative effect on semen traits.

STORAGE CONDITIONS

Temperature

Regarding the storage temperature, generally 15-18 °C represents a good condition to store rabbit semen. Nevertheless, optimal temperature could depend on the extender used.

Extenders

One important factor to have good survival rate of sperm is the medium used in semen dilution and the dilution rate. The comparison between different media has shown that any physiologic buffered saline solution is adequate for very short storage period. However, for longer storage there are differences in the ability to support sperm survival (Seed *et al.*, 1996). Tris-buffer is enough to allow storage from 24-48h.

The high dilution rate (more than 1/100; Castellini *et al.*, 2000b) has a detrimental effect on motility and the excessive dilution of seminal plasma, which in rabbit plays a relevant role (Minelli *et al.*, 2001), which reduces kinetic characteristics of spermatozoa.

Incubation

In accordance to specific demands of semen for further laboratory analysis or for AI, an adequate procedure has to be chosen to maintain vitality and physiological capability of fertilisation over a defined time interval. Thus, semen has to be diluted or spermatozoa have to be separated and put into a final medium. For semen dilution as well as for the spermatozoa containing medium, optimal conditions must be assured by selecting buffers that are adapted to the reproduction organs; CO₂ (5%) incubators preferentially will support long term spermatozoa vitality by simultaneously reducing excessive contact with oxygen.

RABBIT SEMEN AS MODEL FOR TOXICOLOGICAL OR METABOLIC STUDIES

Exposure to some chemical compounds can alter semen quality and fertility. Since the prevalence of human infertility in industrialized countries has increased over the past decades, the assessment of the risk to the human reproductive system associated with exposure to compounds due to pollution is of major concern.

Mice are widely used for toxicological studies because of the massive reported information on its development and functions, along with responses to many toxicants. Even for rabbit, the main physiological traits have been studied, both as a laboratory animal and as a component of the food chain. The rabbit is the smallest laboratory and least expensive animal model, wherein almost all the reproductive and toxicological endpoints of humans can be measured. The rabbit semen can be easily collected by artificial vagina and the fertility of sperm tested (Foote and Carney, 2000; Foote, 2002).

For *in vivo* models, a very useful method could be the use the spermatozoon as a toxicological target. The mature spermatozoon is a cell that is eliminated of almost all the normal biological functions but retains those dedicated to provide motility and the fertilisation ability. Damages to one of such structures are easily detectable because the ejaculated spermatozoon is unable to activate a repairing process. Following these considerations, it is sound to use the spermatozoon as a good target for metabolic (Kamp *et al.*, 1996) and toxicological studies (D'Cruz *et al.*, 2000).

According to such assumptions, some authors (Young *et al.*, 1992; Foote, 2002) hypothesized the use of rabbit spermatozoa as a model for toxicological studies and proposed motion-based indices as toxicological endpoints. For several chemicals, motion-based endpoints of spermatozoa may be useful for the *in vitro* assessment of chemical cytotoxicity.

It is quite evident that to develop a repeatable *in vitro* spermatozoa model for *in vitro* toxicity tests, different preliminary steps of standardization are required. Accordingly, the variability factors are reduced (Seed *et al.*, 1996) and the discriminating power of the model increased (Williams *et al.*, 1990).

Renieri *et al.* (2002) and Sartini *et al.* (2006), within a research program of the European Centre of the Validation of Alternative Methods, developed an *in vitro* rabbit spermatozoa model of spermiotoxicity for some metal ions. It involves the use of integrated strategies based on data derived from human and animal studies, as well

as *in vitro* toxicity test, which also lead to a reduction in the use of laboratory animals that is very advantageous for ethical and economic reasons.

It appears that some endpoints of this rabbit spermatozoa model involving motility rate and the integrity of the acrosome are very sensitive traits for detecting metal ions damages and could be useful in assessing toxicity.

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SHORT TERM EFFECTS OF DIFFERENT DIETS ON OVARIAN FUNCTION AND OOCYTE MATURATION OF RABBIT NULLIPAROUS DOES

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Determination of the nutritional needs in reproductive does has been intensively studied, but the direct influence of nutrition on the ovarian physiology is poorly known in rabbits. Some nutritional factors may influence oocyte maturation that is the first step affecting successful fertilization and preimplantational embryo development. In this context, leptin is a possible link between the nutritional status and the reproductive function. The goal of this work was to study the short term effects of feeding strategies during rearing period (from 11 to 16 weeks of age) on the endocrine and reproductive parameters of nulliparous rabbit does. A total of 40 New Zealand x California white rabbits were randomly allocated to two experimental groups (HL and SL) fed with rich fibrous diets with a high (HL: NDF 50% DM, ADL 16% DM) or a standard lignin content (SL: NDF 41% DM, ADL 5% DM). Ten does per group were euthanized, five before (G1 group) and the other five (G2 group) after artificial insemination (AI). The remaining rabbits were used to measure the conception rate. Serum leptin concentrations, ovary and body weight, number of follicles ≥ 1 mm in size in the ovarian surface, ovulation rate, and blastocyst recovery rate were recorded. Cumulus oocyte complexes (COC) were aspirated from ovarian follicles ≥ 1 mm in size of one ovary and were matured *in vitro*. A total of 113 COC were treated progressively for cortical granules (CG) and nuclear staining and observed under a confocal laser-scanning microscope. Leptin levels were higher in SL than in HL group (5.49 vs. 4.53 ng/ml; $P < 0.04$). However, the average weight of does (HL: 3526 g; SL: 3641 g) and ovaries (HL: 182.1 g, SL: 218.1 g), mean of total ≥ 1 mm follicles per ovary (HL: 7.3, SL: 5.8), and ovulation rate (HL: 14.4, SL: 16.6) were similar between nutritional groups at time of the first insemination (16 week). In addition, there were no significant differences in both nuclear and cytoplasmic *in vitro* maturation measured as metaphase II (HL: 67.5%, SL: 59.3%) and CG migration rate (HL: 26.1%, SL: 39.2%); blastocyst recovery rate (HL: 80.5%, SL: 71.0%), and fertility rate (HL: 75.5%, SL: 86.6%). In conclusion, both fibre-rich diet with low and elevated content of lignin during rearing period did not seem to exert any effect on the reproductive parameters of the nulliparous does.

PITUITARY GONADOTROPINS AND RECEPTORS FOR ESTROGEN AND GNRH IN FASTED DOES

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The present work examined the expression for both estradiol-17 β subtype α (ER α) and GnRH receptors (GnRH-R) and that for FSH β as well as their regulation after two-days of fasting in the anterior pituitary gland of control and estrogen-primed rabbits, together with the LH dynamic secretion following GnRH stimulation. Sexually mature unmated NZW female rabbits were randomly assigned to the following groups: control does fed *ad libitum* (AL), AL implanted with 2.0 mg estradiol-17 β (AL-E), 48 h fasted does (F), and F implanted with estrogens (F-E). Whereas control does were fed *ad libitum*, treated does were fasted for 48 h before killing or i.m. GnRH injection. Immediately after GnRH treatment, fasted does were re-fed *ad libitum*. ER α immunoreactivity was evidenced in the nucleus of most pituitary cells in both control and treated does. In F-E rabbits, the intensity of ER α signal and the number of positive cells were markedly reduced. Positive staining for GnRH-R was evidenced in the nucleus and in the cytoplasm, although with weaker signal, of many pituitary cells of both control and treated does. In pituitaries of F-E does, GnRH-R signal intensity was markedly reduced. Fasting and oestrogen did not affect basal plasma LH concentrations. In all the groups, the LH peak surge was observed 30 min after GnRH injection. The LH magnitude was lower ($P \leq 0.01$) in F than in AL rabbits, but higher ($P \leq 0.01$) in F-E than in AL-E does. In all the groups, plasma LH levels declined close to basal values 4 h after GnRH. Fasting as well as oestrogen priming of both AL and F rabbits reduced ($P < 0.01$) the levels of pituitary ER α mRNA expression. The lowest ER α gene expression was found in F-E does. GnRH-R mRNA relative abundance was down regulated ($P < 0.01$) four-fold in F-E does. FSH mRNA expression was down-regulated ($P < 0.01$) only in F-E rabbits. In conclusion, the rabbit anterior pituitary responds to changes in nutritional status, as provoked by 48 h fasting, and to gonadal steroid through adjustments of ER α , GnRH-R, and FSH β at the level of gene expression as well as through regulations of LH release into the blood stream in order to adjust the reproductive system to metabolic condition. Complete deprivation of food for a short period of time could be a useful model for analysing the interrelationships between nutritional factors and reproductive function in rabbits.

THE PHYSIOLOGICAL DILEMMA OF THE HIGH PROGESTERONE SYNDROME IN RABBIT DOES

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This work focused on the mechanisms that may cause multiple asynchronous ovulations and alter normal ovarian function in order to characterize the high progesterone (P+) syndrome in rabbit does, that, having abnormally high plasma progesterone concentration at the time of insemination, fail to become pregnant. At different luteal stages, at either days 4, 9, or 13 of pseudopregnancy, induced by GnRH injection (d-0), two groups of rabbits (n=5/group) were treated with saline or 0.8 µg GnRH. Blood samples were collected from d-0 to d-26 of pseudopregnancy. At d-4, GnRH injection prolonged (P<0.05) the functional CL life span by 3 to 4 d over that of controls. At d-9, GnRH caused a transient decline (P<0.01) of progesterone for the following 3 d but, thereafter, increased again and remained higher (P<0.01) than controls up to d-26. At d-13, progesterone fell to 1 ng/ml within one day following GnRH, but then gradually increased. Based on these progesterone profiles, it can be argued that, at both mid- and late-luteal phase, GnRH triggered luteolysis and induced ovulation followed by the formation of a new generation of CL. For the *in vitro* study, CL, collected at days 4, 9, and 13 of pseudopregnancy, were incubated with GnRH, GnRH-antagonist, PLA₂ inhibitor, and PLC inhibitor. GnRH decreased (P<0.01) progesterone secretion by d-9 and d-13 CL cultured *in vitro*; by converse, GnRH antagonist increased (P<0.01) progesterone release from d-4 CL. Co-incubation of GnRH with GnRH antagonist increased (P<0.01) progesterone release in d-4 CL, but had an opposite effect (P<0.01) on d-9 and d-13 CL. PLC inhibitor reversed the GnRH effects in both d-9 and d-13 CL, while PLA₂ inhibitor did not change progesterone release. These data suggest that rabbit CL express a functional receptor for GnRH, likely of type II, that utilizes the PLC post transductional cascade. Luteal FSH-R and LH-R mRNA relative abundances did not differ between d-4 and d-9 CL, but were two- to three-fold (P≤0.01) higher, respectively, at d-13. StAR mRNA was highly expressed at d-4 of pseudopregnancy, but then markedly declined (P≤0.01) at d-9 and d-13. Taken together, our results show that GnRH triggers i) functional regression when CL acquire luteolytic capacity from d 9 of pseudopregnancy onward, and ii) multiple asynchronous ovulations, thus partly explaining the P+ syndrome associated with the simultaneous coexistence of two population of “fresh” and “old” CL, although not yet the underlying causes.

BODY CONDITION SCORE AND RELATED PRODUCTIVE RESPONSES IN RABBIT DOES

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A simplified 3-point scale method for *in vivo* scoring body condition is proposed as an useful and rapid tool to support experimental and on-farm management of nutrition and reproduction of rabbit does. The productive responses of lactating rabbit does scored and inseminated at 11 days *post partum* (pp) are verified, as well as performance of non-pregnant does scored and re-inseminated at 32 days pp when non-lactating. After their first parturition, 96 New Zealand White rabbit does were checked for 126 days over three successive 42-day reproductive cycles. The body condition scoring was based on feel by hand the loin and the rump for bone protrusions and muscle fullness. The loin was evaluated for poor, intermediate and wide level, the rump for poor and wide level. The body condition was scored “0” with poor loin; “1” with intermediate loin and poor rump; “2” with intermediate and wide loin and wide rump. The highest fertility was obtained with intermediate body condition score (BCS) for both lactating (52.0, 84.9 and 58.6% for BCS 0, 1 and 2; $P<0.001$) and non-lactating does (53.1, 84.4 and 64.5% for BCS 0, 1 and 2; $P<0.05$), indicating the negative effect of poor or excessive body fatness. A poor BCS led to lower litter size at birth in lactating (7.2, 8.2 and 8.3 for BCS 0, 1 and 2) and non-lactating does (8.4, 11.1 and 9.6 for BCS 0, 1 and 2; $P<0.05$), and higher losses of kits from day 1 to 11 of nursing (10.3, 2.2 and 4.5% for BCS 0, 1 and 2; $P<0.001$). The main part of does culled for infertility (44.4%) or death (46.7%) showed the BCS 2. When lactating does resulted non-pregnant at an 11-day artificial insemination (AI), their frequency in the BCS 2 showed a higher increase from parturition to AI (from 30.8 to 52.2%) than they were pregnant (from 26.8 to 37.7%). The lactating does that were non-lactating in the previous cycle showed a higher incidence at BCS 2 (54.8%) than they were lactating (43.8%) or nulliparous (39.6%). These latter indications suggest that failures in fertility at 11-day AI and culling rate are imputable more to the excessive body fatness of does than to their poor body condition.

PHEROMONE IN RABBITS: PRELIMINARY TECHNICAL RESULTS ON FARM USE IN FRANCE

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The results obtained when a pheromone, the Rabbit Appeasing Pheromone (RAP), was applied on farms between July 2005 and February 2007 are shown and discussed. The authors present briefly the pheromones and refer to the following definition: a pheromone is a chemical substance produced by one animal to communicate by olfactory route with other congeners of the same specie, and to induce on them some innate stereotyped behaviour patterns. The same protocol of RAP application was implemented in 9 farms differing for size, location, reason of RAP use and season when the product was applied. All the farms were under all in-all out system of management. RAP devices were introduced in the reproduction compartment 2 to 3 days before parturition, and let for 42 days until the next parturition. So, the litters grew under action of the product until weaning (35 days). As the pheromones spread in the air, it was not possible under field conditions to have contemporary placebo treated control, thus, technical results obtained with RAP were compared to the results of the previous cycles. Only farms with regular technical monitoring and properly recorded data were included in this trial. The data were transmitted by the technician responsible for the farmer economical results. In most of the cases, the farmer observed that the animals seemed quiet quickly after the implementation of the product (from a few hours to 1 day). Does were less stressed, and technical actions (as sweeping, nest manipulation, introduction of external personal) were easier. At least, technicians often noticed by additional comments on the technical documents that the treated rabbits were heavier and healthier at weaning. Fertility (percentage of parturitions per artificial insemination (AI), live born kits per litter and kits viability at birth improved in 3 farms. A global analysis, performed from data of all the farms (13090 does of reference groups and 8915 of treated groups) confirmed this trend. The results of this pooling were quite comparable to individual data recorded in each farm, confirming the interest of the use of this RAP for reproductive does. In conclusion, these trials brought new information regarding the use of pheromones on a mammal species: does are quieter with RAP, and manipulations are easier, fertility, litter size and viability at birth improve.

EFFECT OF A REPRODUCTIVE RHYTHM BASED ON RABBIT DOE BODY CONDITION ON FERTILITY AND HORMONES

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Productive protocols based on standard (Control: artificial insemination (AI) 11 days *post-partum*) or Conditioned rhythm (AI only when the does reach a perirenal fat depot weight estimated between 15 and 30 g) were compared. Two groups of fifty New Zealand White females were inseminated for seven consecutive cycles. The kits were weaned at 26 days. On the day of AI, all the does were submitted to ultrasound scanning of the perirenal regions to measure fat thickness and to BCS evaluation. Live weight, perirenal fat weight estimated and cumulative BCS were higher ($P<0.01$) in the Conditioned Group. These does, compared to the controls, showed higher sexual receptivity and fertility rate ($P<0.01$) as well as number of live born ($P<0.05$). The higher progesterone value found in control rabbits ($P<0.05$) confirms the negative effect of lactation on both sexual receptivity and fertility ($P<0.01$). Control does had lower ($P<0.05$) T3 blood concentrations, reflecting their worst body status. Multiparous does had higher ($P<0.01$) leptin levels, that only in Conditioned group reflected a greater body condition. Insulin and glucose concentrations were slightly increased in control does compared to conditioned rabbits. Further investigations are needed to analyze other important metabolites and hormones and to confirm the effect of body status condition on long-term performance of rabbit does. In conclusion, compared to fixed insemination at 11 days *post partum*, the conditioned rhythm seems more adapted to the reproductive physiology of rabbit does as testified by their higher sexual receptivity and fertility rates and better body condition. It is also evident that excessive fatness of non-pregnant does should be controlled by reducing feed ingestion during the dry period. Both BCS evaluation and measurement of perirenal fat by ultrasound technique permit to manage properly the energy balance and to improve body status, reproductive performance and welfare of rabbit does. Hormonal and metabolite analyses represent a good tool for understanding the physiological mechanisms required to meet these objectives.

IN VITRO VIABILITY OF SPLIT RABBIT EMBRYOS BEFORE AND AFTER VITRIFICATION

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Of 125 embryos (category I and II) recovered from 18 does, 25 were maintained intact as control, 25 bisected using a simplified splitting protocol, 25 frozen thawed according to a vitrification method, 25 bisected-vitrified, and 25 vitrified-bisected. The survivability of intact embryos and demiembryos were evaluated after 24 hours of *in vitro* culture by development ability, morphological assessment, and cell count with double staining method. The development rate after *in vitro* culture of intact control embryos (96%) was higher ($P<0.001$) than vitrified embryos (36%). The development rate of demiembryos was higher (74%) than bisected-vitrified embryos (34%), and vitrified-bisected embryos (10%) as well ($P<0.001$). A higher number of cells and percentage of surviving cells was observed in control embryos (91%), followed by fresh demiembrios (84%), vitrified embryos (74%) ($P<0.01$), bisected-vitrified demiembryos (67%), and vitrified-bisected demiembryos (61%). Embryos and demiembryos originated from category I embryos showed higher development rate than those obtained from category II. The reduced survivability for the vitrified-bisected embryos might be due to the combined effects of loss of cohesion and reduction of cells as a result of the bisection and to the crioprotectant toxicity during hydration. In conclusion, bisection and vitrification can be used together without micromanipulation and freezing complex systems, but the embryo quality is the main factor in the success of these procedures.

MANUAL INDUCTION OF LORDOSIS AND DETECTION OF OESTRUS IN THE DOMESTIC RABBIT (*Oryctolagus cuniculus*)

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Receptive female mammals adopt the lordosis posture to facilitate fertilization during copulatory interaction. The present paper shows the results of experiments carried out to find mounting-associated stimuli that actually activate the lordosis in female domestic rabbits. The involvement of estrogens in such activating stimuli was tested. Eight females were ovariectomized (experimental condition 1) and subsequently estrogenized with estradiol benzoate (experimental condition 2). In each condition, females were subjected to two kinds of stimulus: a) pressure upon the rump, sacs filled with sand (weighting either 0.5, 1.0, 2.0, or 3.0 kg) simulated the bodyweight of the male during mounting; b) perineal beatings, subtle finger-beatings (3 per second) simulated the penile exploration of a male attempting penetration. Pressure exerted upon the rump is capable of activating the lordosis response when it is ≥ 1.0 kg. The lordosis response to the pressure stimulus is entirely dependent on body levels of estrogens. Seemingly, the effectiveness of this stimulus is strongest when the pressure approximates the weight of a young semental male: the difference between responses to 1.0 kg and 2.0 kg was significant ($P=0.003$). On the other hand, the lordosis response is induced by the beating stimulus in a particularly accentuated manner. Interestingly, the effectiveness of the beating stimulus is estrogen-independent. Similar responses were obtained with ovariectomized and estrogenized females. Our findings suggest that different nervous mechanisms induce the lordosis response. We propose the induction of the lordosis response as an alternative to current indicators of sexual receptiveness, which are either ambiguous or relatively difficult to notice. Induction of lordosis can be achieved by subtly pushing the rump of females with an open hand.

EFFECT OF GELATIN ADDITION TO FREEZING EXTENDER ON RABBIT SEMEN PARAMETERS AND REPRODUCTIVE PERFORMANCE

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An improvement in reproduction management has been associated to the wide use of artificial insemination in rabbits. The use of frozen semen in this species is greatly limited due to its low fertility rates. Moreover, in polytocous species, prolificacy is a very important trait and it is particularly affected when semen is frozen. Since gelatine addition to fresh semen extenders improves and prolongs motility and viability sperm parameters, its use in semen freezing could enhance sperm efficiency after thawing.

The aim of this study was to assess the effects of gelatine addition to freezing extender using a commercially available extender (MIII) and a tris-based extender (TGC). Four experimental extenders were used: TGC extender with gelatine, the same without gelatine and MIII extender with and without gelatine. In order to evaluate seminal parameters, motility was assessed using the Computer Assisted Sperm Analysis, while viability rate was calculated using flow cytometry. To evaluate *in vivo* frozen-thawed semen, 273 females were inseminated. No significant difference was found among the four freezing extenders when motility, viability fertility or prolificacy were considered. Motility rates ranged between 22.4% and 35.1%, while viability varied between 22.5 and 30.9%. Fertility rates oscillated from 80.8% to 86.2% and the total born ranged from 7.9 to 8.5. While *in vitro* results were lower than those obtained in other studies, *in vivo* results were quite favourable. In conclusion, gelatine addition did not improve sperm motility and viability after thawing nor fertility and prolificacy after insemination with frozen-thawed semen, using MIII or TCG extender.

IMMUNE RESPONSE TO REPEATED rhFSH SUPEROVULATION TREATMENT IN RABBIT DOES

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Some studies have demonstrated that, when superovulation is induced more than once in the same animal, the response to treatment may be reduced. This reduced response may be related to an increase of anti-gonadotrophin antibodies. The aim of this study was to evaluate the effect of repeated recombinant human (rh) FSH superovulation treatments on the ovulation rate and anti-FSH antibodies production. For this purpose, 34 females were treated i.m. with rhFSH (0.6 µg every 24 h, for 3 days) in order to induce superovulation four consecutive times. Control does were injected with the vehicle at the appropriate time. The interval between the first three treatments was one month; and three months between the third and fourth treatment during which the females were inseminated without superovulation treatment. Ovulation rate was checked by laparoscopy and blood samples were collected after each treatment. An indirect ELISA was used to detect sera anti-FSH antibodies. The ovulation rate was significantly higher in does treated with rhFSH than in control group. The ovulation rate was significantly higher in does treated for the first time with rhFSH than in those treated two, three, or four times (8.7±1.42, 19.3±1.36, 13.5±1.26, 13.0±1.28, 14.3±1.31 for control and superovulated females, respectively, P<0.05). On the other hand, results obtained after four consecutive rhFSH treatments indicate that there was a significant difference in immune response of does after the second treatment (P<0.05), none of the treated females presented immune response in the first or second treatment, on the contrary, in the third and fourth treatment the 40 to 60% of females presented high antibody levels. The results of the present study clearly demonstrate that repeated rhFSH superovulation treatments in rabbit does induce an immune response and have a negative effect on ovulation rate. Although anti-FSH antibodies induce a decrease in superovulation response, the ovulation rate of females superovulated twice, three and four times was significantly higher than control females. The immune response developed has an important individual variability and may be related with the reproductive response decrease after repeated treatments. Nevertheless, since there were females in which ovulation rate diminished without an increase in sera antibodies, it is clear that reproduction failure after consecutive superovulation treatments can be caused by different reasons, which have to be studied in future.

MOTILITY AND ACROSOMAL INTEGRITY OF FROZEN RABBIT SPERMATOZOA AS AFFECTED BY DIFFERENT EXTENDERS, CRYOPROTECTANTS AND PACKAGING METHODS

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In a first trial semen from twenty NZW rabbit bucks was used to study the effects of different extenders (Tris–yolk fructose, lactose–yolk citrate and sucrose–yolk citrate), type and level of cryoprotectant (glycerol or dimethylsulfoxide) on motility and acrosome integrity percentages of frozen rabbit spermatozoa. In a second trial the effect of packaging method (straws or pellets) of frozen rabbit semen on sperm motility and acrosome integrity was also studied. The results showed that sucrose–yolk citrate and tris–yolk fructose extenders were significantly ($P<0.01$) better than lactose–yolk citrate extender in maintaining high motility and acrosomal integrity. The 2% glycerol or 4% dimethylsulfoxide levels gave significantly ($P<0.01$) higher motility and acrosomal integrity for frozen rabbit bucks spermatozoa. Post–thaw motility and acrosomal integrity were significantly better when semen of rabbit bucks was packaged in straws rather than in pellets.

ESTIMATION OF MILK PRODUCTION OF RABBIT DOES BY CROSS SECTIONAL DIGITAL IMAGING

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Several researchers have measured the milk yield of rabbit does. Daily milk yield can easily and accurately be measured by determining the weight difference of the doe before and after nursing; there is an estimation possibility of milk yield based on the kits' growth during the suckling period. Computer Tomography (CT) is used for *in vivo* body composition analysis in rabbits. The aim of this study was to develop an indirect computer tomography-aided method to predict the milk production of lactating does. Five lactating does were weighed by weight-suckle-weight method four times at days 7, 14, 21, 28 of lactation. At the same time they were scanned by spiral CT before and after suckling to estimate their milk yield. During the examination, the animals were fixed with belts in a specially designed cradle without using anaesthetics. Spiral CT scans were adjusted to acquire 10 mm thick overlapping slices from the whole body. Data collection was performed on the basis of whole mammary gland tissues. To determine the rabbit milk density, some milk samples were collected for digital imaging. The density interval of milk was approximately +20-60 Hounsfield Unit (HU). The mammary gland density range was 0-60 HU; the mean density slightly changed before and after nursing. The linear regression between measured and mammary gland density based CT estimated milk yield showed the highest coefficient of determination ($R^2=0.963$; SE 17.03; $P<0.01$). The estimated milk yields of the first, second, third and fourth pair of glands were 20, 33, 25 and 22%, respectively. The applied *in vivo* CT examination method seems to be suitable in estimating milk yield depending on pairs of mammary glands.

RESAZURIN REDUCTION TEST AS A TOOL FOR ASSESSMENT OF RABBIT SEMEN QUALITY

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In the present investigation, spectrophotometric evaluation of resazurin reduction test (RRT) to assess the color changes of resazurin reduction in butanol extracted color was used to evaluate rabbit semen quality. One hundred samples of rabbit semen were included in this study and the absorption was read at 580 nm and 615 nm. Results indicated that RRT ratios decreased as the preservation time increased and the highest correlation was observed with sperm motility ($r=0.975$, $P<0.0001$) and acrosomal integrity ($r=0.864$, $P<0.0001$). In conclusion, RRT could be used as a tool for evaluating the quality of rabbit semen.

LINE AND BIRTH SEASON EFFECTS ON OXIDATIVE STRESS PARAMETERS IN TESTIS OF MATURING RABBITS

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Oxidative stress is a factor related to the male reproductive function. Differences between breeds have been found for testis size, seminiferous tubule diameter, number and size of interstitial and germ cells, etc. Traits related to the redox system could also be affected by genetic factors. The existence of differences between lines for these traits could lead to differences in reproductive maturation and fertility.

The present paper has investigated the age-related changes of the superoxide anion ($O_2^{\cdot-}$) radical formation, superoxide dismutase activity (SOD), catalase activity (CAT) and thiobarbituric acid reacting substances (TBARs) level in testis of rabbits. The existence of differences between lines selected for different aptitudes (Caldes for growth rate and Prat for litter size) and the effect of birth season for these traits were assessed.

Major changes in the oxidative metabolism were observed at an early age and could be explained by the concomitant changes in testicular structure and function. Both lines showed similar developmental profiles and levels for all the variables studied. Environmental conditions affected both lines in the same manner. Significant seasonal variations were found in $O_2^{\cdot-}$, SOD and CAT. Future studies should take into account differences between seasons for a more precise analysis.

**INFLUENCE OF ENVIRONMENTAL TEMPERATURE
AND RELATIVE HUMIDITY ON QUANTITATIVE
AND QUALITATIVE SEMEN TRAITS OF RABBITS**

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In rabbits, it has been shown that the comfort zone temperature is around 21°C and that their productive and reproductive performance could be impaired when temperature-humidity index (THI) is over 27.8, value that implies the beginning of heat stress. However, no information is available about the optimum range of THI and the value of THI which indicate the starting of cool stress.

The present paper investigated the effect of low to moderate THI index on semen traits of rabbits. Three THI classes were defined: THI=16 (ranged from 14.0 to 17.4), THI=20 (ranged from 18.6 to 20.6) and THI=22 (ranged from 20.7 to 23.1); and the following variables were recorded: absence of gel plugs, urine, calcium carbonate deposits and agglutinated and dead spermatozoa in the ejaculate, individual motility, pH, volume and cell sperm concentration. Differences between THI-class have been found for absence in the ejaculate of gel plugs, urine, calcium carbonate and agglutinated and dead spermatozoa in the ejaculate, for ejaculate volume and for ejaculate pH. Differences were relevant and favourable to THI-22 for ejaculate volume and absence of agglutinated spermatozoa, and important and favourable to THI-16 for absence of urine, calcium carbonate and dead spermatozoa.

EFFECT OF COLOUR OF LIGHT ON THE REPRODUCTIVE PERFORMANCE OF RABBIT DOES

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The effect of light colour on the rabbits' production has not yet been analyzed. According to the literature the rabbits perceive the red light (its wavelength) less compared to other light colours. The objective of our experiment was to analyze the effect of the blue light on the rabbits' production. The experiment was carried out at the University of Kaposvár using Pannon White rabbit does. The does were housed in two identical rooms. The rooms only differed in the applied light colour. In the first room white colour was applied (W group, n=59), in the second group blue colour was used (B group, n=63). In both rooms a 16L/8D lighting regime was used throughout the experiment. Luminous intensity, independently of the light colour, measured at the middle of the cages ranged between 40-70 lux. The blue light significantly reduced the feed consumption of rabbit does during the third week of pregnancy and through their whole period of first pregnancy, and their body weight measured at parturition and day 23 *post partum*. Individual and litter weight measured at 23 days of age was significantly higher in the B group (3498 vs. 3611g and 435 vs. 451 g; $P<0.05$). Number of rabbits born per insemination and litter weight measured at 23 days of age showed a 6.4% and 7.9% difference (W: 7.37, B: 7.84; W: 2.91 kg, B: 3.14 kg) respectively. Results suggest that evaluation of the light colour's effect on the rabbit production can be perspective.

EFFECT OF A LIGHT STIMULATION ON THE REPRODUCTIVE PERFORMANCE OF RABBIT DOES

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In order to replace the PMSG treatment used for oestrus synchronisation, the effects of the increased lighting from 8 to 16 hours were examined. The experiment was carried out at the University of Kaposvár using Pannon White rabbit does. Prior to the experiment does were kept using a 16L:8D lighting program and each doe had 2-3 parturitions. The does were randomly housed in two identical rooms. Rooms differed only in lighting regime. In the first room a 16L:8D lighting regime was used throughout the experiment (16-16L group). In the other room a lighting system of 8L:16D was used during the days (lighting: between 6.00 and 14.00) after parturition, then the light period was increased to 16 hours per day 8 days prior to insemination (lighting: between 6.00 and 22.00). After insemination the lighting period was modified to 8 hours per day (8-16L group). Light intensity measured in the cages at the height of rabbit does ranged between 40 and 70 lux. In the 16-16L and 8-16L groups, 153 and 154 inseminations (94 and 111 parturitions) of 55 and 54 rabbit does were evaluated, respectively. Based on the results, the increased lighting period prior to insemination favourably affected pregnancy rate (16-16L: 61.4% vs. 8-16L: 72.0%, $P < 0.05$). Although the differences were not significant, number of kits born alive and litter size at 3 weeks of age were higher in the 8-16L group, while slightly higher individual and litter weights were recorded at the age of three weeks in the 16-16L group. No differences were found for mortality between the groups. Comparing the pooled results per 100 inseminations, the 8-16L does produced 16% more kits alive at birth (620 vs. 522, $P < 0.05$). Light stimulation could be an alternative to PMSG treatments. Nevertheless, further experiments will be necessary to determine the cause of the decreased rabbit weight when applying longer daily lighting program and to test the durability of the positive effects.

OOCYTE GLUTATHIONE CONCENTRATION IN A RABBIT LINE SELECTED FOR OVULATION RATE

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The present study was designed to determine glutathione concentration in rabbit oocytes and to establish its relation to ovulation rate. Glutathione concentration is used to assess oocyte quality. A total of 59 does belonging to a line selected for ovulation rate during seven generations were mated with vasectomized males to induce ovulation. Females were slaughtered fifteen hours later and oocytes were collected by flushing the oviducts. Oocytes were processed and glutathione concentration was determined by the enzymatic recycling assay of the 5,5-dithio-bis (2-nitrobenzoic acid)-glutathione disulfide reductase. Glutathione concentration ranged from 4.7 to 10.3 pmol/oocyte. Ovulation rate was classified into three levels: low (10-14 corpora haemorrhagica), medium (15-16 corpora haemorrhagica) and high (17-24 corpora haemorrhagica). Oocyte glutathione concentration was significantly lower in oocytes from females with high (6.6 ± 0.3 pmol/oocyte) and medium (7.3 ± 0.4 pmol/oocyte) ovulation rates than in oocytes from females with low ovulation rates (8.4 ± 0.3 pmol/oocyte). It seems that high ovulation rates could be associated with poorer oocyte quality in comparison with low ovulation rates.

CORRELATION BETWEEN CASA AND ASMA PARAMETERS IN RABBIT SEMEN

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Computerized motility analysis (CASA) and quantitative, morphologic and morphometric analyses (ASMA) were performed in 187 semen samples from 23 adult males selected for daily weight gain between 28 and 63 days of age (Line R). The following traits were recorded: volume (VOL), concentration (Cn), normal apical ridge (NAR), abnormal sperm (ANR), length of sperm head (L), width of sperm head (W), area of sperm head (A), perimeter of sperm head (P), percentage of motile cells (MOT), average of curvilinear velocity (VCL), average straight-line velocity (VSL), average path velocity (VAP), linearity index (LIN), straightness coefficient (STR), wobble (WOB) and amplitude of lateral head displacement (ALH). Phenotypic correlations between sperm traits were estimated, and Bonferroni's correction was applied. Several significant correlations between volume, concentration, motility, morphological and morphometric parameters were also observed.

Positive correlations between sperm concentration and motility ($r=0.22$) and negative correlation with volume ($r=-0.28$) were evidenced. In this study we observed a significant correlation between some morphometric parameters like width and area of the sperm head and the percentage of motile cells ($r=0.42$, $r=0.37$, respectively) and abnormal sperm percentage ($r=-0.25$, $r=-0.24$, respectively) indicating the relationship between morphometric parameters and sperm quality in rabbit males. The quality of motion, measured by different indexes (LIN, STR, WOB), was positively correlated with width sperm and/or area head ($r=0.31$, $r=0.26$, $r=0.24$, respectively for LIN, STR, WOB, with W, and $r=0.27$, for LIN with A, respectively). Significant positive correlations were observed between different sperm velocity parameters and the amplitude of the lateral head displacement of sperm ($r=0.50$, $r=0.78$, $r=0.60$ for VSL, VCL, VAP), indicating that rapid sperm motion is associated with a greater lateral displacement of the sperm head. These results indicate that morphometric parameters of sperm head, in combination with sperm concentration, morphology analyses and motility parameters determined by CASA systems, can provide complementary information about the kinematic and morphometric characteristics of rabbit semen, and their inclusion in the spermogram could improve the prediction of potential fertility.

OPIOID INHIBITION OF THE PULSATILE LUTEINIZING HORMONE RELEASE AS ASSESSED BY NALOXONE TREATMENT IN THE LACTATING RABBIT

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Since in nursing rabbits sexual receptivity and fertility achieved after AI appear to be depressed during lactation, the effect of endogenous opioid peptides (EOP) on serum LH were investigated in lactating rabbits (n=30) by administration of the opiate antagonist naloxone (nal). Blood samples were collected every 15 min for 4 h via an indwelling catheter inserted in the central ear artery. After the first h of sampling, the rabbits received i.v. saline (SAL, n=10); nal (LN, 0.5 mg/kg, n=10); or nal (HN, 1.0 mg/kg, n=10). The pulsar algorithmic procedure for the study of pulsatile hormone secretion was used to calculate mean and basal LH concentrations, frequency and amplitude of LH pulses. Data from the statistical analysis are presented as mean \pm SE of area under the curve (AUC) units of LH response. Mean LH concentration during the 60 min pre-challenge period was 0.24 ± 0.03 ng/ml. The AUC of LH during that period did not differ among treatment groups (LN=16.6 \pm 4.1, HN=11.8 \pm 2.9 and SAL=14.3 \pm 4.0 area units). In the SAL group LH secretion remained low during the 4 h sampling period. Mean and basal LH concentrations were 0.21 ± 0.05 ng/ml and 0.03 ± 0.02 ng/ml (LN=0.59 \pm 0.13 ng/ml and 0.20 ± 0.04 ng/ml; HN=0.38 \pm 0.09 ng/ml and 0.13 ± 0.03 ng/ml). The mean pulse peak was 0.70 ± 0.15 ng/ml (LN=1.95 \pm 0.37 ng/ml; HN=1.26 \pm 0.23 ng/ml), and the mean pulse amplitude was 0.67 ± 0.14 ng/ml (LN=1.89 \pm 0.36 ng/ml; HN=1.22 \pm 0.21 ng/ml). A lower number of pulses (1.88 \pm 0.35) was also detected for the 4 h period (LN=2.57 \pm 1.13; HN=2.34 \pm 1.11). Nal treatment increased LH release. A greater AUC was observed during the 60 min post nal period in both nal-treated groups (LN=55.0 \pm 15.5 and HN=38.8 \pm 10.3 vs. SAL=5.9 \pm 2.7 area units; $P < 0.01$). Rabbits receiving 0.5 mg/kg nal had an increased ($P < 0.05$) AUC (LN=105.9 \pm 26.1 area units) through 180 min after nal administration compared with the saline-treated group (SAL=34.9 \pm 9.2 area units). The group LN differed from the SAL group through the 180 min post-nal period, while the HN group differed only for 60 min. Nal-treated groups (LN and HN) did not differ in either of the post-nal periods (60 and 180 min). Since all the experimental rabbits presented a clear LH surge after the nal challenge, the suppression of pituitary LH release has been shown to be associated with EOP activity. EOP may have modulated hypothalamic secretion of GnRH, resulting in a tonic inhibition of LH secretion. However, further studies are necessary to better clarify EOP-cortisol interaction in the lactating rabbit doe at the moment of AI and after an i.m. injection of a GnRH analogue to induce ovulation.

ULTRASOUND EVALUATION OF OVARIAN FOLLICULAR DYNAMICS DURING EARLY PSEUDOPREGNANCY AS A TOOL TO INQUIRE INTO THE HIGH PROGESTERONE (P+) SYNDROME OF RABBIT DOES

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The investigation of the underlying causes for the high progesterone (P+) syndrome should presuppose further studies to more precisely characterise pseudopregnant rabbit does. The purpose of the present research was to examine follicular dynamics in the postovulatory rabbit ovary by means of real-time B-mode ultrasound scanning. A particular attention was focused upon the population of large-sized follicles present through the first 6 days after induced pseudopregnancy, since this follicular category, being steroidogenically active, may be crucial to the survival of the developing CL. Pseudopregnancy was induced in twenty-four hybrid rabbit does caged individually by injection of 100 IU human chorionic gonadotropin (hCG). Sequential monitoring of dynamic changes in the follicular population was performed by ultrasonography on day 0, 2 and 6 of pseudopregnancy (day 0 being the day of hCG injection). The follicle distribution in ovaries of untreated does (day 0) was marked by approximately equal number of small follicles ($46.1\% \pm 6.7 < 2.0$ mm) and large follicles ($54.4\% \pm 6.1 \geq 2.0$ mm). However, at 2 days after injection of an ovulatory dose of hCG, the large follicles population was markedly depleted with only $10.2\% \pm 0.9$ of the follicles present classified as large. The ovulation rate averaged 10.8 ± 0.7 per rabbit. By day 6 of pseudopregnancy the size distribution showed that a repopulation of the ovary with large follicles occurred with $71.3\% \pm 6.8$ in this category. The large follicles lost at ovulation had apparently been replaced by the time of luteal estrogen dependence at day 6 of pseudopregnancy. The results presented in this report, demonstrating that the rabbit ovary is depleted of large follicles following ovulation but that large, steroidogenically active follicles are again present by day 6, could be considered as a preliminary step to understand what mechanisms protect the CL from luteolysis until day 6 of pseudopregnancy, when CL shift from complete refractoriness to partial and complete responsiveness to PGF₂ α treatment. Since little is known about what factors effectively trigger luteolysis in normal, physiological conditions as well as what mechanisms protect CL from luteolysis in the first days of pseudopregnancy, the innovative and non-invasive approach supplied by the real-time ultrasonography could represent an additional tool for investigation.

EFFECT OF FEEDING REGIME DURING REARING AND AGE AT FIRST MATING ON THE REPRODUCTIVE PERFORMANCE OF RABBIT DOES

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Effects of feeding restriction, associated to a later age at first artificial insemination (AI), on the reproductive performance of rabbit does were examined. Half of the Pannon White (PW n=222) and larger body size (LB n=197) does were fed *ad libitum* and inseminated at the age of 15.5 weeks (AD15, n=203). The remaining does were fed 130 g/day of a commercial pelleted diet from the age of 11 weeks till 8 days prior to the first AI and were inseminated at the age of 19.5 weeks (RES19, n=216). At the first AI the AD15 groups showed significantly ($P<0.05$) lower conception rate (PW: 81.3%; LB: 73.0%) than the does from the RES19 groups (PW: 90.1%; LB: 88.5%). Mortality of kits in the first litter of the LB-AD15 does was significantly higher than that of the kits of the LB-RES19 does (19.8% vs. 15.6%, $P<0.05$) or that of the kits born in latter parities regardless of the does' group (pooled average of the groups: 10.6%). Number of kits born alive, total number of kits born, litter size at day 21, litter and individual weight at day 21 were not significantly affected by the treatment (rearing method and age at first AI) in both genotypes. Number of kits born alive per 100 inseminations was higher in the RES19 group does in both genotypes (754 vs 718 for PW; 721 vs 688 for LB). It can be concluded that inseminating larger adult size does at the age of 15.5 weeks is not advisable even by *ad libitum* feeding. Regardless of the does' genotype feeding restriction during rearing and insemination at a later age are advantageous.

INFLUENCE OF FSH, LH AND PROLACTIN ON THE COMPONENTS OF LITTER SIZE IN RABBIT DOES

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The aim of this study was to investigate whether the concentrations of luteinizing hormone (LH), follicle-stimulating hormone (FSH), and prolactin (PRL) around mating were related to the components of litter size. Data from 60 primiparous females were used. Blood was taken 48 h before, and 2 and 48 h after the natural mating. Sera were analyzed by radioimmunoassay for LH, FSH, and PRL. Laparoscopy was performed at day 12 of gestation to record ovulation rate (OR) and number of implanted embryos (IE). The litter size (LS) at birth was recorded. There were significant differences among LH concentration measured 48 h before or after mating (5.1 and 5.4 ng/ml, respectively) vs. 2 h after mating (29.0 ng/ml), but not for FSH and PRL. Non lactating females and females at day 18 of lactation showed higher LH and PRL concentrations than females on days 11 and 25 of lactation. All the hormones were influenced by the season, LH showing higher values in autumn than in summer, in opposition to both PRL and FSH. The females with high OR (>15 ova), IE (>12 embryos), and LS (>8 kits) showed higher plasma LH concentration (15.1, 14.9, 15.5 ng/ml, respectively) than females with low OR, IE, and LS (11.1, 9.6 and 9.7 ng/ml, respectively). The level of FSH influenced OR and LS, but PRL affected only OR.

REPRODUCTIVE PERFORMANCE OF RABBITS FED *MORINGA OLEIFERA* AS A REPLACEMENT FOR *CENTROSEMA PUBESCENS*

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In a twenty-week trial, forty does of heterogeneous stocks of rabbits (derived primarily from New Zealand White, California White and Chinchilla breeds) aged one to two years and weighing between 1.5 and 2 kg were allotted to five experimental diets in a completely randomized design to evaluate the reproductive performance of rabbits fed *Moringa oleifera* as a replacement for *Centrosema pubescens*. Freshly harvested *C. pubescens* and *M. oleifera* leaves were offered to the animals at 2% of their live weights at the ratios of 100:0 (M0), 75:25 (M25), 50:50 (M50), 25:75 (M75) and 0:100 (M100), respectively in addition to the concentrate feed offered to the animals.

There were significant differences in the total DM intake of does on the different treatments ($P<0.05$). The M25 does had the highest DM intake (131.6 g/day) followed by M50 does (125.5 g/day) and M0 does (122.5 g/day), while M100 does had the lowest (112.1 g/day). However, there was no significant difference in CP intake among the groups.

M0 and M100 does had the highest litter size at birth of 5.12 and 5.81 respectively (though not significantly different). M50 does had the lowest litter size at birth (4.06) and this was significantly different from that of M100 does (5.81). There was no significant difference in the initial average body weight and gestation length of the does on the different treatments as well as in the litter weight at birth. Both litter size and litter weight at weaning were highest in M0 and M100 groups recording a litter size of 5.00 for both treatments and a significant ($P<0.05$) difference in litter size at weaning across all treatments. The average daily weight gains per kid were 6.99, 8.06, 8.64, 8.13 and 6.78 g/day for M0, M25, M50, M75 and M100 treatments, respectively. There was a significant difference ($P<0.05$) in average daily weight gain per kit and in milk yield across all the treatments. Milk yield was higher in M0, but not significantly different in M75 and M100 does. It can be concluded that *M. oleifera* can be used to replace *C. pubescens* without adverse effect on the reproductive performance of rabbits.

THE INFLUENCE OF DIFFERENT CONTACT LEVELS WITH MALE ON THE VAGINAL CYTOLOGY IN RABBITS UNDER THE TROPICAL HUMID CONDITION

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This study aimed to determine the influence of the contact with the male on vaginal cyclicity in the female rabbit through the evaluation of the cellular content of the vaginal lumen. Twelve rabbit does of mixed parity were divided equally into three treatments: Auditory and Olfactory contacts (AUD-OLF); Auditory, Olfactory and Visual contacts (AU-OL-VI); No contacts (NO-COT) with the male. Animals were housed in pair. In AUD-OLF and AU-OL-VI treatments the male animal was housed in a separate compartment between the females, while visual contact between the male and females was blocked with a screen in AUD-OLF. NO-COT was housed in another pen about 200 m away. Vaginal smears were daily collected from all the females for 29 days and the cells classified into superficial, intermediate and parabasal epithelial cell. From 10 different fields, each cell type was counted and then expressed as percentage of total. The rectal temperature of the animals was measured just prior to each smear collection. Intermediate cells appeared dominant (5.63-72.82%) in the AUD-OLF treatment, whereas parabasal cells predominate (20.24-90.66%) in the NO-COT group. Parabasal and intermediate cells occurred at equal proportions in the AU-OL-VI animals. The type of contact had no clear cut effect on the appearance of the superficial cells with only slight differences among treatments (9.20, 11.05 and 13.52% for AUD-OLF, AU-OL-VI and NO-COT, respectively; $P < 0.05$). On the contrary, the intermediate cell numbers were highest in AUD-OLF, followed by AU-OL-VI and NO-COT (59.44, 40.07 and 28.82%; $P < 0.05$). The reverse was the case with parabasal cells with values of 31.37, 48.87 and 57.64% ($P < 0.05$), respectively. Rectal temperature was significantly ($P < 0.05$) higher in NO-COT animals (38.20°C). Rectal temperature had significant ($P < 0.01$) positive (0.40) and negative (-0.44) relations with parabasal and intermediate cell count, respectively. Superficial and intermediate cells were significantly ($P < 0.01$) and negatively related (-0.30) as also were intermediate and parabasal cells (-0.91). The association between superficial and parabasal cells was also negative (-0.11), but not significant. In conclusion the vaginal smear from normal adult rabbit shows no distinct pattern of occurrence of superficial, intermediate and parabasal epithelial cells as in spontaneous ovulators; female rabbit isolated completely from the influence of male shows more parabasal cells in the vaginal lumen which is characteristics of anoestrous animals and auditory, olfactory and visual contacts with the male appear determinant to prepare the female rabbit for sexual receptivity.

EFFECT OF LHRH ANALOGUE INCLUDED IN SEMINAL DOSE ON KINDLING RATE AND PROLIFICACY OF RABBITS ARTIFICIALLY INSEMINATED

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The objective of our work was to influence positively the induction of ovulation and kindling rate in female rabbits and fertilization capacity of rabbit's spermatozoa by means an intravaginal application of a superanalogue GnRH-Lecirelinum (Supergestran) included in the insemination dose. Experiments were performed on adult female rabbits. Control females were given an intramuscular dose of GnRH-Lecirelinum (Supergestran) (2.5 µg per doe) immediately after the artificial insemination. In experimental females, the insemination doses (I.D.) had different quantities of GnRH-Lecirelinum (Supergestran): 2.5 µg/I.D. (group S2.5), 5.0 µg/I.D. (group S5), 7.5 µg/I.D. (group S7.5) and 15.0 µg/I.D. (group S15), which were incubated 30 minutes at laboratory temperature. Obtained parameters were statistically evaluated by one-way variance analysis. Significance of differences among groups was determined by t-test (LSD) and Duncan's test. Kindling rate (%) in the control group of females was 62.74±13.70. The lowest value of kindling rate was obtained in the group S2.5 (42.99±5.64) and the highest (72.09±2.96) in females of group S7.5. Medium values of kindling rate were reached in females of group S5 (59.97±11.56) and S15 (52.77±3.92). We noticed significant differences in kindling rate of group S2.5 in comparison with those of control, S5 and S7.5 groups only. It would seem that the intravaginal dose in group S2.5 was not sufficient to cause suitable ovulation. Intravaginal application of GnRH-Lecirelinum in the dose 7.5 µg/I.D. influenced positively the induction of ovulation and interactions spermatozoon – zona pellucida of the ovum, with subsequent benefit for the kindling rate in comparison with the control (+9.35%). The average number of kits born alive and kits mortality at birth were not significantly different among the experimental groups of females. Therefore, intravaginal application of GnRH-Lecirelinum in insemination dose did not influence negatively the litter size at birth.

OVULATION INDUCTION IN RABBIT DOES BY INTRAVAGINAL ADMINISTRATION OF THE GnRH ANALOGUE [DES-GLY10, D-ALA6]-LHRH ETHYLAMIDE: FIELD TRIAL

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In the rabbit doe, ovulation does not occur spontaneously, but it has to be induced through a neurohormonal reflex, which is initiated during mating. So, when using AI, in the absence of a male, ovulation has to be induced by artificial methods. The ovulation inducing method most frequently used is an intramuscular injection of a synthetic analogue of GnRH or gonadorelin. An alternative way, demonstrated in previously studies, is the intravaginally administration of the hormone.

This study aimed to evaluate the efficacy of GnRH analogue [des-Gly10, D-Ala6]-LHRH ethylamide, administered intravaginally, to induce ovulation in rabbit does submitted to AI. In this experiment, a large scale field trial was done to test the use of 25 µg of GnRH analogue [des-Gly10, D-Ala6]-LHRH ethylamide vehiculated in the seminal dose (n=270) against 20 µg of gonadorelin via intramuscular (n=270). Fertility was higher (P<0.05) when ovulation was induced by intravaginal administration of [des-Gly10, D-Ala6]-LHRH ethylamide (91.1% vs. 85.6%). Prolificity or mortinatality were never affected by the ovulation induction treatments.

It was concluded that GnRH analogue [des-Gly10, D-Ala6]-LHRH ethylamide can be used for ovulation induction in rabbit does vehiculated in the seminal dose, with better AI results as those obtained with gonadorelin administered intramuscularly.

YOUNG RABBIT DOES FED WITH FIBROUS DIET DURING REARING: SERICAL AND PRODUCTIVE PARAMETERS

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The aim of this work was to determine the effect of different feeding systems during rearing on serum leptin, non esterified fatty acids (NEFA) and total protein levels and some productive parameters of rabbit does. Thirty six animals 11-weeks old were randomly distributed in three experimental groups: group AL-C and group R-C were fed *ad libitum* and restricted (150 g/day) respectively a commercial diet (NDF 38% DM). One week before first artificial insemination (AI), R-C group was fed *ad libitum*. AL-F group fed an experimental diet with a higher level of fibre provided *ad libitum* (NDF 50% DM). First AI was made in AL-C does at 16 weeks of age and in AL-F and R-C does one week later. After first parturition, all animals fed *ad libitum* the commercial diet. During the rearing period, animals of group R-C had the lowest daily feed intake ($P<0.001$), digestible energy intake ($P<0.03$) and digestible protein intake ($P<0.001$), delaying the onset of puberty ($P<0.001$), decreasing their fertility at first AI ($P<0.02$) and having the lowest pre-puberal leptin concentration ($P<0.001$) in comparison with the other two groups. During pregnancy, AL-F does had a tendency to a higher feed intake with respect to other groups, and their fertility on day 11 *post partum* (pp) tended to be higher ($P<0.09$). All animals showed physiological high levels of total serum protein at 16 weeks of age. Serum NEFA level was high around parturition in all groups indicating mobilization of energy reserves, even though in AL-F group this mobilization was lower. *Ad libitum* feeding system with fibrous diets during rearing allowed to obtain similar reproductive performances in nulliparous rabbit does than feeding system with lower fibre inclusion.

REPRODUCTIVE AND PHYSIOLOGICAL RESPONSES OF RABBIT DOES UNDER DIFFERENT NUTRITIVE LEVELS BEFORE THE FIRST PARTURITION

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The objective of the research was to study the effect of different feeding levels before the first kindling on the reproductive performance and blood plasma profile of rabbit does and on the litter performance during two reproductive cycles. The trial was carried out on Grimaud female rabbits (15 weeks old) reared according to three nutritive treatments: the control group (C) was fed *ad libitum* a commercial pellet (CF=18.7% DM; DE=11.7 MJ/kg DM), the restricted group (R) was fed an 80% *ad libitum* intake of commercial feed and the fiber group (F) was fed *ad libitum* a high-fiber pellet (CF=24.6% DM; DE=9.8 MJ/kg DM). The experimental diets were offered for 8 weeks until the first kindling, after which the does were fed the control pellet *ad libitum*. A blood sample was taken from each doe five days after delivery. At the first reproductive cycle, the live body weight of the females at the end of the treatment was significantly different, as C rabbits had a higher ($P<0.01$) body weight than those R and F groups. The growth rate was higher ($P<0.01$) in C rabbits than in R females and the F group was intermediate. Feed intake was higher ($P<0.01$) in F rabbits than in C subjects; the R females showed the lowest ($P<0.01$) consumption. After first delivery, the C and F rabbits exhibited higher ($P<0.05$) body weights than R does, but at weaning the females of the three groups had similar live weight. The F group had a higher ($P<0.05$) number of inseminations/pregnancy and a higher ($P<0.05$) insemination-pregnancy interval compared to the other groups. The *interpartum* interval did not exhibit different values among the groups. The F group produced litters with higher ($P<0.05$) birth weight because of lower litter size than the C and R groups. At weaning the litter weight was similar among the groups. The number of rabbits at weaning was similar for the three groups as well as the mortality rate. Blood triglycerides levels were higher ($P<0.05$) in F and R does than in C females. Neither total proteins nor urea levels differed. Regarding mineral content, plasma calcium was higher ($P<0.05$) in R does than in the other two groups and phosphorus was higher ($P<0.05$) in R and F females. The results indicate that feed restriction on pubertal females up to one week before the first parturition affects the growth and has significant effects on certain reproductive and physiological parameters during the first reproductive cycle. It does not, however, negatively influence the productive response of litters. In the second reproductive cycle no relevant difference among the groups was observed for does and litter performance.

INFLUENCE OF DOE EXPOSURE AND SEASON ON REACTION TIME AND SEMEN QUALITY OF MALE RABBITS

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The objective of the present research was to evaluate the influence of doe exposure on reaction time (RT) and five semen attributes of male rabbits in winter and spring. Fifteen-month-old selected sexually experienced fertile New Zealand White male rabbits (n=18) were randomly allocated to one of two treatments: (1) doe exposure; and (2) no doe exposure (control). Doe exposure was done with prepubertal replacement does separated by an adjacent wire-mesh wall; females were changed in an every other week schedule. Semen collection lasted 14 weeks, 7 in winter and 7 in spring; collection was twice a week using an artificial vagina and a teaser female, and two ejaculates were obtained at each collection. In total, 728 ejaculates were analyzed statistically. Analyses of variance were under a mixed model where fixed effects were treatments, season and ejaculate, rabbit was the random effect. No significant ($P \geq 0.05$) interaction of treatment with season was found. Doe exposure improved ($P \leq 0.05$) RT and four of the five semen attributes: RT was shorter (-0.9 sec) and volume (+0.21 ml), sperm motility (+17.4 percent units), concentration (+49.7 x 10⁶) and total live normal motile sperms (+44.5 x 10⁶) were higher. Spring showed better ($P \leq 0.05$) RT and semen attributes than winter. First ejaculate was better ($P \leq 0.05$) than the second except for sperm motility, which in the second ejaculate was 15.3 percent units higher than in the first one. There was a significant effect ($P \leq 0.05$) of the interaction treatment x ejaculate on total live normal motile sperms (TLNMS): bucks with doe exposure had a higher TLNMS in the first ejaculate (126.3±9.2 x 10⁶) than in the second (107.8±9.2 x 10⁶), while in bucks isolated from females the second ejaculate showed higher TLNMS (84.5±9.2 x 10⁶) than the first (58.4±9.2 x 10⁶). Doe exposure can enhance sexual behaviour and semen attributes of male rabbits in winter and spring.

EFFECT OF CRYOPRESERVATION ON ATP CONTENT OF OVULATED RABBIT OOCYTES

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Oocytes cryopreservation represents a very promising tool for medicine and preservation of animal genetic resources. Assessment of oocytes viability after cryopreservation is often based on structural and developmental criteria whereas metabolism of thawed oocytes is not frequently studied. The aim of this study was to evaluate the impact of slow freezing and vitrification methods on the ATP concentration in ovulated rabbit oocytes. ATP contents of oocytes were evaluated by measurement of light emission intensity during the ATP-dependent oxidation of luciferin by luciferase. ATP levels were compared between frozen and vitrified oocytes after 2 hours of *in vitro* culture and fresh oocytes as a control. Independently of the cryopreservation method used, ATP content per cell was significantly ($P < 0.001$) lower for cryopreserved oocytes as compared to fresh ones (6.34 ± 0.39 pmol of ATP per fresh oocyte). Frozen oocytes showed a significantly ($P < 0.001$) lower ATP metabolism than the vitrified ones (2.93 ± 0.23 and 4.88 ± 0.21 pmol of ATP per frozen and vitrified oocyte respectively). In conclusion, this study shows that ATP assay is an attractive tool to evaluate the viability of cryopreserved oocytes. Moreover, according to these results, vitrification seems to be a more suitable method than slow freezing to cryopreserve ovulated rabbit oocytes.

COMPARISON OF TWO REPRODUCTIVE RHYTHMS OF RABBIT DOES

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The objective of the experiment was to compare the reproductive rhythm of 42 with that of 56 days. One hundred twenty two does were randomly halved and the two groups (42D and 56D) were inseminated 11 and 25 days after parturition, respectively. During the last 3 days prior to the insemination controlled nursing was applied (instead of free nursing) in group 42D and kits were weaned at the age of 35 days. In group 56D rabbits were weaned at the age of 23 days, two days prior to the dams' insemination. Does' production was monitored for 336 days. During this period the maximum parity was 8 and 6 in 42D and 56D groups, respectively. Nearly significant differences were found between groups 42D and 56D for the number of inseminations per kindling (1.22 vs. 1.12), does' body weight at parturition (4188 vs. 4474 g), kindling interval (46.6 vs. 59.5 days), E-value of TOBEC measurements at 4th and 5th parturitions (2770 vs. 2434) and survival rate at 336 days (13 vs. 26%; P=0.07). No significant differences were found in litter size (total and alive) and in individual and litter weight at day 23. From the viewpoint of animal welfare inseminating does 25 days after parturition is favourable because their survival and condition was superior although the performance of group 56D was poorer compared to the 42D group for number of annual parturitions (7.8 vs. 6.1) and for the number of kits born alive (69.2 vs. 51.9) which makes efficient production impossible.

HIGH PLASMATIC PROGESTERONE LEVELS AT INSEMINATION DEPRESS REPRODUCTIVE PERFORMANCE OF RABBIT DOES

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The aim of this experiment was to quantify the frequency of the high progesterone syndrome at the moment of insemination and to study the influence of progesterone level (P4) on receptivity and reproductive performances of primiparous and secondiparous rabbit does. A total of 422 primiparous INRA 0067 were inseminated twice at an interval of 42 days. Prior to artificial insemination (AI), sexual receptivity of the does was tested in the presence of a vasectomised buck and, just after AI, blood samples were collected to determine progesterone concentration by RIA. The mean plasma progesterone concentration was 1.8 ± 3.4 ng/ml and significantly depended on parity (primiparous: 2.2 ± 3.7 ng/ml, secondiparous: 0.9 ± 2.2 ng/ml, $P < 0.001$). At the following AI, 78% of pseudopregnant rabbit does returned to the basal level of oestrous condition, demonstrating that the pseudopregnancy is a reversible process. The overall percentage of pseudopregnant does ($P4 \geq 1$ ng/ml) was 25.9%, but it was differently ($P < 0.001$) distributed between primiparous (31.2%) and secondiparous does (12.2%). Moreover, primiparous lactating females were more frequently pseudopregnant than non-lactating ones (36.5 vs. 18.9% respectively, $P < 0.001$). The progesterone level of primiparous does was related to the lactation status (2.5 ± 3.8 vs. 1.5 ± 3.2 ng/ml, respectively for lactating and non-lactating, $P < 0.001$). The receptivity was highly related to the level of progesterone ($P4 < 1$: 74.1%, $1 \leq P4 < 6$: 79.1% vs. $P4 > 6$: 56.3%, $P = 0.006$). Also the kindling rate was significantly influenced by progesterone concentrations. Non-pseudopregnant does ($P < 1$ ng/ml) had the highest fertility rate (79.0%) but when the progesterone concentration increased from $1 \leq P4 < 6$ to $P4 > 6$ ng/ml, the fertility decreased from 68.1 to 37.4%, respectively ($P < 0.001$). Consequently, the productivity at birth was highly depressed when the progesterone level was over 6 ng/ml (9.5 and 8.6 vs. 4.5 number of born alive rabbits/AI, for $P4 < 1$, $1 \leq P4 < 6$ and $P4 > 6$ respectively, $P < 0.001$). The productivity of pseudopregnant and non-receptive females was very poor in comparison with pseudopregnant receptive does (0.4 vs. 9.5 born alive rabbits/AI). The productivity at birth was also highly ($P < 0.001$) influenced by the physiological status of the does. Primiparous non-lactating does produced the highest number of born alive rabbits/AI, whereas primiparous lactating does had the lowest productivity at birth (9.9 vs. 5.6), secondiparous lactating being intermediate (7.1). In conclusion, the high progesterone syndrome evaluated at the moment of insemination occurred in 25.9% of females and had a strong negative impact on receptivity and reproductive performance of rabbit does.

INFLUENCE OF PHOTOPERIOD ON THE SEXUAL BEHAVIOUR OF NON-LACTATING RABBIT DOES: PRELIMINARY RESULTS

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The aim of this experiment was to study short term and long term effects of different lighting programmes on the sexual behaviour of rabbit does maintained without production for 18 weeks after their first weaning. Sixty INRA 0067 rabbit does were equally divided into three groups (groups 8, 816 and 16). Four days before the first insemination, they were placed into three identical rooms under a constant 16L:8D lighting programme. The day following the first weaning, three different lighting programmes were applied. For does of groups 8 and 816, the lighting programme suddenly changed from 16L:8D to 8L:16D. Only for group 816, at the beginning of the 10th week, a sudden change was applied from 8L:16D to 16L:8D. During the whole experiment, does of the control group were under a constant 16L:8D light programme (group 16). The experiment lasted 18 weeks. For each group, the sexual behaviour of rabbit does was tested in the presence of a vasectomised buck. The tests were done two times a week for two weeks at different phases during the experiment. For each phase, the receptivity test was considered on day 0 (D0) and 5 days (D5), 7 days (D7) or 12 days (D12) days later. Whatever the phase, under a 16L:8D photoperiod, does were significantly more receptive than under a 8L:16D one (phase 1: 91.3 vs. 77.5%; phase 2: 83.8 vs. 70.0%; phase 3, 76.3 vs. 62.5%). After the light stimulation of group 816, the percentage of receptive does increased from 55% (D0) to 90% (D12) and remained above 80% for one week (D7: 85%, D12: 90%). At the end of the experiment (17th and 18th weeks), the sexual behaviour of rabbit does was significantly ($P < 0.001$) related to the lighting programme. For group 816, even seven weeks after a light stimulation, does were more receptive than under a constant 16L:8D photoperiod whatever the testing day. Further studies are necessary to precisely conclude the delay between the light stimulation and the optimal sexual behaviour response and the duration of these effects. Moreover, a better knowledge of subjacent physiological mechanisms is necessary to progress in the control of rabbit reproduction.

INFLUENCE OF A PHOTO-STIMULATION ON OVARY AND EMBRYO RECOVERY IN NULLIPAROUS RABBIT FEMALES

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The goal of the present experiment was to estimate the effect of a photo-stimulation following previous one month maintenance of the young rabbits within short-day (8L:16D) conditions and applied during 10 days before artificial insemination (AI). Forty 17-weeks old nulliparous rabbit does were divided into four experimental groups (10 does/group). Group L was kept in constant long-day (16L:8D) photoperiod until insemination, imitating commercial situation. Groups S+4, S+6 and S+8 were first submitted to a short photoperiod (8L:16D) then the duration of the light was increased with 4, 6 and 8 hours, respectively, 10 days before insemination. AI was performed with standard techniques using fresh heterospermic pools. Doe rabbits were sacrificed at 48 hours after AI. Ovarian function was evaluated, oviducts were flushed to collect embryos and evaluate them after 48 hours in *in vivo* conditions. Increasing daylight hours did not influence receptivity, as assessed by the vulva state at the moment of insemination, nor weight of the two ovaries and average ovary length. Only one doe did not ovulate in group S+4. The mean number of corpora lutea in ovulated females was 13.2, 5.7, 10.6, 12.0 in groups L, S+4, S+6, S+8, respectively ($P < 0.001$). Proportion of embryo donor does was different ($P < 0.10$) being 1.0 the highest value in the group S+6 and 0.71 the lowest in the group S+4. The highest number of collected embryos for inseminated does was in group S+6 with 9.2 and the lowest in S+4 with 3.4 per doe ($P < 0.001$). Embryo recovery rate was the highest (0.89) in S+6 group and this was significantly ($P < 0.001$) different from the results of the other groups (0.56, 0.64 and 0.52, respectively for S+4, S+8 and L). Proportion of 96 hours old good quality embryos within the total washed amount was the highest in group L and S+8 (0.84 and 0.87, respectively) and lower in S+4 and S+6 (0.69 and 0.71, $P < 0.05$). Ovarian follicle anomalies (follicle cysts and haemorrhagic follicles) were present in each group: the average value (1.4 per inseminated doe) was the lowest in group S+8 and was more than 3 times higher in group L (4.7 per inseminated doe, $P < 0.001$). Higher proportion of the receptive females gave an embryo and the embryo recovery rate was also higher in these does compared to the non-receptive females. It is concluded that switching light hours from 8 to 14 per day leads to the same proportion of the embryo donor does, embryo recovery rate, number of collected embryos and similar number of blastocysts appropriate for transfer per inseminated female in comparison with the constant long day illumination. Smaller change of daylight hours is probably insufficient. Long days either by 8 hours supplemental lighting or constant however can result in lower embryo recovery rate.

**NUTRITION AND
DIGESTIVE PHYSIOLOGY**

NEW CONCEPTS AND OBJECTIVES FOR PROTEIN-AMINO ACID NUTRITION IN RABBITS

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ABSTRACT

In the European context, the new legislation to avoid mineral contamination and the ban of antibiotics as growth promoters has led to the definition of new objectives with respect to nitrogen supply. The present study summarizes the state of nitrogen nutrition in rabbits and reviews the role of protein and amino acids on rabbit health and the new nitrogen value of protein sources based on the true ileal digestibility (TID) for future recommendations. The main sources of nitrogen for microbial growth are ammonia, urea and protein (endogenous and dietary). The surplus of nitrogen flow to the caecum increases mortality rates during fattening by favouring the growth of potential pathogenic bacteria. Accordingly, feeding strategies to reduce the ileal nitrogen flow has been reviewed. A large reduction of dietary protein level might have negative consequences on growth performances and mortality. In order to formulate balanced low-protein diets, data on ileal and faecal amino acid digestibility of 14 raw materials are summarized. Furthermore, the use of this different unit for amino acid digestibility is discussed.

INTRODUCTION: A HISTORICAL PERSPECTIVE

Knowledge on nitrogen nutrition, including protein and amino acid requirements, is essential for formulating productive and cost-effective diets for domestic animals. In rabbits, there is a paucity of knowledge relative to that reported in ruminant (cattle or sheep) or non ruminant species (pigs or poultry). Also, rabbits are relatively less important with respect to other livestock species, and combined with a slow rate of industrialization, might have contributed to this fact, despite the historical wide contribution of rabbit to the protein supply of the human diet.

From 1940's to early 1970's, the rabbit was considered a laboratory animal and consequently, research was focused to investigate the "qualitative" aspects of nitrogen and amino acids utilization. The role of caecotrophy and caecal metabolism on nitrogen digestion and body retention (Tacker and Brant, 1955, Yoshida and Kandatsu, 1964; Yoshida *et al.*, 1968, 1971, 1972; Hoover and Heitman, 1975;

Proto, 1976), or the essentiality of some amino acids for growth (McWard *et al.*, 1967; Gaman and Fisher, 1970; Cheeke, 1971; Adamson and Fisher, 1971, 1973) has been determined by several authors. From these studies, it was concluded that caecal microbiota are able to use non-protein compounds (as urea) and that the caecotrophy contributes to improve N digestion and retention. However, these studies also affirm that this extra N can not compensate for a low dietary protein level or the use of low quality protein sources (with unbalanced amino acid composition) to meet growth requirements.

Consequently, in the 1970's and 1980's, research focused on "quantitative" aspects in order to determine the optimal concentration of some amino acids (arginine, lysine and methionine) and the protein needs for productive purposes (growth) using practical instead of purified diets. Studies performed at INRA (Lebas *et al.*, 1973; Colin 1974, 1975a, 1975b) and those of Davison and Spreadbury (1975) and Spreadbury (1978) led to confirm the essentiality of these amino acids and the high requirements of arginine, in contrast to that observed in poultry. Furthermore, they also suggested that the optimal level of an amino acid depends on the balance with other amino acids and the level of energy in the diet, so the recommendation for lysine is given in grams per 1000 kcal of digestible energy. The above mentioned works constituted the base of the NRC recommendations in 1977 (NRC, 1977) (Table 1).

With respect to optimal dietary levels of protein for growth, in the early 1980's, our research at UP Madrid was focused to set the best nutritive unit for energy and protein (crude, digestible or net), and the requirements of these nutrients for growth. These studies considered a wide range of protein and energy levels, different slaughter weights (2.0, 2.25 or 2.5 kg) or weaning age (25 vs. 35 days) (de Blas *et al.*, 1981, 1985; Fraga *et al.*, 1983). From these results it was concluded that Digestible Energy (DE) to Digestible Protein (DP) ratio is a more reliable unit as it has a higher and direct impact on body nitrogen and energy retention than the dietary content of fibre, which is inversely related with digestible energy. Therefore, the optimal level for crude protein in a diet depends on its digestibility and the DE content. A recommended ratio of 23.5 kcal DE/g DP (or 10 g DP/MJ DE) was suggested to optimize the growth rate and the mortality.

Table 1: Protein and amino acid recommendations according to several authors (as-fed basis).

	NRC (1977)		INRA (1984)		de Blas and Mateos (1998)	
	Growing rabbits	Lactating does	Growing rabbits	Lactating does	Growing rabbits	Lactating does
Energy (MJ/kg)	10.5	10.5	10.5	11.0	10.5	11.1
CP (%)	16.0	17.0	16.0	18.0	15.3	18.4
Digestible protein (%)					10.7	12.9
Lysine:						
Total (%)	0.65		0.65	0.75	0.75	0.84
Digestible (%)					0.59	0.66
Sulphur aa:						
Total (%)	0.60		0.60	0.60	0.54	0.65
Digestible (%)					0.41	0.50
Threonine:						
Total (%)	0.60		0.55	0.70	0.68	0.70
Digestible (%)					0.47	0.48
Arginine (%)	0.60		0.90	0.90		
Histidine (%)	0.30		0.35	0.43		
Leucine (%)	1.10		1.05	1.25		
Isoleucine (%)	0.60		0.60	0.70		
Phenylalanine and Tyrosine (%)	1.10		1.20	1.40		
Tryptophan (%)	0.20		0.18	0.22		
Valine (%)	0.70		0.70	0.85		

The protein requirements for reproductive does were studied by several researchers in America and Europe (Partridge and Allan, 1982; Adams, 1983; Sanchez *et al.*, 1985; Partridge *et al.*, 1986; Parigi Bini *et al.*, 1990, 1991, 1992; Xiccato *et al.*, 1992). These works determined higher requirements for protein (around 20%) to optimize reproductive performances than those needed for growth. The lack of specific studies on amino acid requirements for lactating or pregnant does has led several authors to consider analogous requirements as for growth (Lebas, 1988; Partridge, 1989) despite the fact that differences among the amino acid profile of milk and muscle protein (Moughan *et al.*, 1988; Partridge, 1989) may compromise such analogy. In the 1990's, the most important practical advance was the recommended nutritional needs of growing rabbits and does for the most frequent limiting amino acids (lysine, methionine and threonine) (Maertens and de Groote, 1988; Taboada *et al.*, 1994, 1996; de Blas *et al.*, 1998) as presented by de Blas and Mateos (1998) (Table 1). It is noted that current recommendations are higher for lysine and threonine (from 12 to 23%). These differences might be explained by the higher productivity observed in newly developed breeds, but also by differences in the digestive utilization of the diets used in the experiments where these recommendations were determined. In fact, these studies led to consider a new unit for amino acid supply, addressing the need to determine not only "crude" but also "digestible" (at faecal level) amino acid requirements. This unit brings into a better adjustment of dietary supply and

requirements as it considers the high variability of protein digestibility observed in raw materials (Villamide *et al.*, 1998). The utility of digestible units for protein characterization was also largely recognized by the feed industry. However, the lack of information about the faecal digestible amino acids content of raw materials (at that moment only data on alfalfa hay was available from García *et al.*, 1995) limited the practical use of this unit. This raised the question for a further digestible amino acid content evaluation by doing the balances at faecal or at ileal level. The preliminary results presented at the 7th World Rabbit Congress (Carabaño *et al.*, 2000) suggested that faecal balance is not correct to characterize the absorption of amino acids at ileal level, and consequently to meet the amino acid requirements. Consequently, recent studies have been carried out to characterize the digestible amino acid content in the main raw materials used in rabbit diets. In the following sections, the results of these studies will be summarized.

Therefore, until the 21st century, the dietary supply of protein and amino acids had the primary and traditional objective to meet rabbit requirements for production. As crude or total units have been widely used in practical diet formulation, an excess of protein is typical in commercial diets. Furthermore, the tendency in the last decade to increase the dietary fibre level and reduce the starch to avoid digestive problems has favoured increased inclusion levels of alfalfa hay and cereal by-products, resulting in higher dietary protein levels than recommended (>15%). However, in the European context, the new legislation to avoid mineral contamination and the ban of antibiotics as growth promoters has led to define new objectives with respect to the nitrogen supply. In all livestock species, the current trend is to maximize N retention by adjusting dietary protein levels accordingly to a balanced amino acid supply and thus avoiding any N excess in the diet. According to Maertens *et al.* (2005), 2/3 of N excretion is explained by nitrogen ingestion and has been recently reviewed by Xiccato *et al.* (2006). Therefore, the reduction of dietary protein level could be an effective strategy to satisfy new laws. Furthermore, this reduction may also help to control the growth of pathogenic bacteria that promote higher mortality in the post-weaning period. The experience in pigs and poultry has confirmed the utility of this strategy, but also has brought up the important role of some essential or semi-essential amino acids on gut health.

In the following sections, the current knowledge on the role of protein and amino acid nutrition in the prevention of digestive disorders and on productive traits will be reviewed.

THE ROLE OF NITROGEN IN MICROBIAL GROWTH

The caecum is the main reservoir for microorganisms in the intestinal tract (10^{10} - 10^{12} bacteria/g of caecal content; Penney *et al.*, 1986). The last segment of the small intestine (the ileum) and the proximal colon also contains a great proportion of total intestinal microbiota. Residues of intestinal digestion and the nutrients recycled through the blood are the potential substrates that allow the growth of microbiota. At the end of the ileum, fibre is the main component of the digesta (about 70% of total

DM) while nitrogen is the second in importance (about 15% of total DM) (Table 2). This latter figure may be a relatively poor indicator of nitrogenous components as potential substrate for microbial growth. However, taking into account the low fermentability of the fibre (30% of digestibility for NDF components) and the high content of endogenous substances in the nitrogen residues (about 65%, see the following sections), both components may equally contribute to resident intestinal microbiota maintenance.

Table 2: Ileal balance in adult rabbits fed alfalfa based diets (adapted from Gidenne, 1992; Merino and Carabaño, 1992; Carabaño *et al.*, 2001).

	Intake (g/d)	Ileal flow (g/d)
DM	150	60
CP	24	9
Starch	22	0-2
NDF	52	42

There is little information about the qualitative and quantitative importance of nitrogenous components utilization by caecal microbiota. However, early studies on caecal metabolism indicate that microbiota is able to utilize the nitrogen that enters into the caecum. When germ-free animals are compared with conventional ones, studies (Yoshida *et al.*, 1972; Rerat, 1978) observed that caecal content is enriched in different nitrogenous compounds as urea, free amino acids, peptides and other nitrogen sources of endogenous origin (mucoproteins, pancreatic enzymes or desquamated cells). On the contrary, in conventional animals the caecum contains more ammonia and lower quantities (up to 10-fold) of endogenous components. Further studies confirmed the previous conclusions. Some of the most frequent isolated caecal bacteria (*Bacteriodes* spp.) are the most active genera in mucin digestion (Hill, 1986; Sirotek *et al.*, 2003). Also, Emaldi *et al.* (1979) observed that the main activities of caecal microbiota were in decreasing order: ammonia-user, ureolytic, proteolytic and cellulolytic. Figure 1 shows a tentative scheme of the caecal nitrogen metabolism. The proteolytic activity of the caecal bacteria would be particularly responsible for total volatile fatty acid and ammonia production in the caecum. These end products are partially absorbed through the caecal and colon walls, and ammonia is also used by caecal bacteria as the main substrate for protein synthesis. However, the extent of these processes has not been quantified. Other nitrogen source for microbial growth is the urea recycled into the caecum through the blood.

When protein intake exceeds the requirements for body protein synthesis, it is catabolised producing urea as an end product. Then urea is partially recycled to the caecum (Forsythe and Parker, 1985). As previously mentioned, one important urease activity has been detected in the caecum. The hydrolysis of urea produces ammonia that can be used for microbial growth to increase the ammonia concentration of caecal contents, provided that there is not enough energy for bacterial protein synthesis. Fraga (1998) observed a negative correlation ($r = -0.88$) between the DP/DE ratio and caecal ammonia concentration in a review involving 25 experimental diets.

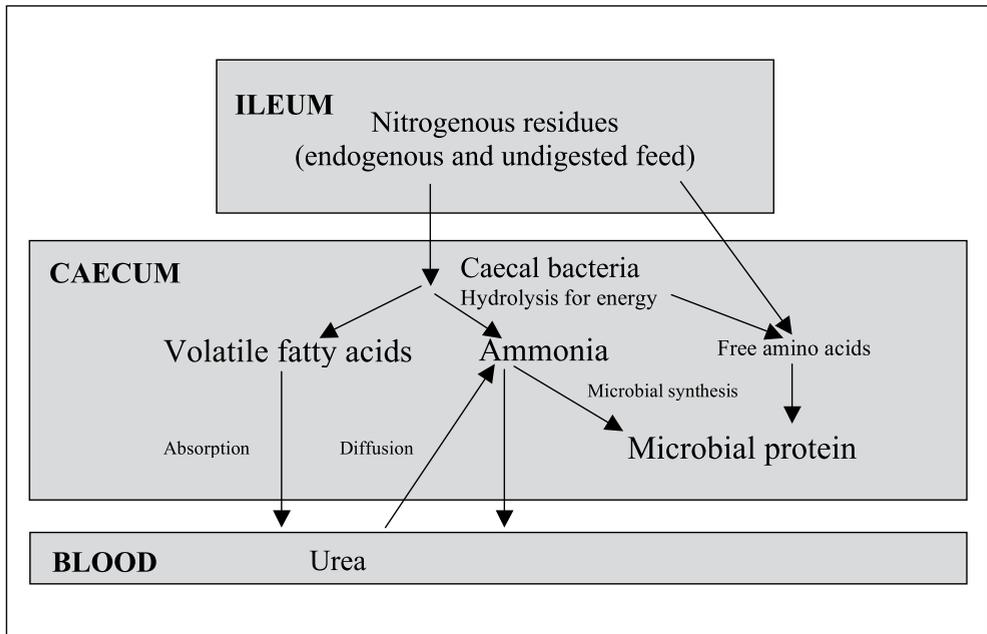


Figure 1: Caecal nitrogen metabolism.

THE ROLE OF NITROGEN ILEAL FLOW ON INTESTINAL HEALTH

The resident caecal microbiota seems to be able to flourish from nitrogenous substrates. However, when unbalanced diets are offered to the animals, some potential pathogens can also be favoured. According to de Blas *et al.* (1981), there is a quadratic relationship between mortality and DP/DE ratio, with a minimum for a ratio of 10 (Figure 2). Extreme diets with low (12%) or high CP (18%) content showed the highest mortality rates. The reason for this relationship was unknown because there was no control of the microbiota. Some genera with potential pathogenic effect, such as *E. Coli* or *Clostridia*, can use protein or amino acids as substrate for growth. So, an increase of the nitrogen flow into the caecum could favour these changes in microbial growth. Haffar *et al.* (1978) observed an increase of *Clostridium* in animals fed diets containing excessive protein concentration.

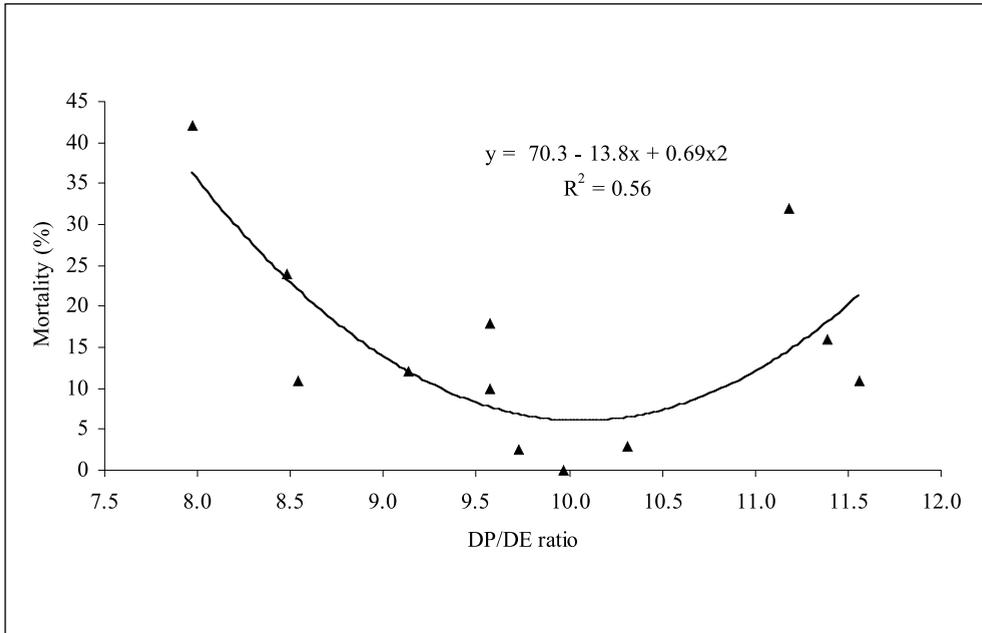


Figure 2: Relationship between mortality (%) and the DP/DE ratio in the growing period (from de Blas *et al.*, 1981).

This hypothesis has now been confirmed with the development of ileal digestibility techniques in the post weaning period (Blas *et al.*, 2000; Carabaño and Merino, 2005). Chamorro *et al.* (2007a) observed that a reduction of dietary CP level (by dilution of the diet with starch and without modifying ileal CP digestibility) from 18 to 16% led to a reduction of ileal CP flow (16%), the presence of potential pathogens bacteria (*Clostridium perfringens*) and mortality (10 points) due to Epizootic Rabbit Enteropathy (Table 3).

Table 3: Effect of the level of protein in isofibrous diets (30% NDF) on pathogenic flora and mortality in early (25 d) weaned rabbits (Chamorro *et al.*, 2007a).

	18% CP	16% CP	SEM	P <
Ileal CP flow (g/d)	6.0	5.0	0.25	0.05
Frequency of animals with <i>C. perfringens</i> (%)	47.2	18.0	-	0.05
Fattening mortality (%)	21.2	11.0	-	0.05

Further reductions of protein (from 16 to 14% CP; Chamorro *et al.*, unpublished) followed the same tendency but the effects were of smaller magnitude, and the reduction of mortality was only observed in the postweaning period. The great contribution of endogenous losses at the ileal level may limit the reduction of N overflow. Large-scale studies conducted on French commercial farms confirmed the beneficial effect of a reduction (from 18 to 14%) of the dietary CP level on mortality (5 points) and morbidity due to different pathologies including ERE (Gidenne and García, 2006).

Another way to reduce the nitrogen flow is by including in the diet highly ileal digestible sources. Gutierrez *et al.* (2003) observed that the source of protein affected CP ileal digestibility in diets with the same level of CP (18%) and faecal digestibility (80%). Sunflower meal or soybean protein concentrated (ethanol treated) showed higher ileal digestibility, lower ileal flow and lower mortality than soybean meal or a mixture of soybean meal with potato protein (Figure 3). The results of García-Ruiz *et al.* (2006) confirm the higher mortality of diets with soybean with respect to those with sunflower (11 vs. 4%, respectively).

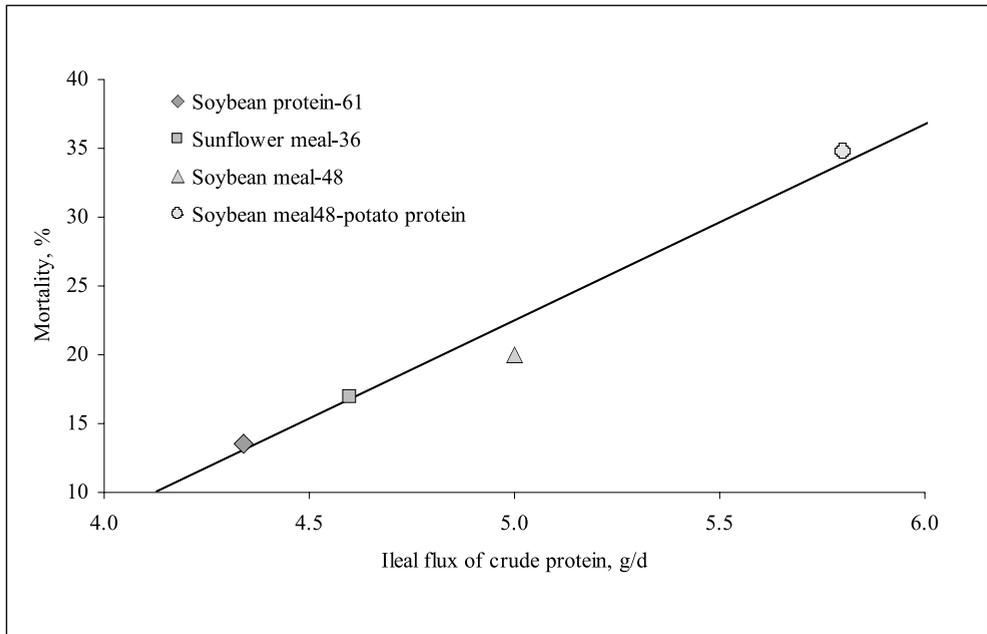


Figure 3: Effect of ileal flux of crude protein on mortality from 25 to 60 days of age (Gutiérrez *et al.*, 2003).

The dietary addition of proteases could also help to reduce nitrogen flow but mainly in the post weaning period when animals have a limited enzymatic capacity to hydrolyse the protein (Dojana *et al.*, 1998). Accordingly, the results of García-Ruiz *et al.* (2006) showed that the dietary supplementation with proteases was effective in the reduction of nitrogen ileal flow both for sunflower or soybean based diets. However, this reduction only improved the intestinal health in animals fed the sunflower diets. The presence of anti-nutritive factors or allergenic compounds in soybean based diets might exert an additional effect on the mortality. The source of protein may also affect the mucosal integrity of small intestine and its functionality by modulating local immune responses and possibly triggering an inflammatory response, as shown in piglets (Vente Spreeuwenberg *et al.*, 2004). Limited works have been carried out on rabbits on this topic and without conclusive results. Cano *et al.* (2004) reported that rabbits fed a soybean meal-rich diet had a lower feed intake around weaning, associated with a higher serum anti-feed IgG, hypothesising that it provokes a sub-

chronic inflammation process, and consequently increases the sensitivity of young rabbits to digestive diseases. Gutiérrez *et al.* (2000) observed that inclusion of animal plasma instead of soybean meal improved intestinal mucosal morphology in early weaned rabbits. However, other studies did not find differences in mucosal integrity (Gutiérrez *et al.*, 2003) or in the phenotypic distribution of lymphocytes in the duodenal lamina propria (Campín *et al.*, 2003) when soybean meal is included in the diet.

Endogenous nitrogen (e.g., digestive enzymes, mucoproteins, desquamated cells, urea) is another relevant source of protein for microorganisms in the gut, and in rabbits it may represent about 64% of the total ileal protein flow (García *et al.*, 2005; Llorente *et al.*, 2006, 2007b). However, this contribution is variable and is mainly influenced by DM intake, but also by the diet composition, depending on fibre type and inclusion level or anti-nutritional factors (ANF). The relevance of this nitrogen supply for microbial growth (pathogen or saprophyte) and their consequences on the mortality is unknown. The interpretation of actual results on this subject is difficult, mainly due to the effect of these dietary factors on other gut barrier mechanisms that also contribute to maintain intestinal health (Carabaño *et al.*, 2008). In addition, the presence of tannins and other phenolic compounds in the diet increases the nitrogen flow towards the caecum (Merino and Carabaño, 1992). However, tannins can protect the intestinal mucosa against oxidative damage and pathogens, and inhibit microbial activity in caecum (Fraga *et al.*, 1991; Motta *et al.*, 1996; García *et al.*, 2002). Maertens and Štruklec (2006) reported a reduction of mortality (due to ERE) in rabbits fed diets supplemented with tannins. Also, the inclusion of soluble fibre might increase the endogenous nitrogen as suggested in recent works (Chamorro *et al.*, 2007a). Moreover, the soluble fibre may also protect intestinal mucosa and reduce the presence of *C. perfringens* and hence mortality (Gomez-Conde *et al.*, 2007). Indeed, in-depth studies are necessary to verify the role of endogenous nitrogen on intestinal health.

Above mentioned results are summarised in Table 4. According to these results, feeding strategies that minimize the ileal nitrogen flow can help to reduce the incidence of digestive disorders. However, more studies are needed to track ileal nitrogen origin and other characteristics.

Table 4: Effect of level and type of dietary CP on intestinal health and ileal CP flow.

Diets	Ileal flow	<i>Clostridium perfringens</i>	Other bacteria	Mortality	Authors
CP level:					
18 vs. 16 % CP	▼ 16%	▼		▼ 10 points	Chamorro <i>et al.</i> (2007)
16 vs. 14 % CP	▼ 10%	▼		▼ 5 points	Chamorro <i>et al.</i> (unpublished data)
16 vs. 14% CP	ND ¹	ND	▼ total anaerobic bacteria	No mortality	García-Palomares <i>et al.</i> (2006a)
18 vs. 14% CP	ND	ND	ND	▼ 5 points	Gidenne and García (2006)
CP type:					
Soybean proteins vs. sunflower vs. potato protein	▼ 35%	ND	ND	▼ 20 points	Gutierrez <i>et al.</i> (2003)
Soybean vs. sunflower meal	No effect	ND	ND	▼ 7 points	García-Ruiz <i>et al.</i> (2006)
Sunflower meal + proteases	▼ 15%	ND	ND	▼ 7 points	García-Ruiz <i>et al.</i> (2006)
Alfalfa vs. soluble fibre + soybean isolated	No effect	No effect	No effect	No effect	Chamorro <i>et al.</i> (2007)

¹ND= not determined

PRACTICAL CONSEQUENCES OF A REDUCTION OF DIETARY PROTEIN SUPPLY

Accordingly, a reduction of protein supply in the diet might be an effective feeding strategy to reduce nitrogen load to the environment or intestinal disorders. Current commercial levels of dietary protein for fattener and reproductive does averages from 16 to 18% CP. These levels exceed the recommendations in several circumstances, as final phases of growth or lactation (Xiccato *et al.*, 2006).

Protein levels around 14% fed to rabbits from weaning (at 35 days old) to slaughter (2 to 2.7 kg) did not impair growth performance (up to 55 g/d), if DP/DE is around 9.5 and the amino acid supply is correct (de Blas *et al.*, 1981; Trocino *et al.*, 2000; García-Palomares *et al.*, 2006a). With this level of protein it is possible to reduce up to 38% of N-excretion in the fattening period (Maertens *et al.*, 1997), and also reduce the mortality (see Table 4). However, this level may not be enough to meet the growth requirements in postweaning diets fed to very young animals (21 to 35 days old) (Maertens *et al.*, 1997; Feugier *et al.*, 2006). Protein and amino acid requirements are relatively high in young rabbits, not only for tissue accretion but also because of the high needs for intestinal growth (Lebas and Laplace, 1972; Trocino *et al.*, 2000), and maintenance of the intestinal mucosa functionality. From 21 to 42 days of age, there is an exponential growth of the enzymatic and immunological mechanisms that allows the nutrient assimilation and the protection against pathogens (Lebas and Laplace,

1972; Knight and Crane, 1994; Dasso *et al.*, 2000; Lanning *et al.*, 2000; Campín *et al.*, 2003). Due to the relatively slower daily gains after weaning, the higher weight of gut maintenance on total requirements can increase significantly the relative needs for certain essential and non-essential amino acids with respect to advanced stages of growth. In addition, the defence mechanisms of the intestinal barrier can have specific needs for amino acids. Thus, threonine is a major component of mucin proteins, whereas glutamate is the main amino acid used by enterocytes as an energy source which plays an essential role in the repairing mechanisms of mucosa tissue (Le Floch and Séve, 2000; Reeds *et al.*, 2000). Recent studies in rabbits (Chamorro *et al.*, 2007b, 2007c; Baylos *et al.*, 2008) indicate that dietary supplementation with glutamine reduced the mortality caused by ERE, modified ileal microbiota (with a decrease of the frequency of detection of several pathogens such as *C. perfringens* and *Helicobacter* spp), and diminished the presence of *Eimeria* spp in the jejunum. Therefore, a reduction of the protein level, even when the supply of most limiting amino acids for growth is maintained (lysine, sulphur and threonine), may reduce the supply of other essential or non-essential amino acids that could also affect to growth performance or mortality. Accordingly, very low levels of protein (12%) have been related with low growth performance and incremental increases in the mortality rate (de Blas *et al.*, 1981) (Figures 2 and 4).

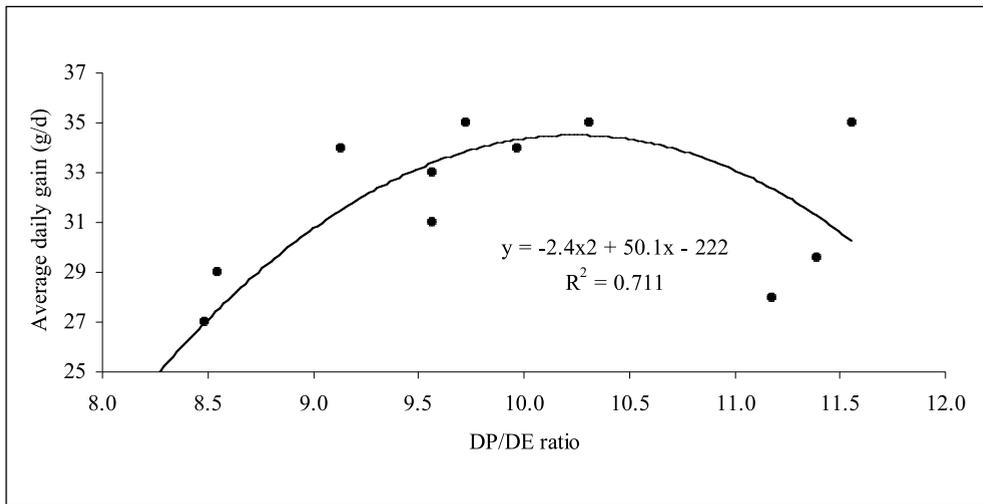


Figure 4: Relationship between average daily gain (g/d) and the DP/DE ratio in the growing period (from de Blas *et al.*, 1981).

For lactating does, a reduction of protein level (from 18 to 16%) with a DP/DE ratio of 11.5 g/MJ in the late lactation (21 d to 35 d) did not affect performance of rabbit does and their litters (García-Palomares *et al.*, 2006b). The protein supply to lactating does after the 21st d of lactation could be decreased to 11.5 g DP/MJ DE, taking into account the decrease of milk yield, which corresponds to the lowest value recommended by Xiccato (1996) and de Blas and Mateos (1998) for highly

productive does. As Xiccato *et al.* (2006) suggested this might also contribute to reduce the N-excretion as nitrogen intake during the lactation, accounting for one-third of the total nitrogen amount.

In practical situations, the proposed dietary CP reduction is not easy to accomplish. As mentioned above, some conditions are necessary to obtain adequate performance. A better knowledge of amino acid requirements and improving the characterization of protein and AA value of raw materials and diets are necessary to minimize the risk of CP reduction. Furthermore, the use of synthetic amino acids is necessary to avoid the excess of protein.

NITROGEN AND AMINO ACID EVALUATION

The first step to formulate balanced diets with a reduced CP content is the characterisation of the protein value of the feedstuff in terms of nitrogen and amino acid availability. Furthermore, ileum is the last segment where the amino acids are absorbed, so in other non ruminant species, the ileal digestibility is considered the most precise unit to estimate the real availability of the amino acids for animal protein synthesis. However, up to now faecal digestibility has been the unit used for feedstuff evaluation in rabbits.

Ileal and faecal digesta contains important amounts of protein of endogenous origin (3.8 and 2.5 g/100 g DMI, at ileal and faecal level, respectively) (García *et al.*, 2004; Llorente *et al.*, 2006) originating from digestive secretions, epithelial cells and mucins or micro-organisms. This endogenous protein represents about 64% of the total nitrogen flow both at ileal and faecal level. The relative importance of endogenous protein varies with the DM intake, but also varies with the type of diet and the CP origin. Thus, for a diet based on peas or soybean hulls with the same intake and similar chemical composition, the endogenous protein at the ileal level represents 65 and 55%, respectively. The amino acid composition of endogenous protein at ileal and faecal level is shown in Table 5.

Table 5: Amino acid composition of endogenous flow at ileal and faecal level.

	García <i>et al.</i> (2004)	Llorente <i>et al.</i> (2006)	
	Ileum	Ileum	Faeces
Cystine	3.1	2.7	3.3
Histidine	1.6	1.3	1.2
Isoleucine	3.7	3.8	3.2
Leucine	4.5	4.3	4.7
Lysine	3.2	3.6	3.3
Methionine	0.9	0.8	1,1
Phenylalanine	1.7	4.1	4.1
Threonine	4.9	5.6	5.3
Tyrosine	1.7	3.5	3.4
Valine	5.3	5.1	4.7
Alanine	3.1	3.4	3.7
Arginine	4.1	3.6	4.3
Aspartic acid	7.0	7.2	7.0
Glutamic acid	12.6	12.5	9.1
Glycine	6.1	8.0	4.1
Proline	4.8	4.7	3.3
Serine	6.6	5.8	4.4

The endogenous protein at ileum or faeces contains high concentrations of some essential (thr, val, leu, ile and lys) and non-essential (gln, gly and asp) amino acids. Therefore, for a more reliable analysis of digestible protein and amino acids of feedstuffs, a correction for endogenous losses must be performed. When this correction is done, a new unit arose and is referred as “standardised” or “true” (TID) instead of “apparent” (AID) ileal digestibility in non-ruminant species (Carabaño *et al.*, 2000).

The variation of each amino acid with respect to the CP ileal digestibility for soybean meal is shown in Figure 5. In particular, some amino acids (glycine and threonine) are considerably less digestible than protein whereas others, such as methionine or isoleucine, are more digestible (from -14 to +6 points for AID, and from -8 to +5 points for TID). Therefore, using the same digestibility value for all amino acids leads to major errors, mainly when apparent values are used, because the endogenous correction, which further leads to a decrease in the variation of amino acid digestibility with respect to protein digestibility.

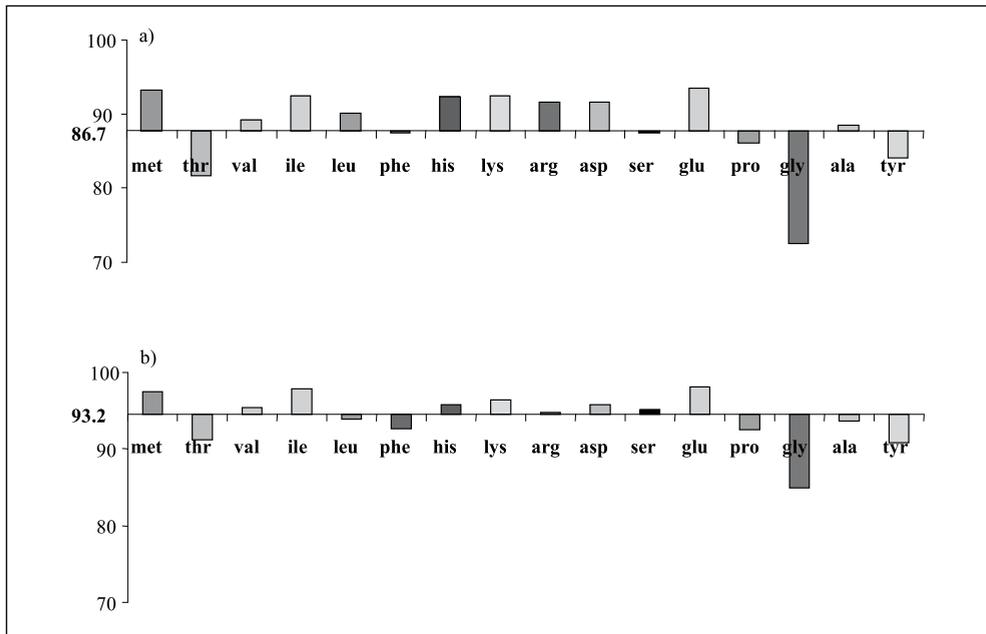


Figure 5: Apparent (a) and true (b) ileal amino acid digestibility (%) of soybean meal with respect to apparent (86.7%) and true (93.2%) crude protein digestibility.

García *et al.* (2004, 2005) conducted several studies in collaboration with feed companies aimed to evaluate the main protein sources used in rabbit diets. Ileal (apparent and true) and faecal (apparent) digestibility of CP and of limiting amino acids of these raw materials are shown in Table 6. True ileal digestibility of CP is relatively high (average of 80.8%), while the apparent values (average of 65.7%) are 15 points lower due to the great importance of endogenous losses. Apparent faecal digestibility (AFD) of CP shows intermediate values (average of 75.6%), indicating an important disappearance of protein in the large intestine, although at a different rate for each amino acid. Threonine seems to disappear to a larger extent (from 4 to 46 points) than methionine, while showing higher ileal than faecal values (0.2 to 5.2 points) for some feedstuffs. The important microbial activity in the caecum leads to wide changes in the amino acid composition of digesta, and, consequently, the faecal amino acid balance leads to an unclear interpretation (García *et al.*, 2005). Using fistulated does, values for CP faecal digestibility agree with average dietary values (73.2%, $n = 164$, Xiccato *et al.*, 2003) as determined in growing rabbits.

There are important differences among feedstuffs both in protein and amino acid digestibility, being mainly related to their CP content ($r = 0.91$, 0.81 and 0.61 for AFD, AID and TID, respectively), and with the type of protein (concentrates vs. forages or fibrous by-products). Apparent ileal digestibility of CP and threonine for the different feedstuffs determined in rabbits are lower than those obtained in pigs (INRA, 2002), whereas lysine and methionine AID values are similar. However, both CP and limiting amino acids TID values are higher for almost all feedstuffs than the

standard ileal digestibility as determined in pigs, due to the higher importance of endogenous protein in rabbits. The TID evaluation is time consuming and expensive because of the use of semi-purified diets supplied to cannulated animals and the amino acid analysis. Therefore, we attempted to predict them from easier and less costly methods. In this sense, encouraging results were obtained using an *in vitro* method (Llorente *et al.*, 2007b) as developed for pigs and adapted to rabbits (Ramos *et al.*, 1992). Although the *in vitro* CP digestibility was higher than the corresponding *in vivo* values (22.5, 11.9 and 5.8 points as averages for AID, AFD and TID, respectively), the precision of their estimation was high (i.e., the coefficient of variation was lower than 5.5% for TID) even when the *in vitro* CP digestibility was used as predictor.

Table 6: Digestibility of the most important sources of protein in rabbit diets.

	Apparent Faecal Digestibility				Apparent Ileal Digestibility				True Ileal Digestibility			
	CP	Lys	Met	Thr	CP	Lys	Met	Thr	CP	Lys	Met	Thr
Soybean meal (47% CP)	95.9	96	97	91.9	86.8	92.5	93.2	81.7	93.2	96.4	97.4	91.1
Full-fat soybean	91.4	94.4	94.1	88.0	82.3	90.3	91.0	76.4	90.1	95	96	87.6
Soybean hulls	65.3	75.2	72.7	66.9	31.3	62.2	60.1	20.7	52.9	72.9	71.7	49.2
Sunflower meal (28% CP)	82.9	86.4	91.4	79.8	75.6	88.4	91.6	74.3	87	98	95.3	89.8
Sunflower meal (34% CP) ¹	85.2	79.6	92.1	77.1	80.7	84.5	93.8	73.8	86.1	91.5	96.7	74.4
Sunflower meal (36% CP)	84.9	85.4	92	80.7	79.8	85.3	92.7	76.5	88.1	93.1	95.5	88.4
Peas	81.7	88.6	83.5	72.7	75.5	90	87.5	63.4	87.9	97.1	97.2	83.8
Wheat	75.9	75.7	86.4	56.4	67	74.6	88.7	44	89.1	94.2	99.3	84.3
Maize	65.8	63.5	78.4	42.1	49.4	63.2	81.8	15.8	78.2	92.2	94.0	62.0
Barley ¹	68.3	59.7	73.5	53.3	61.9	61.6	80.3	45.7	79.6	79.2	89.8	72.5
Wheat shorts	68.1	74.2	81.2	51.3	65.8	78.0	86.8	44.1	84.4	93.5	96.6	77.2
Wheat bran ¹	56.1	48.0	60.2	52.6	52.9	47.2	69.0	43.9	69.8	65.2	79.1	74.4
Gluten feed	75.7	82.6	85.3	65.9	65.5	78.1	85.0	49.8	78.1	88.5	92.2	71.5
Alfalfa hay	68.3	76.2	75.5	60.7	54.3	71.8	76.9	43.5	73.4	85.8	87.3	69.3
Alfalfa hay ¹	69.1	55.0	69.6	50.3	59.1	59.4	74.4	56.2	74.2	71.7	84.2	75.2

From Llorente *et al.* (2005, 2006 and 2007a); ¹From García *et al.* (2005).

Once the amino acids of the raw materials are evaluated for ileal digestibility, the other objective that must be approached in the near future is their specific requirements. As previously mentioned, amino acid requirements, both for growing and doe rabbits, have been expressed on the basis of total or AFD (see Table 1). There are no recommendations of apparent or true ileal digestible amino acids, but the values should be different. In an attempt to study the practical effect of formulating with these units, the AID and TID threonine requirements were estimated from the raw material composition of experimental diets used to obtain threonine requirements (de Blas *et al.*, 1998). The “estimated” AID and TID threonine requirements should

be 0.37 and 0.51%, respectively. When ileal values were used instead of total ones (Table 6) in a practical formula, the price decreased (3.3 and 2.8%, for AID and TID, respectively), favouring the inclusion of concentrates and cereal by-products instead of forages.

CONCLUSIONS

For past several years, many advances have been achieved on the nitrogen nutrition of rabbits; however, there already are many opportunities to further expand the knowledge on amino acid metabolism in order to meet specific requirements for improving health and welfare of rabbits.

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MYCOTOXINS AND OTHER CONTAMINANTS IN RABBIT FEEDS

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ABSTRACT

Animal-based food products derived from rabbit constitute a measurable portion of the human diet. Data from animal-production research demonstrate that the quality of these products is directly related to animal feeding practices. For that reason, the ingredients used in animal feed and their contamination with undesirable substances are fundamentally important for both the quality of the resulting food products and the potential human health impacts associated with the animal-based food-production chain. Alternatively, inclusion of feed ingredients, which are potentially toxic or contaminated with toxic substances, may result in a range of biological or toxicological effects in the production of animals. Animal feed ingredients that constitute complete feed products are derived from different raw materials, such as plant and animal origin, as well as pharmaceutical and industrial sources. Additionally, the contamination of feed materials from the environment would also be important as a potential hazard. The present review summarizes some of the toxic effects of some potentially hazardous ingredients of rabbit feed, such as mycotoxins (aflatoxins, ochratoxin, citritin, patulin, *Fusarium* mycotoxins – including trichothecenes, zearalenone, fumonisins, moniliformin and fusaric acid), fats and fatty acids, amino acids and coccidiostats. There are also some data about contaminants, such as heavy metals (arsenic, aluminium, cadmium, lead, mercury, molybdenum), and chlorinated dioxins and dibenzofurans.

MYCOTOXINS

Mycotoxins are invisible, highly corrosive, secondary metabolites of moulds which may persist in feed and even hay, when the moulds that produced them are no longer present (Scott, 1990). Nearly all of the mycotoxins are cytotoxic, disrupting various cellular structures such as membranes, and interfering with vital cellular processes such as protein, RNA and DNA synthesis (Guerre *et al.*, 2000). Mycotoxins destroy the tissues by oxidizing proteins and most of them have immunosuppressive effects. Some of them produce acute toxicity, evidenced by digestive disorders or dermatitis, but many more are carcinogenic, resulting in genetic mutations, or causing deformities in developing embryos. Mycotoxins can have very pervasive, yet sub-clinical, effects on the health of rabbits that more often go unnoticed. When the clinical symptoms of mycotoxin poisoning are observed, significant damage has occurred already. Improper

harvesting, packaging and storage or prolonged shipping may enhance the potential for mould growth. Dirty harvesting, manufacturing/pelleting equipment and storage bins may contribute to mycotoxin contamination (Houssein and Brasel, 2001).

The symptoms are similar to more well-known ailments. Mycotoxins may cause fever (Cannon *et al.*, 1982), gastrointestinal problems, internal bleeding, haemorrhages or bruising, stomach ulcers (Aziz *et al.*, 1995), mouth sores, kidney, liver damages (Szilágyi *et al.*, 1994), central nervous system problems (Gabal *et al.*, 1986), immune-suppression (Richard *et al.*, 1991), tumour-genesis, eye, lung problems, hypertrophy of the adrenal cortex, reproductive organ problems (Szilágyi *et al.*, 1994), damaged heart muscle, tachycardia, skin problems (Fairhurst *et al.*, 1987), bone marrow and spleen problems (Niyo *et al.*, 1988), blood abnormalities (Mizutani *et al.*, 1997), rectal prolapses, and increased vascular fragility, respectively.

The poisoning may manifest chronic or acute episodes, depending on the amount of toxic feed ingested. The damage to organs is cumulative over a period of time. A high incidence of gastrointestinal upset and of diseases associated with depressed immune function (e.g. *Pasteurella*) may be clues for a mycotoxin problem exists (Richard *et al.*, 1991). There are some clinical signs, which may appear in rabbit, such as severe pain in the abdomen, while radiograph series may reveal gut shutdown, but no physical blockage, and sometimes severe bloating, hypothermia, several blood abnormalities, e.g. high urea and creatinine levels, calcium-phosphorus imbalance, abnormal levels of liver enzymes (AST, ALT, GGT), low hematocrite and RBC levels, ulcers in the mouth, stomach and oesophagus, feed refusal, weigh loss, presence of mucous in the faeces, rough hair coat, sometimes paralysis or twitching in hind limbs.

The European Commission has made recommendations (2006/576/EC) for the maximum level of several mycotoxins in complete diets (European Commission, 2006b) and regulation (2003/100/EC) for aflatoxins (European Commission, 2003), but only in some cases, particularly in rabbit feeds (Table 1).

Table 1: Recommended maximum amount of mycotoxins in complete feed.

Mycotoxin	Maximum content (mg kg ⁻¹ feed with 12% moisture)
Aflatoxin B ₁	0.02
Ochratoxin A	5.00
Deoxynivalenol	5.00
Zearalenone	0.50
Fumonisin B ₁ +B ₂	5.00

Aflatoxins

Rabbit is a highly susceptible species to aflatoxins produced by *Aspergillus* moulds. The LD₅₀ of aflatoxin B₁ (AFB₁) in rabbits was determined as single oral dose 300 µg kg⁻¹ b.w. (FAO, 2000). However AFB₁ as low as 15 µg kg⁻¹ feed caused high level of morbidity and mortality (Makkar and Singh, 1991) and caused haemolytic anaemia, and strong cytotoxic effects were also observed (Verma and Mehta, 1998). Feeding diet naturally contaminated with 50 µg kg⁻¹ AFB₁ has caused lesions in the liver, absence of lobular architecture (Abdelhamid *et al.*, 2002). Bilirubin UDP-

glucuronyltransferase activity was dramatically decreased, whereas cholestasis occurred as an effect of aflatoxicosis. An exponential dose-dependent increase in plasma bilirubin concentration was also observed. Both the simultaneous exponential increase in bilirubinemia associated to the reduced bilirubin UDP-glucuronyl-transferase activity and the absence of cholestasis suggested that the hyperbilirubinemia is more probably related to increased heme catabolism than to altered bile duct permeability (Guerre *et al.*, 1997). AFB₁ toxicity also caused damage of other tissues, such as kidney, testicles, brain and thyroids (Lakkawar *et al.*, 2004). The teratogenic effects of AFB₁ were described as enlarged eye sockets and enlarged liver of embryos (Wangikar *et al.*, 2005).

Beside the feed as the primary route of AFB₁ intake, inhalation of contaminated dust particles may result in high local exposure of the nasal mucosa. Larsson and Tjälve (2000) assessed the bio-activation and toxicity of AFB₁ in the nasal mucosa after intranasal administration of AFB₁, and also examined whether translocation of the mycotoxin occurs from the nasal mucosa to the brain along olfactory neurons. The data indicated that intranasal administration of AFB₁ resulted in formation of tissue-bound metabolites in subtentacular cells, in some cells of Bowman's glands, and in a population of neuronal cells in the olfactory mucosa, whereas in the respiratory nasal mucosa, there was selective bio-activation of AFB₁ in mucous cells. The data indicated materials transported in the olfactory nerves represent AFB₁ and/or some of its non-reactive metabolites. It is concluded that application of AFB₁ on the nasal mucosa results in high local bio-activation of the mycotoxin and translocation of AFB₁ and/or its metabolites to the olfactory bulb. The toxic effect of AFB₁ in the nasal mucosa is related to bio-activation of the mycotoxin. To our knowledge, there is no evidence that AFB₁ may induce tumours in olfactory bulbs. Thus, while tumours originating from nasal mucosa are frequently found in livestock exposed to mouldy feed, no such evidence exists for forebrain neoplasm. Lack of CNS carcinogenesis is likely due to the inability of AFB₁ to pass from primary olfactory neurons to secondary or other neuronal connections in the olfactory system (Larsson and Tjälve, 2000).

As previously mentioned, AFB₁ is a potent hepatotoxic and hepato-carcinogenic mycotoxin that requires bio-activation to AFB₁-8,9-epoxide for activity (Essigmann *et al.*, 1982), which binds to DNA. Both endo- and exo-stereoisomers of AFB₁-8,9-epoxide exists, and although they are both produced in a variety of tissues, only exo-AFB₁-8,9-epoxide binds efficiently to DNA (Eaton and Gallagher, 1994). The International Agency for Research on Cancer has classified AFB₁ as a group I human carcinogen (IARC, 1993). Besides that, AFB₁ can contaminate respirable grain dust and thus the respiratory system is also a potential target for carcinogenesis. In addition to epoxidation, microsomal mono-oxygenases transform AFB₁ to the less toxic metabolites, aflatoxin M₁ (AFM₁) and aflatoxin Q₁ (AFQ₁). The rate of bio-transformation of AFB₁ depends on tissues, for instance values for AFM₁ formation in liver microsomes were greater than in lung, but the rate of AFQ₁ formation is the same in the above mentioned tissues (Daniels *et al.*, 1990). Bio-activation-related toxicity of AFB₁ has also been observed in tracheal mucosa following intra-tracheal instillation of AFB₁ in rabbits (Coulombe *et al.*, 1986). These results indicate that besides liver, lung and trachea are capable to activate AFB₁ and that rabbit lung and

tracheal microsomes contain high activity for this reaction (Daniels *et al.*, 1990). Interestingly, some other toxic, and potentially carcinogenic constituents in the rabbit feed, like polycyclic aromatic hydrocarbons (PAH), such as α -naphthoflavone, induce the cytochrome P-450 system in the pulmonary and hepatic microsomes and consequently induces the detoxification of AFB₁, namely AFM₁ and AFQ₁ formation, and as well increases the DNA binding of AFB₁ (Daniels and Massey, 1992). Different rabbit lung cell types have different abilities to bio-activate AFB₁. Daniels *et al.*, (1993) found that it was the highest in the microsomes of non-ciliated bronchiolar epithelial (Clara) cell-rich fraction.

Epoxide hydrolase and glutathione-S-transferase (GST) are both involved in hepatic detoxification of activated AFB₁, but the GST-catalyzed conjugation of glutathione to AFB₁-8,9-epoxides is thought to play the more important role in preventing epoxide binding to target macromolecules (Eaton and Gallagher, 1994). The glutathione-aflatoxin conjugate is transported from the cells with an ATP-dependent multidrug-resistance protein through an accelerated process (Loe *et al.*, 1997). Despite a preference for conjugating the more mutagenic AFB₁ exo-epoxide isomer, the relatively low capacity for GST-catalyzed detoxification of bio-activated AFB₁ in lung may be an important factor in the susceptibility of the lung to AFB₁ toxicity (Stewart *et al.*, 1996).

Ochratoxin

Ochratoxin A (OTA) is produced by several *Aspergillus* and *Penicillium* moulds. The International Agency for Research on Cancer has classified OTA as group 2B human carcinogen (IARC, 1993). In a study of Diwedi *et al.* (2004) OTA from *A. ochraceus* was given by gastric intubation to rabbits during 6-18 days of gestation at 0.025, 0.05 and 0.1 mg kg⁻¹ body weight levels, respectively. Teratogenic effects were found at the 0.1 mg kg⁻¹ dose group as a significant increase in the incidence of gross anomalies (wrist drop, rudimentary tail, knuckling of fetlock and agenesis of tail), skeletal (agenesis of caudal vertebrae, incomplete ossification of skull bones and wavy ribs), and soft tissue (internal hydro-cephalus, microphthalmia and kidney agenesis). The same embryo development abnormalities were observed by Wangikar *et al.* (2005). The number of live foetuses in the 0.1 mg kg⁻¹ dose group was significantly lower than those of the 0.025 mg kg⁻¹ dose group. The mean foetal weights and mean foetal crown to rump lengths of the 0.1 mg kg⁻¹ OTA group were significantly lower than the 0.025 mg kg⁻¹ dose group (Diwedi *et al.*, 2004).

The effective transfer of OTA from the blood to the milk of lactating rabbit does and subsequently the exposure of their kits is also possible if the lactating rabbit does were fed a naturally-contaminated diet throughout the lactation period. However, approximately 99% of the plasma proteins bound to OTA (Chu, 1974), had the highest concentration of OTA accumulated in the body of the rabbit does were found in kidney followed by liver, mammary gland and muscle. A linear relationship was found between the OTA concentrations in milk and in the plasma of the suckling kits, indicating an effective transfer of the toxin (Ferrufino-Guardia *et al.*, 2000). OTA is also extremely cytotoxic and may cause red blood cell haemolysis in rabbits (Zofair *et al.*, 1996). The proximal tubule of the kidney is the primary site targeted

in OTA-induced nephrotoxicity (Suzuki *et al.*, 1975). The basolateral membrane organic anion transport pathway is involved in OTA accumulation in the renal cell (Groves *et al.*, 1998). However, at the same site, effective excretion of OTA was also found in an *in vitro* model system by Groves *et al.* (1999). They found that the basal-to-apical flux of OTA was increased with time in proximal tubular epithelial cells, and it was about eight-fold more than the apical-to-basal flux reabsorption. The above mentioned study also indicates that the secretion of OTA of rabbit renal proximal tubules is limited to the organic anion secretory pathway. In summary, the secretion of OTA represents a substantial avenue for removal of this mycotoxin from the systemic circulation (Groves *et al.*, 1999).

Citrinin

Rabbit production is important in tropical and subtropical agricultural systems (Cheeke, 1986), but some low-cost rabbit feed constituents, such as maize-milling waste might be infected with moulds, mainly *Aspergillus* and *Penicillium spp.*, and consequently might contain mycotoxins such as citrinin. Citrinin is a mycotoxin isolated originally from *Penicillium citrinum*. It has been found to be produced by a variety of other moulds, such as other *Penicillium* and *Aspergillus* species. Citrinin acts as a nephrotoxin in all farm animal species, including rabbit, but its acute toxicity varies (Bennett and Klich, 2003). The intraperitoneal LD₅₀ value is calculated to be 19 mg kg⁻¹ b.w. (Lakkawar *et al.*, 2004). Citrinin induces mitochondrial permeability pore opening (Da Lozzo *et al.*, 1998) and inhibits respiration by interfering with complex I of the respiratory chain (Chagas *et al.*, 1995). In the experiment of Hanika *et al.* (1984), a single oral dose of citrinin was given by gavages at dose levels of 20, 80 or 100 mg kg⁻¹ b.w. The highest dose caused azotaemia and metabolic acidosis with haemo-concentration and hypokalaemia within one day, while as the effect of the two lower doses blood urea nitrogen and serum-creatinine levels increased, and creatinine clearance decreased indicating renal failure. Urine analysis indicated tubular dysfunction and necrosis with glucosuria, isosthenuria and cylindruria. Another possible consequence of low dose of citrinin toxicosis is the impairment of reproductive performance of rabbits in both genders as was found by Ajayi *et al.* (2005).

Patulin

Patulin is a mycotoxin produced by a variety of moulds, particularly *Aspergillus* and *Penicillium*. It is commonly found in moulded corn. It is not a particularly potent toxin, but some earlier studies have shown that it may be a carcinogen (Dickens and Jones, 1961), though studies have remained inconclusive. Effects of sublethal doses of patulin on the immune system were investigated in rabbits (Escuela *et al.*, 1988). They found significant suppression of the chemiluminescence response of peritoneal leucocytes. The mitogenic response to phorbol myristyl acetate, concanavalin A and, in particular, pokeweed mitogen was also depressed by patulin. This was parallel with decreasing serum immunoglobulin levels. The immune-suppressive effect of patulin is reversible and is probably due to interaction with cellular-free SH groups since the action of patulin can be circumvented, at least partially, by the prior administration of cysteine.

Fusarium mycotoxins

Fusarium fungi are commonly found in temperate climates and their mycotoxins are likely the most economically significant grain mycotoxins on a global basis (Wood, 1992). The numerous *Fusarium* mycotoxins are very diverse in chemical structure and characteristic mycotoxicosis. *Fusarium* moulds produce trichothecenes, such as T-2 toxin, HT-2 toxin, diacetoxyscirpenol (DAS), monoacetoxyscirpenol (MAS) and deoxynivalenol (DON), and also fumonisins, moniliformin, zearalenone (ZEN), fusaric acid, and verrucarin A (De Nus *et al.*, 1997). The main effect of *Fusarium* mycotoxins is to inhibit protein synthesis (trichothecenes), sphingolipid biosynthesis (fumonisins) and have nephrotoxic (moniliformin) and estrogenic effects (zearalenone). Clinical signs of *Fusarium* mycotoxicosis often remain unclear because of the immune-suppressive effect of some *Fusarium* mycotoxins, mainly trichothecenes, which may cause decreased resistance to infectious diseases (Ueno, 1983).

Trichothecenes

Trichothecene mycotoxins are produced by *Fusarium* moulds mainly in fields and cause intoxication through consuming contaminated cereal crops in the compound feed (Placinta *et al.*, 1999). About 150 different, but structurally related, trichothecenes have been chemically identified. The International Agency for Research on Cancer has classified trichothecenes as non carcinogens (IARC, 1993). Most of trichothecenes cause severe toxicosis in rabbit with relatively low LD₅₀ values as shown in Table 2 (Wannemacher and Wiener, 1997).

Table 2: Relative acute toxicity of the most abundant trichothecene mycotoxins in rabbits.

Trichothecene mycotoxin	Route of administration	LD ₅₀ (mg kg ⁻¹ body weight)
T-2 toxin	Intramuscular	1.1
T-2 toxin	Dermal (in dimethylsulfoxide)	10.0
4,15-diacetoxyscirpenol	Intravenous	1.0
Verrucarin A	Intravenous	0.54

However, most of the trichothecenes are partially metabolised by the microsomal xenobiotic transforming enzyme system. For instance, microsomal non-specific carboxyesterase produces C-4 acetyl residues of diacetoxyscirpenol, T-2 toxin, fusarenon-X and diacetylnivalenol. Some other trichothecenes, such as neosolaniol, HT-2 toxin, acetyl-T-2 toxin and tetraacetyl-nivalenol were unaffected by this hydrolysis (Ohta *et al.*, 1978). As an effect of metabolism of trichothecenes mainly in the liver, their accumulation in rabbit meat is moderate or negligible. The rate of metabolism of trichothecenes, e.g. T-2 toxin, depends on the duration of exposure and decreases over a long period of time (Ványi *et al.*, 1989). T-2 toxin causes lipid peroxidation in liver microsomes (Guerre *et al.*, 2000), which also impairs the amount and/or activity of the xenobiotic transformation (Mézes *et al.*, 1996). Guerre *et al.* (2000) found that a daily dose of 0.25 mg kg⁻¹ b.w. of T-2 toxin results in decreased monoxygenase activity in rabbit liver. Total liver microsomal P450 content, and the activity of aminopyrine and benzphetamine *N*-demethylases, pentoxoresorufin *O*-depentylase, glutathione *S*-transferases accepting 1-chloro-2,4-

dinitro-benzene and 1,2-dichloro-4-nitro-benzene as substrates, were decreased. By contrast, activity of ethylmorphine and erythromycin *N*-de-methylases, ethoxyresorufin and methoxyresorufin *O*-dealkylases, aniline hydroxylase, and UDP-glucuronyltransferase accepting *p*-nitrophenol as substrate, were unaffected. Gene expression of P450 1A1, 1A2, 2A1, and 2B4, but not P450 2C3 and 3A6, were also decreased. Beside those effects, microsomal oxidative damage was also proven by the significant increase of microsomal conjugated dienes, fluorescent substances, and malondialdehyde content. At a lower daily dose of T-2 toxin (0.1 mg kg⁻¹), neither significant effects on drug metabolizing enzymes, nor microsomal oxidative damages were observed. Taken together, these results suggest that a short exposure of time to the mycotoxin would not be associated with significant changes in the normal metabolism of xenobiotics in the liver (Guerre *et al.*, 2000).

Some nutritional effects such as deficiency or excess of some fat-soluble vitamins, namely vitamins A and E (Tutelyan and Kravchenko, 1988) increase the toxic effects of trichothecenes because of impairment of the activity of xenobiotic transforming enzyme system. For that reason, a decrease in the rate of conjugation and excretion of trichothecenes and their metabolites.

Trichothecenes inhibit cellular protein synthesis, a property which is probably the cause of many of the symptoms associated with trichothecene toxicoses. For instance, T-2 toxicosis results in hyper-aminoacidemia (Wannemacher and Dinterman, 1983), due to the inhibition of hepatic protein synthesis (Meloche and Smith, 1995). Subsequent elevations in blood tryptophan can result in increased concentrations of tryptophan in the brain. Tryptophan is the precursor of the neurotransmitter serotonin and the serotonergic neurons are thought to be important mediators of behaviours, such as appetite, muscle coordination and sleep. Serotonin synthesis in the brain is poorly regulated and can be promoted by increased concentrations of tryptophan (Leathwood, 1987). Increased amount of brain serotonin is thought to cause loss of appetite and sleepiness.

Among the trichothecene mycotoxins, T-2 toxin caused feed refusal, a first symptom of toxicosis. At a single oral dose of 4 mg kg⁻¹ b.w., T-2 toxin was lethal for rabbits within 48 h and proposed as LD₅₀ value for rabbits (Glávits *et al.*, 1989). Faecal, caecotroph and urine toxin concentrations were related to toxin consumption (Fekete *et al.*, 1989a). It is suggested that the high toxin level of the caecotroph can play role in the high sensitivity of the rabbit, and because of coprophagy, the animal will consume the toxin-containing caecotroph (Fekete *et al.*, 1989b).

The main toxic effect of T-2 toxin is inhibition of protein synthesis which was proven by Ueno *et al.* (1973) when rabbit reticulocytes were treated with low concentrations of T-2 toxin and marked degradation of polyribosomes was observed. Feed containing sublethal amounts of T-2 toxin (12.5 and 25 mg kg⁻¹ feed) caused emaciation, subacute catarrhal gastritis, necrosis of the lymphoid cells of the intestinal mucosa, depletion and necrosis in the lymphoid follicles of the ampulla ilei, spleen and lymph nodes (Fekete *et al.*, 1989a). Niyo *et al.* (1988) described leukopenia, marginal anaemia, and increased number of morphologic changes in nucleated erythrocytes followed by a regenerative haematological response, centrilobular hepatocellular swelling portal and periportal fibrosis as an effect of T-2 toxicosis. Necrosis of lymphocytes,

cells of the mononuclear phagocyte system, and myeloid haemocytogenesis were characteristics in most rabbits treated with T-2 toxin (Niyo *et al.*, 1988; Glávits *et al.* 1989). T-2 toxin and its metabolites also decrease the spermiogenesis and libido in bucks, possibly because of the inhibition of conversion of pregnenolone to testosterone (Fenske és Fink-Gremmels, 1990).

Testing the effects of dermal exposure to T-2 mycotoxin showed moderate oedema and erythema at the site of T-2 mycotoxin exposure for two hours. T-2 toxin induces significant dermatitis and folliculitis characterized by infiltration of the superficial and deep dermis, epidermis, and follicular root sheaths by high numbers of heterophils, thickening of the superficial dermis due to separation of collagen fibres in oedema, and presence of intra-epidermal pustules. The cutaneous injury may be due to ischemia caused by microcirculatory failure (Yarom *et al.*, 1987). Seven substituted trichothec-9-enes and six substituted trichothec-9-en-8-ones were tested for dermatotoxicity in rabbits, as estimated by induction of alopecia. Of these 13 variants, four showed the greatest toxicity: DAS, T-2 toxin, HT-2 toxin and fusarenon-X, especially the first two (Leonov *et al.*, 1990).

Toxic effects of deoxynivalenol (DON) or vomitoxin is very rare in rabbits. However, rabbit producers have been usually concerned with higher than normal deaths due to diarrhoea in rabbits. DON levels of commercial diets, particularly that contain wheat by-products contaminated with greater than 1 mg kg⁻¹ DON has been blamed by some rabbit producers for this problem. The effects of DON on gastric emptying and intestinal propulsion in mice and rats and gastrointestinal myoelectrical activity in rats have been investigated (Fioramonti *et al.*, 1993). Gastric emptying and intestinal transit were evaluated after gavages application of DON (50 to 1000 µg kg⁻¹ b.w.). The myoelectrical activity of the antrum, duodenum and jejunum was measured 10 minutes after application. DON was found to inhibit gastric emptying in a dose-related manner. Intestinal propulsion was reduced only for the highest dose (1000 µg kg⁻¹). It was concluded that, in rodents, DON inhibits gastric emptying by inducing intestinal migrating motor complexes through a peripheral action at the serotonin-3 receptors.

Pregnant does fed a DON contaminated diet showed marked weight loss, but no teratogenic effect was found (Khera *et al.*, 1986). In an *in vitro* erythrocyte model system extremely high doses of DON caused haemolysis (Rizzo *et al.*, 1992), but that effect was not proven *in vivo* at naturally occurring contamination levels.

Zearalenone

Like the trichothecenes, zearalenone (F-2 toxin) is produced by *Fusarium* fungi. It is, however, chemically unrelated. Zearalenone (ZEN) has estrogenic properties (Koch, 1981); although it is not chemically an oestrogen and ZEN toxicity is more readily recognized, than trichothecene toxicity, because the symptoms are more specific. There are also data on its capability to induce adverse liver lesions with subsequent development of liver carcinoma (National Toxicology Program, USA, 1982), however, the International Agency for Research on Cancer has classified ZEN as non carcinogen (IARC, 1993).

The effects of low (10 µg kg⁻¹ b.w.) and high (100 µg kg⁻¹ b.w.) oral doses of ZEN on some blood serum enzyme activities of AST, ALT, ALP, GGT, and total LDH of rabbits

were studied by Čonková *et al.* (2001). Low doses resulted significant increase in ALP activity, while significant increases in activities of AST, ALT, AP, GGT, and LDH were observed, indicating possible liver toxicity due to chronic effects of the toxin.

In rabbit bucks, ZEN impairs spermatogenesis and decreases libido, however only at extremely high dose levels (Fenske and Fink-Gremmels, 1990).

The *in vitro* reduction of ZEN by subcellular fractions from rabbit hepatocytes was investigated by Pompa *et al.* (1986). They found that in the presence of NADH, ZEN enhanced the reducing activity of the microsomal fraction. Furthermore, it was observed that hepatocytes produce α -zearalenol as the major and more uterotrophic metabolite. This means that rabbit has high sensitivity to the estrogenic effects of ZEN at dose levels of 0.1, 1 and 2 mg kg⁻¹ b.w.

*Fumonisin*s

Fumonisin are a family of mycotoxins that were first isolated in South Africa from cultures of *Fusarium verticillioides* (Gelderblom *et al.*, 1988), followed soon thereafter by elucidation of the structures of the prevalent isoforms fumonisin B₁ (FB₁) and B₂ (FB₂) (Bezuidenhout *et al.* 1988). It has been shown that the biochemical mode of action of the fumonisins is due to their chemical structure. They act as inhibitors of sphingolipid biosynthesis (Wang *et al.*, 1991) in most livestock species. In addition, the International Agency for Research on Cancer has classified fumonisins as a group 2B human carcinogen (IARC, 1993).

FB₁ was found to be nephrotoxic and hepatotoxic in rabbits (Gumprecht *et al.* 1995), and it has also been shown to exert deleterious effects on the haematopoietic organs (Mariscal-Quintanar *et al.* 1997). Histological examinations of liver showed centrilobular lipid infiltration, discrete cell necrosis, while nephrosis of the proximal tubuli was observed in the kidney. Lung oedema was found only in a low number of rabbits fed a FB₁ contaminated diet (Orova, 2003). Gumprecht *et al.* (2001) investigated the effects of FB₁ on the transport processes of the endothelial cells of lung capillaries of rabbits in an *in vitro* model system, but they did not detect measurable differences.

The teratogenic effect of FB₁ was also described using a dose of 300 mg day⁻¹ for 14 days (Kovács *et al.*, 2003). In addition, changes of water distribution in the brain and lung of embryos in pregnant rabbit does fed the FB₁ contaminated diet was investigated using magnetic resonance spectroscopy by Orova (2003) and significant changes were found in both tissues as a consequence of FB₁ toxicosis even during intrauterine development.

In rabbit bucks, FB₁ (24.6 mg kg⁻¹ feed) had no significant effect on testicular histometry. However, the weight distribution in the epididymides of the experimental animals demonstrated that the caput and caudal segments were significantly depressed in rabbits fed the highly contaminated diet. Results suggest that FB₁ may provide some protection against potential reduction in the sperm produced and stored in the testes and epididymides, but may elicit degenerations in the caput and caudal segments of the epididymides (Ogunlade *et al.*, 2006).

Moniliformin

Moniliformin is produced mainly by *Fusarium moniliforme*. It acts as an inhibitor of

the tricarboxylic acid cycle in intermediary metabolism. This differs from the mode of action of the trichothecenes. No data are reported on the moniliformin toxicosis in rabbits, which might be due to its rare occurrence and low concentrations in feed (Thiel *et al.*, 1986).

Fusaric acid

Fusaric acid may act synergistically with the trichothecenes to reduce feed intake and cause lethargy in sensitive species (Smith, 1992), but there are no data reported in rabbit. *In vitro* studies support the concept of a toxicological synergism between fusaric acid and the trichothecenes (Dowd, 1988) and Bacon *et al.* (1995) reported an interaction between fusaric acid and fumonisin B1. Fusaric acid, like the fumonisins and moniliformin, is produced mainly by *Fusarium moniliforme* (Burmeister *et al.*, 1985).

OTHER TOXIC FEED CONSTITUENTS AND CONTAMINANTS

Fats and fatty acids

The developmental toxicity of a 20% lipid emulsion that contains medium chain triglycerides to long chain containing lipid emulsion was investigated in rabbits (Henwood *et al.*, 1997). This emulsion was administered by intravenous infusion at dosages of 1 and 4.28 g lipid kg⁻¹ b.w. once daily during organogenesis to assess the potential developmental toxicity. The results showed lower feed consumption of rabbit does but no other test article-related gross necropsy was found. However, embryo and foetal toxicity and skeletal abnormalities were found in rabbits received medium-chain triglycerides at the higher dose.

Fats, particularly which contain polyunsaturated fatty acids, are susceptible to rancidity. Vilas *et al.* (1976) found that feeding rancid fats decreases the growth rate and significantly increases the weight of intestinal epithelial layer, possibly because of inflammation in the intestine. Absorption of lipid hydro-peroxides from rancid fat is questionable. Some research support that reactive metabolites of the oxidised fats or fatty acids are reduced by the antioxidant enzymes, such as glutathione peroxidase, in the intestinal epithelial cells (Reddy *et al.*, 1974), while other studies have shown lipid hydroperoxides in the chylomicrons (Aw *et al.*, 1992) which reached the liver and caused impaired antioxidant defense of the liver cells (Mézes *et al.*, 1996), but severity of liver damage depends on the fat content of the diet (Slim *et al.*, 1995).

Amino acids

Excessive dietary methionine is known to exert the most toxic overall side effects as compared with all other protein amino acids (Benevenga and Steele, 1984). Liver plays a major and unique role in methionine metabolism and for that reason this organ appears to be exposed specifically to excessive methionine. A methionine enriched diet was reported to cause liver enlargement, fatty liver, and decreased liver ATP and glycogen levels (Hardwick *et al.*, 1970). The mechanisms of methionine hepatotoxicity are poorly understood, but several methionine metabolites are capable of injuring hepatocytes. For example, methanethiol, a product of the methionine

transamination pathway, has been proposed to bind to cellular membranes and affect activities of the sulfhydryl-sensitive enzymes (Finkelstein and Bevenga, 1986). A growing body of evidence suggests that thiol compounds may be involved in free radical/lipid peroxidation processes (Munday, 1989). This was proven by Toborek *et al.* (1996) who found that long-term feeding of methionine enriched diet caused a significant increase in the amount of thiobarbituric acid reactive substances in liver that paralleled with increased activity of antioxidant enzymes and also induced atherosclerosis in rabbits (Toborek *et al.*, 1995).

Contaminants

An extensive, long-term analysis on feed toxicosis in rabbit farms in China was published by Gu *et al.* (2005). A total of 34,558 rabbits were poisoned and 8,551 died for a total mortality of 24.74%. Death rate depends on the toxic ingredients and the absorbed quantity, as well as the physiological condition of the rabbits. Among the total cases, more than 50% were caused by diet. In addition, a high percentage of abortion or stillbirth was found, mainly because of mycotoxins (Zilin, 2001). However, a relatively low number of cases, about 15%, were caused by mycotoxins. Plant toxins, such as gossypol (Zhengming, 1999), alkaloids, glycosides and oleandrin caused the same rate as mycotoxins, but with approximately 90% mortality. Contamination of feed ingredients with organic phosphorous insecticides also caused a high rate of mortality (Xianhua, 2002). Some additional cases caused by other contaminants, such as nitrite (Zhaojiang, 2000) or urea (Zhengxian, 1998) with lower rate of mortality have been reported.

In another extensive study (Coleman and Tardiff, 1979), commercial rabbit feed samples originated from USA and Canada were collected and analyzed qualitatively and quantitatively for selected antibiotics, trace metals, pesticides (organophosphates and chlorinated hydrocarbons), natural agents, and polychlorinated biphenyls (PCBs). The results indicated that metals, chlorinated hydrocarbon pesticides, and polychlorinated biphenyls were continually present.

Coccidiostats

There is a general practice in rabbit feeding to use preventive veterinary drugs, such as coccidiostats. Rabbit is extremely sensitive to maduramycin (Tao, 2001) and flavomycin (Hanxiang, 1994). The well-known toxic coccidiostat in rabbits is narasin, which is a polyether carboxylic ionophore, generally used in poultry nutrition. Narasin poisoning in rabbits is mainly caused by cross-contamination of the compound feed. The cause of cross-contamination, as several studies have shown, is that the completely contamination-free production of premixes and compound feeds in the existing multi-product plants is impossible in practice (Strauch, 2003). Novilla *et al.* (1994) found that when rabbit bucks were treated with narasin by gavages with 30 or 100 mg kg⁻¹ b.w., decreased locomotor activity, weakness in the extremities, and ataxia were observed 3 hours after administration. In addition, relaxation of the abdominal muscle, prone position, ptosis, decreased respiration, and unusual breathing were present. Narasin poisoning was also reported in rabbit warrens (Ósz *et al.*, 1988). Clinical symptoms started with a significant decrease in feed intake, followed

by un-coordinated movement, weakness and flaccid paralysis of the extremities, especially in the posterior body half. Nervous symptoms (tonic-clonic convulsions, affecting the entire body, as well as torti-collis) were also observed. Death occurred one to four days after the onset of the clinical symptoms, frequently accompanied by significant malnutrition. In some acute cases, sudden deaths were also observed without any clinical signs. Enteritis and signs of circulatory disturbances were found in many cases. The histological lesions were characterised by moderate to severe Zenker's myofibrillar degeneration with lympho-histiocytic infiltration in the myocardium and skeletal muscle. Increases in serum enzyme activities (CPK, AST and ALT) showed a positive correlation with morphological damage of the muscular tissues. Narasin concentrations varying between 35 and 150 mg kg⁻¹ feed caused acute intoxications and death. However, maternal toxicity was shown in rabbits at levels above 1 mg kg⁻¹ b.w per day (EFSA, 2004).

Metals

Metal, particularly heavy metal toxicity in livestock production is a worldwide problem mainly in the industrialised countries. For that reason, the European Union regulated the maximum content of some heavy metals in complete feed as shown in Table 3 (European Commission, 2003, 2005a, 2005b)

Table 3: Maximum permitted content of some heavy metals in complete feed.

Heavy metal	Maximum content (mg kg ⁻¹)	EU directive
Arsenic	2.00	2003/100/EC
Lead	5.00	2003/100/EC
Mercury	0.10	2005/8/EC
Cadmium	1.00	2005/87/EC

There are some data that lead content of kidneys and liver, cadmium contents of kidneys, liver, heart and muscles are several times higher in rabbits kept in industrial areas as compared to the tissue values of those rabbits which are kept far away from industrial areas (Krelowska-Kulas *et al.*, 2006). The same heavy metal accumulation was also found in wild rabbits (*Oryctolagus cuniculus* L.), the highest quantity of lead was found in muscle (3.81 mg kg⁻¹), while the highest cadmium (1.02 mg kg⁻¹) and mercury (0.08 mg kg⁻¹) values were found in kidney (Eira *et al.*, 2005).

The excess of metals may be harmful to the immune system, even slight exposure to heavy metals (lead, mercury and cadmium) alters immune-competence, although the exact mode of action is not known, but the mentioned heavy metals impair the resistance against infections in rabbits (Fekete and Kellems, 2007).

Long-term cadmium toxicity caused splenic atrophy, and liver and kidney injury in rabbits (Stowe *et al.*, 1972). The oral lethal dose of cadmium was found to be 43 mg kg⁻¹ b.w. (Fairchild *et al.*, 1977). Dietary cadmium load – e.g. carrots grown in cadmium contaminated soil – caused its accumulation in different tissues, mainly in the kidney (Bersényi *et al.*, 1999). Among the blood parameters, gamma-glutamyl-transferase and cholinesterase activities decreased significantly due to kidney and

liver damage as an effect of feeding cadmium contaminated carrots. However, activity of alkaline phosphatase increased because of the pathological changes in the kidneys (Bersényi *et al.*, 1999). Long term cadmium exposure through the feed also impairs the reproductive ability of rabbit does because it causes morphological changes in the ovary (decreased volume of growing follicles and increased stroma and number of atretic follicles) also in the oviduct (oedematization), and in the uterus (oedematization); however, alterations were less in uterus in comparison with ovary and oviduct (Massányi *et al.*, 2007).

Metallothionein (MT) is a low-molecular-weight protein involved in detoxification of cadmium, however, results from the cadmium-heme assay showed that rabbits had low hepatic (2-10 $\mu\text{g g}^{-1}$ liver) MT level (Henry *et al.*, 1994).

Aluminium also has been proposed to be an environmental factor that may contribute to some diseases, affecting several enzymes and other biomolecules and inducing free radical-mediated cytotoxicity. Aluminium also induces reproductive toxicity and exerts significant adverse effect on steroidogenesis (Guo *et al.*, 2005). The toxicity of aluminium chloride (34 mg kg^{-1} b.w.), given orally for six weeks daily on lipid peroxidation and enzyme activities in seminal plasma of male New Zealand white rabbits was investigated by Yousef *et al.* (2005). Results showed that aluminium toxicosis significantly decreased libido, ejaculate volume, sperm concentration, total sperm output, sperm motility, total motile sperm per ejaculate and packed sperm volume. Percentage of dead and abnormal sperm cells was also increased and the relative weights of testicles and epididymis were significantly decreased. Concentration of thiobarbituric acid-reactive substances (TBARS) was significantly increased in seminal plasma as compared to the control. Activities of glutathione S-transferase, AST, ALT and acid phosphatase were significantly decreased.

Beside feedstuffs and complete feeds, drinking water is a real source of toxic materials. Veeramchaneni *et al.* (2001) evaluated the effect of long-term (15 weeks) daily exposure of male rabbits to drinking water containing chemicals typical of ground water near hazardous waste sites. Ground water contained (mg l^{-1}) 7.75 arsenic, 1.75 chromium, 9.25 lead, 12.5 benzene, 3.75 chloroform, 8.5 phenol and 9.5 trichloroethylene, respectively. The control rabbit bucks consumed normal tap water. It was found that the ejaculatory capability decreased, but total spermatozoa per ejaculate and daily sperm production were unaffected by waste water consumption. However, treatment caused acrosomal dysgenesis and nuclear malformations. The baseline serum concentrations of LH were lower, but with borderline significance as compared to control bucks. However, testosterone secretion after exogenous human chorionic gonadotrophin (HCG) administration was low. Thus, exposure to drinking water pollutants caused subnormal mating ability, sperm quality, and Leydig cell function.

Other industrial by-products may also cause detrimental effects in rabbits when they are ingested with feed, including plastic residues, such as polyamides. For instance, *N*-butyl benzene-sulphonamide (NBBS), a plasticizer used commercially in the polymerization of polyamide compounds, is neurotoxic. Rabbits showed dose-dependent motor dysfunction characterized by limb splaying, hyperreflexia, hypertonia, gait impairment, and abnormal reflexes as an effect of long-term 4 to 12 months of NBBS intoxication. Histopathological changes consisted of intramedullary

thickening of the ventral horn axons, random neuroaxonal spheroids confined to brain stem nuclei and spinal motor neurons, and swollen dendritic processes of spinal motor neurons were also observed (Strong *et al.*, 1991).

Polychlorinated dioxins and dibenzofurans

Polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs), are ubiquitous toxic contaminants originating mainly from thermal and incineration processes and representing potential risk for animal and human health. The International Agency for Research on Cancer has classified 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) as the most potent dioxin congener as a group I human carcinogen (IARC, 1993). Various studies showed that environmental levels of this agent have decreased during the last decades (European Commission, 2000). In contrast to this trend, several cases of specific contamination have caused high PCDD and PCDF levels in feedstuffs. It is important to monitor the dioxin contamination of feed to avoid large-scale feed contamination and to decrease human exposure to dioxins. Polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) were also found in some additives employed widely as binder and anti-caking agents in feedstuffs, such as kaolin, bentonite, and zeolite (Abad *et al.*, 2002), also carriers of choline chloride, such as pine sawdust (Llerena *et al.*, 2003). The European Union has regulated (2006/13/EC) the maximum permitted amount of polychlorinated compounds in animal feed (European Commission 2006a) as is shown in Table 4.

Table 4: Maximum permitted amount of polychlorinated compounds in complete feed.

Polychlorinated compounds	Maximum content (ng kg ⁻¹)
Dioxins (sum of PCDDs) and PCDFs expressed in WHO toxic equivalents, using the WHO-TEFC factors	0.75
Sum of dioxins (PCDDs and (PCDFs) and dioxin-like polychlorinated biphenyls (PCBs) expressed in WHO toxic equivalents, using WHO-TEFC factors	1.50

Compared to the feeds of other farm animal species, rabbit compound feeds are particularly contaminated with PCDDs and PCDFs (Table 5) as shown by an extensive survey in Italy (Cecil *et al.*, 2004).

Table 5: Average PCDD/PCDFs concentrations expressed as WHO toxic equivalency factor in complete feed samples.

PCDD/PCDF (ng kg ⁻¹)	Poultry	Cattle	Pig	Sheep	Rabbit
WHO-TEQ	0.022	0.035	0.019	0.021	0.319

Toxic effects of PCDDs and PCDFs in rabbits start with the so-called “wasting syndrome” because of feed refusal (Pohjanvirta and Tuomisto, 1994), later immune-depression and some neurologic effects are also manifested (Bursian, 2007). In the case of local skin contact with those reactive compounds, rabbits showed acne-like lesions on ears (Bursian, 2007). The acute oral LD₅₀ value of polychlorinated biphenyls and dibenzo-*p*-dioxins in rabbit is relatively high 15 µg kg⁻¹ b.w. (Safe, 1990).

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EFFECT OF SHORT HEAT EXPOSURE, BALANCED FEED RESTRICTION AND ACETIC ACID SUPPLEMENT AT POST WEANING ON GROWTH AND THERMOREGULATION IN GROWING RABBITS DURING HOT SEASON

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The aim of this study was to examine hypothesis that thermal or other stressors (like nutritional deficiencies and acidosis in the gut) at the moment of weaning may be beneficial in post-weaning growth and thermoregulation in later stage of rabbit life during summer season. Eighty New Zealand White rabbit kits were weaned at 30 days of age with average weight of 606.9 ± 26.6 g. At the moment of weaning kits were divided into 4 groups: 1st group, kits were kept as control (control); 2nd group, kits were fed a restricted diet during the 1st week post weaning (restriction); 3rd group, kits were exposed to high ambient temperature ($34 \pm 2^\circ\text{C}$) for 1 hour (heat); 4th group, kits were supplemented with 0.5% acetic acid in drinking water (acetic).

During the first week post-weaning, feed intake was increased by 10.6% and 1.8% in acetic and heat groups in comparison with the control, respectively. Feed restriction during the first week post-weaning led to reduction in live body weight and body weight gain. Although weaning in rabbit is often associated with lower weight gain, the body weight, and body weight gain in the 1st, 2nd and 4th week post-weaning in kits of heat exposure and acetic acid groups were higher than in the control group. Increases in body weight and body weight gain in the heat group may be attributed to immediate compensatory growth with higher feed intake that followed the reduction in growth during thermal conditioning. Body weight and body weight gain in the 10th week of age under hot summer season in acetic acid and restriction groups were higher than those in control in the 10th week of age during high ambient temperature (41°C). Rectal temperature of rabbits in the control group was greater than that in other groups. Under the same condition, hydrogen peroxide (H_2O_2) and nitric oxide (NO) in plasma increased in heat group but decreased in feed restriction and acetic acid groups compared with the control group. Total antioxidant capacity and catalase in plasma of all treated groups were higher than in the control group. In conclusion, short heat exposure or balanced feed restriction and acetic acid supplement at the moment of weaning led to the improvement in post weaning growth and in thermo-tolerance during summer season in rabbits.

CHANGES IN OXIDATIVE PROFILE, ACTIVITY OF SOME GASTROINTESTINAL ENZYMES AND PERFORMANCE OF GROWING RABBITS DURING HOT SEASON DUE TO NEONATAL HEAT EXPOSURE

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The aim of this study was to examine the effect of neonatal heat exposure treatment on oxidative stress profile, some gastrointestinal enzymatic activities and performance of growing rabbits during hot (summer) season. Fifty New Zealand White (NZW) kits were divided into two groups (25 kits/group). In the control group (C), kits were kept under normal ambient temperature ($21\pm 2^{\circ}\text{C}$). In the neonatal heat exposure group (H), kits were exposed to high ambient temperature ($34\pm 2^{\circ}\text{C}$ for 6 hours) at day 5 post-partum. All kits were weaned at 30 days of age. The total antioxidant capacity in plasma of neonatal heat exposure group was significantly ($P<0.05$) higher than that of control group under hot condition. Nitric oxide (NO) significantly decreased in neonatal heat exposure group. Specific activities of amylase, protease and lipase in the small intestine of H kits were higher compared with those of C group. Neonatal heat exposure improved daily feed intake and feed conversion compared with control group. There was no difference in weight of kits at weaning between groups. The body weights of young H rabbits at post-weaning and at 10 weeks of age were higher than those of C group. In conclusion, neonatal heat exposure improved weight of young rabbits when reared under hot condition. This may be due to increasing total antioxidant capacity with decreasing the nitric oxide at cellular level. Moreover, neonatal heat exposure may improve the efficiency of enzymes activity in the gastrointestinal tract of rabbit.

RESPONSE OF DOE RABBITS TO DIETARY ANTIOXIDANT VITAMINS E AND C DURING PREGNANCY AND LACTATION

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A seven-treatment experiment was carried out to evaluate the response to dietary supplementation with the antioxidant vitamins α -tocopheryl acetate (vitamin E) and ascorbic acid (vitamin C), provided individually or in a combination, on some performance traits of 49 two-year old multiparous New Zealand White (NZW) doe rabbits during pregnancy and lactation. Does were equally assigned to one of the following supranutritional levels of the two vitamins: 1) Control of no supplement (40 mg Vit. E/kg diet); 2) 80 mg Vit. E/kg (E80); 3) 160 mg Vit. E/kg (E160); 4) 200 mg Vit. C/kg (C200); 5) 400 mg Vit. C/kg (C400); 6) 80 mg Vit.E+200 mg Vit. C/kg (E80C200); and 7) 160 mg Vit. E+400 mg Vit. C/kg (E160C400). Results indicate that vitamin C groups - C400, then, alternately C200 or E80C200 - with a few exceptions were ranked first, second and third as had the highest values for feed intake during pregnancy ($P<0.01$), and lactation ($P<0.05$), litter size at birth ($P<0.01$) and weaning ($P=0.08$), litter weight at birth ($P<0.01$) and weaning ($P<0.05$), and milk production at the 1st ($P<0.01$), 2nd ($P=0.06$), 3rd ($P<0.01$) and 4th ($P<0.01$) week of lactation. The eminent exception was that E80 group came second for litter weight at weaning. Conversely, the E160 and E160C400 treatments negatively affected these traits. Plasmatic concentrations of α -tocopherol and ascorbate, the total antioxidant capacity during lactation, and hematological parameters (PCV % and Hb concentration) were increased ($P<0.01$), especially as the vitamin level was increased either individually or in a combination. In conclusion, it is recommended to boost the diets of pregnant and lactating doe rabbits with extra doses of vitamin C either individually or combined to α -tocopherol at low doses to improve the performance traits during these critical periods.

PERFORMANCE EVALUATION OF GROWING RABBIT FED DIETS CONTAINING VARYING INCLUSION LEVELS OF *CENTROSEMA PUBESCENS* OR *CALAPOGONIUM MUCUNOIDES* IN THE SAVANNAH ZONE OF NIGERIA

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Twenty-five mixed bred weaner rabbits (average initial weight 576 to 602 g) were used to investigate the nutrient potential and digestibility of *Centrosema pubescens* and *Calapogonium mucunoides*. The forages were cut, air dried and milled. Five experimental diets were formulated, diet T₁ contained no forage; diets T₂ and T₃ contained *Centrosema pubescens* at 10% and 20% inclusion level respectively; *Calapogonium mucunoides* was incorporated into diet T₄ and T₅ at 10% and 20% inclusion level respectively, mainly in substitution to maize, palm kernel and ground nut cakes. The animals were randomly distributed among diets. Average daily feed intake and average daily weight gain were monitored. The experiment lasted for ten weeks. Data obtained were subjected to one-way analysis of variance. The highest final weight (1321 g) was observed in T₁ though similar to T₂ (1181 g) (10% *Centrosema pubescens*), T₃ (1247 g) (20% *C. pubescens*) and T₄ (1193 g) (10% *Calapogonium mucunoides*), and the lowest final weight (1017 g) was observed in T₅ (20% *C. mucunoides*). Average daily weight gain also followed the same trend: 11.7, 9.6, 10.4, 9.6 and 8.4 g/d in T₁, T₂, T₃, T₄ and T₅, respectively, the difference between the control or T₃ treatment (20% *C. pubescens*) in one hand and T₅ treatment (20% *C. mucunoides*) in the other hand being significant (P<0.05). For the feed intake, values were 66.0, 62.4, 55.9, 61.3 and 58.8 g/d for treatment T₁ to T₅ respectively, with T₁ and T₂>T₃ and T₅ (P<0.05). The best feed efficiency were observed for T₃ and T₁ (0.19 and 0.18 g gain/g feed intake) and the worst for T₅ (0.14 g/g) (P<0.05). In conclusion, the incorporation of *Centrosema pubescens* at 20% inclusion level gives results similar to the control and better than the incorporation of *Calapogonium mucunoides*.

EFFECT OF DIETARY LEVEL AND SOURCE OF GLUTAMINE ON INTESTINAL HEALTH IN THE POSTWEANING PERIOD

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The aim of this work was to study the effect of dietary glutamine (gln) level and source on rabbit mortality rates induced by Epizootic Rabbit Enteropathy (ERE) and the detection of *C. perfringens* from intestinal isolates. The effect of this amino acid on mucosal barrier mechanisms was also assessed by studying the relative PPAR γ mRNA abundance in ileum and jejunum. Three diets were experimentally tested, a basal diet (C) containing 177 g of CP/kg DM, and 28.8 g of gln/kg DM, and two diets supplemented at 0.5% and 1% above the basal levels (C-G0.5 and C-G1). In addition, to study the effects of including 1% gln as synthetic vs. a natural source, a fourth diet (W) was included in the experiment containing a wheat protein concentrate (VITEN cws). One hundred and ninety-six individually lodged animals (49 animals/treatment) and two hundred and twelve collectively lodged animals (53 cages/treatment) weighing 476 \pm 93 g and 498 \pm 100 g respectively were blocked by litter and randomly assigned to the experimental diets. Animals were fed the experimental diets for 18 d (28 to 46 d of age). During the second period (46 to 60 d of age) all rabbits were fed the basal diet (C). For the microbiological caecal study 60 rabbits with 44 d of age (15 per treatment) weighing 565 \pm 96 g were selected. For the RNA isolation and real-time RT-PCR 7 rabbits per treatment (C, C-G0.5 and C-G1) from the microbiological analysis were selected. The supplementation with 0.5 L-gln resulted in a reduction of 1.1 log unit of *C. perfringens* CFU/g (P=0.05) compared to the basal diet. Also a tendency for a quadratic effect of dietary gln level (P=0.09) was observed. Higher relative abundance of PPAR γ mRNA was observed in ileum than in jejunum, however, no dietary effects were reported either in ileum or in jejunum. No differences were observed between sick and healthy animals. Our results suggest glutamine plays an important role in the intestinal health without effects on growth performances. However, gln level had no effect on PPAR γ mRNA abundance partly explained by the lack of a clear inflammatory process in sick animals. Optimal results were obtained with 0.5% of synthetic gln supplementation. No differences between the two sources of gln were detected for any of the traits studied. Therefore, synthetic gln sources might be substituted by natural ones, as wheat protein concentrate, decreasing feeding costs.

IN VIVO AND IN VITRO STUDY OF CAECAL FERMENTATION PATTERN AND METHANOGENESIS IN RABBITS

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Methane formation and caecal fermentation pattern were studied *in vivo* and *in vitro* in 16 white New Zealand rabbits allocated to four diets formulated with two different sources of fibre (alfalfa hay or sugar beet pulp) and starch (wheat or maize). Animals received the diet for at least two weeks before methane production was measured *in vivo* in a respiratory chamber. Then, animals were slaughtered and caecal contents were sampled for volatile fatty acid (VFA) determination and used as inoculum for *in vitro* incubations performed to determine gas production and methane formation. Total VFA concentration in caecal contents averaged 52.5 mM, with butyrate content higher when alfalfa hay was the main source of fibre compared to sugar beet pulp (9.97 vs. 7.32 mM). Fermentation pattern was also affected by the experimental treatment, acetate being lower (72 vs. 79%) and butyrate higher (20 vs. 14%) when maize was the source of starch in relation to wheat. Fermentation *in vivo* vs. *in vitro* showed some differences (molar proportion of acetate, 75 vs. 72% *in vivo* and *in vitro*, respectively; molar proportion of propionate, 6.9 vs. 9.1% *in vivo* and *in vitro*, respectively), probably due to differences in pH (6.0 vs. 6.7 *in vivo* and *in vitro*, respectively). Only 2 out of 16 rabbits produced a substantial volume of methane *in vivo*, whereas all of them showed some formation in the *in vitro* incubations. No effect of the experimental treatment was observed *in vivo* on methane production due to the high inter-individual variability, whereas *in vitro* an effect of the source of starch was observed, being higher when maize was included in the diet compared to wheat. A similar effect was shown in total gas production, probably due to the stimulation of caecal bacteria in the animals receiving a more resistant starch from maize that cannot be digested in the small intestine and reaches the caecum. H₂ recovery was very low, suggesting the importance of other mechanisms of H₂ disposal than methanogenesis such as reductive acetogenesis, and was again affected by the source of starch in a similar way.

EFFECT OF WATER RESTRICTION TIMES OF 2 AND 4 HOURS PER DAY ON PERFORMANCES OF GROWING RABBITS

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Hydric restriction, which is an indirect feed restriction, is a common practice to reduce post weaning digestive disorders in rabbits. Two hydric restriction times (4 h and 2 h per day) to induce feed restriction in growing rabbits in good sanitary conditions were tested in this study. Thirty six rabbits were divided at weaning (4 weeks) into three groups: T: Control group; R2 with restricted access to drinking water from 35 to 77 days of age of 2 h per day (from 8 to 10 am); and R4 with access to drinking water 4 h per day (from 8 to 12 am). Body weights, mortality, daily food and water consumption were recorded. Once a week, from 41 to 76 days of age, the alimentary compartment was followed by control of water and feed consumptions each half an hour (between 8 am and 12 am). A restricted access to drinking water of 2 or 4 hours/day induced feed restriction in growing rabbits of 25 and 20% respectively. Compared to group T, water consumed by restricted groups R4 and R2 were 71% and 58% respectively ($P < 0.01$). The water intake for R2, R4 and T were respectively: 171, 207 and 291 g/day. Water/feed ratio was lower ($P < 0.01$) with restricted groups compared to group T (1.65, 1.86 and 2.05 for R2, R4 and T respectively). Water restriction ameliorated feed conversion: 3.5 g/g for restricted groups (R2 and R4) and 3.8 for the control group. Weight gain decreased with the degree of restriction ($P < 0.01$): 36.9, 32.8 and 29.9 g/d respectively for T, R4 and R2. Controlling water and feed consumptions once a week (6 times between 41 and 76 days) is a good predictor of global feed consumption for the duration of the trial (35 to 77 day of age), with at most 2% of difference (group R2). The prediction of water consumption differed with groups. It decreased with the degree of restriction: 4%, 12% and 29% for T, R4 and R2 respectively.

EFFECTS OF FEED RESTRICTION ON THE PERFORMANCE AND CARCASS OF GROWING RABBITS

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Seventy two Hyla rabbits were studied from 6 to 11 weeks of age. They received a commercial diet distributed at 3 levels: *ad libitum* (C), 85% (R85) and 70% (R70) of *ad libitum* intake. Restricted animals consumed more quickly their rations. The animals receiving 70% finished their ration after 10 hours, those in 85% in 16 hours. Feed restriction significantly affected daily weigh gain (38.54, 34.58 and 29.42 g/day for C, R85 and R70 groups, respectively), reducing the growth and final weight of rabbits (2115, 1955 and 1740 g, respectively) but improving the feed conversion of diets (by 5 and 8% respectively for R85 and R70). Restricted animals had lower percentages of caecum and presented less fat than those fed *ad libitum* (1.31, 1.68 and 1.85% of reference carcass, respectively for R70, R85 and C).

EFFECT OF DIETARY FAT QUALITY ON THE PERFORMANCE AND HEALTH OF FATTENING RABBITS

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Three experiments were performed with 786 weaned rabbits (28-day old) in order to evaluate the effects of feeding recycled fatty materials on the performance (690 rabbits) and health status (96 rabbits) during the fattening period. Six experimental feeds for rabbits were formulated including 3% of fats with low (L) or high (H) levels of *trans* fatty acids, polycyclic aromatic hydrocarbons (PAHs) or lipid oxidation. Weight gain and feed intake were recorded between 28 and 56 days and between 57 and 63 days. In 63-day old animals, the caecal ambient was assessed by measuring pH, NH₃ and short chain fatty acids (SCFA) in caecal content, and the hepatic and renal functions by determining gamma glutamyl transferase (GGT), glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), alkaline phosphatase (ALP), urea and creatinine on serum samples. Because of Epizootic Rabbit Enteropathy, a very high mortality rate was recorded (varying between trials from 31% to 60%), with no statistically significant differences between L and H treatments in any experiment. No statistically significant differences in performance were linked to the dietary levels of *trans* fatty acids (body weight gain and feed intake in this experiment averaged 46.8 g/day and 121 g dry matter/day, respectively), PAHs (45.1 g/day and 107 g dry matter/day) or lipid oxidation (45.1 g/day and 108 g dry matter/day). Similarly, blood levels of the hepatic or renal function markers were not affected by the dietary levels of *trans* fatty acids (GGT, GOT, GPT, ALP, urea and creatinine averaged 7.29 IU/l, 64.7 IU/l, 65.4 IU/l, 156 IU/l, 20.7 mg/dl and 0.82 mg/dl, respectively), PAHs (6.43 IU/l, 76.0 IU/l, 64.0 IU/l, 148 IU/l, 19.7 mg/dl and 0.73 mg/dl) or lipid oxidation (6.32 IU/l, 69.3 IU/l, 59.4 IU/l, 155 IU/l, 17.5 mg/dl and 0.79 mg/dl). However, some dietary effects on caecal ambient were detected. Thus, a higher level of *trans* fatty acids (and saturated fatty acids) was associated with lower SCFA concentration in caecal content (164 vs. 215 mmol/l, P<0.01). On the other hand, caecal NH₃ concentration was higher in rabbits receiving the diet high in PAHs (11.7 vs. 5.56 mmol/l, P=0.04). No effects of dietary lipid oxidation on caecal parameters were detected.

EFFECT OF INULIN SUPPLEMENTATION ON CAECAL MICROFLORA AND FERMENTATION IN RABBITS

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Oligosaccharide-based prebiotics, like inulin, are intensively examined as alternatives for antibiotics in rabbits. The aim of the present experiment was to study the effect of dietary supplementation with inulin on the caecal microflora, fibrolytic activity, and production of volatile fatty acids. Pannon White rabbits (n=180) were divided into three groups at weaning (28th day of age) and fed *ad libitum*. Experimental diets were already fed one week before weaning. The control group (C) was fed a standard basal diet with no supplementation; group M received the same but medicated (1 mg/kg diclazuril, 500 mg/kg oxytetracycline and 50 mg/kg tiamulin) diet, while 4% inulin (Frutafit, HD, Brenntag, Budapest) at the expense of barley was added into the diet of the Inulin (I) group. The diets were similar in the level of crude protein, fat and fibre fractions, while diet I differed in starch and sugar content. At 28, 35 and 42 days of age, 6 healthy animals from each group were randomly selected and caecal microflora and fermentation pattern were examined. Body weight (BW) at the age of 28 and 35 days was not significantly different, but at 42 d of age a lower ($P<0.05$) LW was measured in group I. Considering the whole experimental period the weight gain (36 ± 13 g/day) was the smallest and the feed conversion was the greatest (2.46 ± 0.46 g/g) in group I. The incidence of diarrhoea was 1, 2 and 6 in groups C, M and I, respectively. Caecal pH was below 7.0 already at weaning and no significant difference between groups was detected. There was a temporary increase in the number of *E. coli* in each group at 35 days of age, but thereafter it decreased except in group I. The highest cellulase activity was found in the control group, while the lowest in the medicated rabbits. On the 35th d of age the greatest activity of xylanase and cellulase was detected in group M, while on day 42, the highest xylanase was found in the control group. Concentration of total VFA decreased in group C and M at 35 days of age, after that it increased except group I, where the decrease was observed at 42 days of age. The highest acetic acid and lowest butyric acid ratio (7%) were found in group I on the 42nd day. Based on our results, a dietary inclusion with inulin does not improve the zootechnical performances or enhance the caecal environment.

EFFECT OF *BACILLUS CEREUS* VAR. *TOYOI* ON CAECAL MICROFLORA AND FERMENTATION IN RABBITS

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In commercial rabbit meat production, a major part of the mortality results from diseases of the digestive system that are related mainly (about 25%) around weaning (from days 18 to 50). Besides economic losses, this disease has a negative influence on animal welfare as well. Antibiotics are still widely used to reduce mortality of the growing rabbit, although there is an increasing human health and food safety concern over drug residues in meat products. As an alternative to in-feed antibiotics, the effect of a probiotic, i.e. *Bacillus cereus* var. *toyoi*, was examined in rabbits. One group of Pannon White does (Group T) was fed a diet containing 200 ppm Toyocerin (2×10^5 *B. cereus* spores/g feed), while the other group (Group K) received an antibiotic free diet with the same chemical composition. Kits consumed the same diet as their mothers (K and T) till weaning. After weaning at 28 days of age, all litters were divided into two groups, one feeding the same diet as before (KK, n=37 and TT, n=46), while the diet of the other two groups changed (KT, n=38 and TK, n=44). Supplementation of the does diet significantly improved the kit weight gain. The weight of K rabbits (389 ± 8 g) was significantly ($P < 0.05$) lower at 3 weeks age compared to treated (T) animals (462 ± 12 g). This could be presumably due to the better milk production of the does. The difference in body weight between the two groups was still marked on week 4. During the whole experimental period (4 to 6 weeks of age) body weight gain (54 g/day) and feed conversion (2.0) proved to be the best in group KT. Morbidity and mortality were lower in animals fed supplemented diet after weaning. The most impressive change was observed in the number of coliform bacteria in the GI tract. Caecal chyme of K rabbits contained significantly higher coliform germ count on day 21 (5.9 CFU \log_{10} /g chyme). On week 5 adding Toyocerin into the diet significantly decreased the bacterial count in groups KT and TT (2.0 CFU \log_{10} /g chyme). For coliforms the count of 10^3 - 10^4 bacteria/g chyme in group TK could be considered as physiological, but the count of 10^5 in KK rabbits is considered to be of high risk from the animal health point of view. Total volatile fatty acid concentration was around 66-80 mmol/kg after weaning in all groups, though in the TK and TT groups it was temporarily raised above 100 mmol/kg during the 4th week.

FEED RESTRICTION DURING SUMMER: EFFECT ON RABBIT GROWTH PERFORMANCE

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The paper aimed to study the effect of feed restriction applied during the summer season and the whole productive cycle on growth performance of rabbits. Two groups, each of them consisting in 684 Hybrid Hyla rabbits of both genders, were fed from weaning to 60 days of age and from 61 days to slaughter age the same “weaning” and “finishing” concentrate. The group indicated as AL, fed the concentrate *ad libitum*, while the group called RES received a restricted quantity of the concentrate. The level of restriction was 80% and 90% of *ad libitum*, respectively from weaning to 60 days of age and from 61 days of age to slaughter. During the trial, mortality rate was recorded daily, while feed intake and live weight of 24 rabbits per group were recorded weekly in order to calculate daily weight gain and feed conversion ratio. Feed intake of *ad libitum* group was about 13% lower than the values recorded in the same farm during the winter season. No statistically significant differences were found between the groups for weight at slaughter (2.42 vs. 2.50 kg, respectively for group restricted and *ad libitum*), while feed conversion ratio calculated for the whole experimental period was significantly ($P < 0.05$) lower for restricted group (3.18 vs. 3.56). Mortality rate during the whole experimental period was significantly ($P < 0.01$) higher for restricted group (21.21 vs. 13.93%, respectively for restricted and *ad libitum* group) due to the higher values recorded in particular during the weeks 56-63 d and 63-70 d, when environmental temperature was very high and the maximum values reached 34.7°C. Our results indicate that feed restriction is not advisable during the summer period, in particular when environmental temperature is very high.

EFFECT OF DIETARY SUPPLEMENTATION OF ORGANIC ACIDS AND ESSENTIAL OILS ON IMMUNE FUNCTION AND INTESTINAL CHARACTERISTICS OF EXPERIMENTALLY INFECTED RABBITS

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To analyze the effect of dietary organic acids and essential oils supplementation 120 young rabbits (28 d old) were divided into three groups and submitted to the following dietary treatments: Control diet; Zinc Bacitracin diet (Control diet+150 ppm Zinc Bacitracine); FormaXol diet (Control diet+0.4% FormaXol - mixture of microencapsulated formic and citric acids and essential oils). At 38 d of age, all rabbits were experimentally infected with *Escherichia coli* O103 and *Clostridium perfringens* type A and clinically monitored for 3 weeks. To evaluate the native serum immunity (lysozyme, haemolytic complement assay and serum bactericidal activity) haematic samples were collected by cardiac puncture at regular intervals. Twenty one days after infection, all rabbits were euthanized and intestinal swabs were collected from different tracts of the small intestine, colon and caecum to investigate the aerobic and anaerobic bacteria. A jejune portion was excised to analyze the height of the villi. The haemolytic complement values showed a drastic reduction after infection in Control and Zinc Bacitracine diet animals; on the contrary, animals of FormaXol diet showed a steady trend in the first period (9 d) and a drastic reduction in the final period (11 d) like the others group. The better immune response in animals of FormaXol diet could be lied to higher values of serum bactericidal activity and lower values of lysozyme. The Control diet showed higher increase of lysozyme values after infection. All the studied immune parameters are in homeostatic equilibrium being subject to opposite phenomenon as production and consume. A similar percentage of *E. coli* O103 and *C. perfringens* type A were found in the Zinc Bacitracin and FormaXol groups. The highest percentage of non-pathogen bacteria was found in the Zinc Bacitracin and FormaXol rabbits. The results obtained measuring the villi height confirmed the previous analyses with a better value when FormaXol diet was used (P<0.05). Considered together these results show that 0.4% integration of microencapsulated formic acid and citric acids and essential oils reduces the damage of both Gram⁻ and Gram⁺ pathogen bacteria permitting to obtain a better serum innate response in experimentally infected rabbits.

EFFECT OF DIFFERENT FEEDING SYSTEMS FOR YOUNG RABBIT DOES ON THEIR DEVELOPMENT AND PERFORMANCE UNTIL FIRST WEANING: PRELIMINARY RESULTS

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One hundred thirteen young rabbit does were used to study the effect of the feeding program during the rearing and first gestation on their development and performance until first weaning. Five experimental groups (CL, CR, FL, FC and FCF) were constituted from 9 weeks of age, following different feeding program during rearing and gestation period. Does of CL and CR groups received C diet (11.0 MJ DE, 114 g DP and 277 g ADF per kg of DM) offered always *ad libitum* in CL group and restricted (140 g/day) from 12 weeks of age to first parturition with *ad libitum* feeding during 5 days before insemination in CR group. Does of FL, FC and FCF groups received F diet (8.7 MJ DE, 88 g DP and 394 g ADF per kg of DM) *ad libitum* until 16 weeks of age; afterwards, FL group continued receiving F diet *ad libitum* until parturition while FC and FCF groups were switched to C diet also offered *ad libitum* until parturition in FC group and until 20 weeks of age in FCF group, that returned to F diet *ad libitum* from 20 weeks until parturition. After kindling, all groups were feed on C diet. Feed restriction affected ($P<0.05$) the live weight and body condition of young does at insemination (-326 g of live weight and -0.57 mm of perirenal fat thickness, CR vs. CL group) and at kindling (-171 g of live weight and -0.26 mm of perirenal fat thickness, CR vs. CL group), but performance of does was not affected. On the other hand, the use of a high fibre diet also induced lower live weight and body condition of does at insemination (-341 g of live weight and -0.44 mm of perirenal fat thickness, FL vs. CL group, $P<0.05$) in spite of their greater feed intake, but the use of C diet after 16 weeks of live (FC and FCF groups) allowed some recovery in live weight and body condition at insemination, as consequence of a higher energy intake (+149 kJ DE/kg LW^{0.75} day, FC and FCF vs. FL group, $P<0.05$), and increased the size and weight of alive litter at birth in the FCF (returned to F diet *ad libitum* from 20 weeks of age until parturition). Feed intake during the first week of lactation was significantly different among groups ($P<0.05$), lower in CL group (78 g DM/kg LW^{0.75} day), higher in FL and FCF groups (98 g DM/kg LW^{0.75} day) and intermediate in CR and FC groups (87 g DM/kg LW^{0.75} day), but no differences were found during the rest of lactation period. Milk yield was similar in all groups, but litter weight at weaning was lower in CR group.

EFFECT OF DIETARY ACIDIFICATION ON GROWTH PERFORMANCE AND CAECAI CHARACTERISTICS IN RABBITS

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The present work aimed to evaluate whether the addition of organic acids to feed could improve growth performance and caecal characteristics, and modify the equilibrium of caecal microbiota population in growing rabbits. For this purpose, 216 rabbits, weaned at 30 d of age, were divided into four experimental groups. Control group (C) was given the basal diet without any additive, while Antibiotic group (A) rabbits were fed the same diet with antibiotics until 55 d of age. The other two experimental groups were T1 (containing a commercial organic acid product characterized by a blend of formic acid, lactic acid and essential oil from rosemary, thyme and cinnamon) and T2 (included only formic and lactic acid). At 30, 55 and 84 d of age the caecum weight, caecal content characteristics in terms of pH, ammonia and volatile fatty acids (VFA), and caecal microbiota composition were determined in 10 rabbits per group. Between 55 and 84 d of age the daily weight gain of T1 and T2 groups were higher (34.0 and 34.5 g/d; $P < 0.05$) than those found in C and A groups (31.2 and 31.2 g/d); in the same period, animals fed T1 and T2 diets showed also a significantly lower feed conversion rate ($P < 0.01$). Before 55 days, T2 group presented significantly lower feed conversion ratio than those of the other groups, suggesting a positive effect of acidification during the weaning period, even if this effect was not significant in animals fed the diet T1. Rabbits given T2 diet had a significantly higher VFA concentration and a lower pH in the caecum in comparison with the other experimental groups, probably due to a higher fermentative activity; also VFA molar proportions were significantly affected by the treatments, with a lower acetate concentration in T2 and A groups, perhaps associated with changes occurred in composition of commensal intestinal microflora, not determined in our study. The data of microbial analysis at 55 days of age showed significantly lower aerobic and facultative anaerobic bacteria counts of C group, while the other bacteriological traits were similar among treatments. The mortality of animals fed the diet supplemented with essential oils (T1) was significantly lower than that of rabbits given T2 diet, including only formic and lactic acid, but not different from that of C and A treatments. In conclusion, in our trial formic acid, lactic acid and essential oils inclusion to the diet stimulated weight gain and increased feed conversion rate in the second phase of fattening, without reducing mortality when enteric disease occurred.

**EFFECT OF DIETARY FAT ADDITION ON GROWTH PERFORMANCE,
NUTRIENT DIGESTION AND CAECUM FERMENTATION
IN 2-3 MONTHS OLD MEAT RABBITS**

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An experiment was conducted to determine the effect of different diet fat addition on growth performance, nutrients digestion and caecum fermentation of 2 to 3 months old New Zealand meat rabbits. Eighty four 2-month old rabbits were allocated in individual cages for seven treatments in which they were fed each diet with addition of fat (lard and soybean oil) at 0, 20, 40 and 60 g/kg. Compared with the control group, the lard addition decreased the average daily intake (ADI) and improved the feed conversion rate (FCR) ($P<0.05$). Compared with the control group, the fat addition increased the coefficients of total tract apparent digestibility (CTTAD) of ether extract (EE) ($P<0.05$) and decreased the pH and acetic acid/propionic acid ratio in the caecum ($P<0.05$), and did not affect the CTTAD of crude fiber and energy ($P>0.05$). The CTTAD of EE of control group, 20, 40 and 60 g/kg fat addition was 0.695, 0.827, 0.840 and 0.851, respectively. When the dietary fat level increased, pH and acetic acid/propionic acid ratio decreased ($P<0.05$) and butyric acid content in the caecum increased ($P<0.05$).

VERY LOW PROTEIN, AMINOACID-SUPPLIED DIET FOR HEAVY BROILER RABBITS: EFFECTS ON GROWTH, FEED EFFICIENCY, CARCASS AND MEAT PERFORMANCES

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Two very low protein diets (VLP), named A1 and A2, were supplied by the four available synthetic Amino Acid (AA) (L-Lysine, DL-Methionine, L-Thryptophan and L-Threonine) and compared to two Commercial Control (C1-C2) standard diets with no antibiotic. Synthetic aminocids were added to A1 and A2 diets at levels such to equalize their content to C1 and C2 feeds: other nutritional parameters (starch, crude fiber, and digestible energy) were the same for all the diets. Ninety three rabbits belonging to “*Macchiata Italiana*” and “*Bianca italiana*” strains, in purebred and crossed, were weaned at 31 d and slaughtered at 94 d for an heavy broiler production; thus *ad libitum* feeding in individual cages was modulated in two phases with a minor crude protein level administration in the first post weaning growth: 14.5 vs. 17.5% DM (-17%) and 19.7 vs. 15.9% (-19%) respectively for the two phases. At the feeding change, on the 66th d, a crossover of treatments (A1->C2 and C1->A2) occurred in a half of the rabbits. Mortality raised to 20% overall, but it was concentrated in the C diets (38% vs. 14%, P=0.01). The feed intake was not modified by the diets or their combinations, but the growth rate of the second period was dramatically enhanced in the rabbits which were previously fed with VLP diet A1 (36.8, 39.1 g/d vs. 30.6, 30.1). The feed conversion ratio, which did not change in the first phase, was strongly improved in relative 24% in the second phase, parallel to the extension compensatory growth extent. The efficiency of the protein fed to rabbits (PER) get strongly advantage for the VLP diets in measure higher than 13%. Genetic factor enhanced significant differences in growth and feed efficiency traits mainly in the first phase. In conclusion, nitrogen restriction in a long post-weaning phase could be beneficial to heavy broiler rabbits production because their prolonged compensatory growth, positive effects on overall health status of the animals, and to maximize N utilization with minimum N output without modification in carcass and meat quality.

INFLUENCE OF THE DISTRIBUTION AT BIRTH OF A HEAT SHOCK PROTEINS BOOSTER ON GROWTH AND MORTALITY OF RABBITS BEFORE AND AFTER WEANING

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Heat shock proteins (HSPs) are produced by the organism following a stress and are active about 2 hours following the stress. A booster of HSP proteins isolated from the prickly pear (*Opuntia ficus india*), the *Tex-oe*®, accelerates this production of HSPs which are secreted within 8-10 minutes. This work presents results of the distribution of *Tex-oe*® to the rabbit kits immediately after birth. Four experiment involving 546 litters corresponding to 5416 one-day-old kits were carried out. Half of the one-day old kits received one drop of *Tex-oe*® diluted in a vitamin solution during the hours immediately following their birth. In these conditions, the *Tex-oe*® intake was about 0.01 ml/animal corresponding to 1.6 ml of *Tex-oe*® for 10 kg live weight. The mortalities and growths of the rabbits were measured from birth to slaughter age at about 70 days. The distribution of this small quantity of *Tex-oe*® immediately after the birth had long term effects for the rabbit and increased the adjusted final weights by 20 g (2273 vs. 2253 g; P=0.088). For mortality, the main observation before and after weaning was a great variability of the results among the experiments: significantly positive effects, significantly negative effects or an absence of effects were observed. The effect of the *Tex-oe*® on the mortality seems to be affected by the pathologic situation of the animals: it was very positive in the first experiment where the dominant pathology was a respiratory one (12.2 vs. 18.5%; P=0.001). In this case, the mortality reduction was particularly important during the finishing stage when the respiratory mortality was the highest. On the contrary, the *Tex-oe*® increased mortality in the 3 other experiments with dominant digestive pathologies (14.0 vs. 11.4% for the control; P=0.034) at the beginning of the fattening period. These different results could probably be explained by an action of the *Tex-oe*® simultaneously protecting the cells against the stress alterations and increasing the feed intake capacity of the animals even after several weeks. This last mechanism may explain the higher growth rate after ingestion of *Tex-oe*®, but may present some inconvenient too in the case of the just weaned rabbit by increasing the digestive problems risks.

**IMPACT OF AN EXCLUSIVE MILK VS. MILK AND DRY FEED INTAKE
TILL WEANING ON INTAKE, GROWTH, AND ON THE CAECAL
BIODIVERSITY AND FIBROLYTIC ACTIVITY OF THE YOUNG RABBIT**

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The effect of an immediate shift from milk to solid feed at weaning (30 d) was studied on intake, growth, caecal ecosystem and health of the young rabbit. Two groups of 11 litters were weaned at 30 d of age: PILA group, where the kits were fed exclusively with maternal milk until weaning, and then immediately fed with a pelleted feed; Control group where the kits were classically bred, with a progressive increase in feed intake before weaning from mother feeder. The adaptation of the young to the solid feed was quick, since their relative intake became similar to that of Control between 32 and 36 d (respectively 89.9 vs. 89.3 g/d/kg LW). From 30 to 49 d of age, the growth of PILA did not differ from Control (meanly 50.7 g/d). From 49 to 70 d, the intake of PILA group did not differ from Control, as well growth rate or feed conversion. The mortality (4/154) and the morbidity (3/154) were low and similar among the 2 groups. An exclusive milk intake till weaning strongly inhibited the pectinolytic and xylanasic bacterial activities at 30 d (respectively 7 and 4 times lower for PILA than for Control), but they were similar to Control at 37 and 44 d. The bacterial biodiversity in the caecum, estimated by Simpson index, did not change significantly between 30 and 44 d of age for the control group. In return, an exclusive milking reduced the caecal biodiversity at 30 d of age (PILA), but it increased rapidly and reached a level similar to control group at 37 and 44 d of age.

ASSESSMENT OF LOOFAH GOURD SEEDS *LUFFA CYLINDRICA* (ROEM) ON PERFORMANCE AND SOME HAEMATOLOGICAL INDICES OF RABBIT WEANERS

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Loofah gourd seeds (LGS) *Luffa cylindrica* (M.J. Roem) were made into a meal and used to replace partially soybean meal (SBM) on weight basis at 0, 5, 10, and 15% inclusion in weaned rabbits diet, in a 56-day feeding trial. Twenty-four rabbits (initial live weight 555 ± 55 g), New Zealand x Chinchilla cross bred, were divided into four groups of three replicates containing two rabbits each, in a completely randomized experiment. Growth performance, nutrient digestibility and some haematological parameters were measured. Daily water intake was significantly ($P < 0.05$) the highest for rabbits fed diets with 5% LGS (183 ± 12 ml) and the lowest for those on 15% LGS (149 ± 11.6 ml). Daily weight gain were 15.0, 19.2, 18.5 and 10.3 g/d respectively with increasing level of LGS. Values observed with 5% and 10% LGS were significantly higher than with 15% level which was lower than observed with control diet ($P < 0.05$). Feed conversion ratio (3.12 and 3.26) and protein efficiency ratio (0.53 and 0.52) were similar for 5% and 10% LGS inclusion and better ($P < 0.05$) when compared to the control diet (3.93 and 0.63, respectively) and 15% LGS inclusion by the test diet (5.56 and 0.89 respectively). Dry matter and nitrogen digestibility values for rabbits fed 15% LGS (57.5% and 56.0%) were significantly lower ($P < 0.05$) when compared to other levels of inclusion in the test diets: 70.7, 73.2 and 71.0% for DM digestibility and 66.6, 65.2 and 66.8% for nitrogen digestibility for the for 0, 5 and 10% LGS, respectively. The crude fibre digestibility was similar for the control diet (64.8) and the 5% LGS (65.5) or 10% LGS (64.0) diet and significantly lower for 15% LGS diet (62.4%). Packed cell volume was highest in rabbits on 5% LGS (43.0) and similar to 10% LGS (38.3), but lower ($P < 0.05$) in 15% LGS (32.3) replacement of SBM. Haemoglobin for rabbits on 5% LGS (12.6 g/dl) was also highest and similar to 10% LGS diet (12.3 g/dl). The red blood cell count ($5.81 \pm 0.34 \times 10^6/\text{mm}^3$) and white blood cell count ($8.44 \pm 0.77 \times 10^6/\text{mm}^3$) were higher ($P < 0.05$) in rabbits fed 5% LGS but lowest for those on 15% LGS ($10.41 \pm 0.99 \times 10^6/\text{mm}^3$ and $4.80 \pm 0.11 \times 10^6/\text{mm}^3$ respectively). Rabbits fed 15% LGS had the highest ($P < 0.05$) blood urea (7.97 ± 0.22 mg/dl) followed by those of on 10% LGS and 5% LGS which were similar (6.09 ± 0.12 mg/dl and $6.05 \pm 0.21 \times$ mg/dl) but lowest for those on 5% LGS (5.91 ± 0.42 mg/dl). This indicates wastage and poor utilization of the dietary protein at 15% LGS replacement level. In conclusion loofah gourd seeds may be used up to 10% in growing rabbit diets.

NEW ADVANCES IN DIGESTIVE PHYSIOLOGY OF RABBITS WITH THE USE OF TROPICAL FORAGE IN CUBA

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The objective of this paper was to determine *in vivo* and *in vitro* nutrient digestibility of different tropical forage sources for rabbits. Results of four experiments conducted at the Instituto de Ciencia Animal were used. In the *in vivo* experiments, twenty-four White New Zealand rabbits of 60 days of age and 1.2 kg live weight were allocated in six individual metabolism cages. They were distributed in a completely randomized design into four groups with six replications. The *in vitro* experiment was conducted with the use of pancreatine-pepsine-chlorohydric method and cecal inoculum for semisynthetic diets. The use of cecal inoculum was compared with the use of *in vivo* method. There was higher digestibility ($P < 0.001$) of dry matter (DM), neutral detergent fiber (NDF) and greater microbial growth using citrus meals diets with bacterial counts of 68.18, 45.17 and 2.56×10^{10} cfu/g for *Medicago sativa*, *Saccharum officinarum* and mulberry, respectively, while no differences in NDF digestibility between these forage sources and the citrus meal were observed. High correlation coefficient ($R^2 = 0.71$; $P < 0.05$) between *in vitro* and *in vivo* NDF digestibility was obtained with the use of cecal rabbit inoculum and mulberry as the substrate. The NDF and ADF digestibilities increased in animals fed with 10 and 20% of dolicho forage meal. The NDF digestibility improved ($P < 0.01$) by 15.21 and 13.29% in comparison with the control at 10 and 20% inclusion, respectively. It can be concluded that the citrus meal has higher nutritional quality than mulberry and sugarcane meal, and that the use of dolicho and mucuna forages at moderate level improves digestibility of diet fiber fractions.

FEED ADDITIVES AS THEY AFFECT THE FATTENING PERFORMANCE OF RABBITS

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This work compared the separate effect of probiotic, prebiotic, organic acids and herbal extract dietary supplementation on growth performance. Rabbits were fed a diet without additives in the Control group (C). The same diet was supplemented with 1000 mg/kg of probiotic bacteria of *B. subtilis* and *B. licheniformis* (B group); 0.3% prebiotic inulin (I group); 0.3% organic acids (O group); or 0.3% tannin (T group). Each diet was antibiotic free, but supplemented with anticoccidial robenidine. New Zealand White rabbits aged 35 d were weighed, weaned and assigned to one of the five dietary groups. A fattening trial was carried out with 150 individually housed rabbits (n=30/group) and 360 housed collectively (3 rabbits/cage, n=72/group) and reared up to 63 d of age. Feed intake was not affected by the diets. The 63-d live weight tended to be higher (P=0.081) both in the I and T groups (2043 and 2051 g), intermediate in the C and B groups (1990 and 1996 g) and lower in the O group (1964 g). The additives exerted a significant influence on 49-63 d growth rate (P=0.006) that was the best in the I and T groups, moderate in the O and C groups and poorer in the B group. The 35-63 d feed conversion was not affected (3.14, 2.99, 2.94, 3.02, 2.95 in the C, B, I, O, T groups, respectively; P>0.05). Health risk was the lowest in the C group and highest in the O group (33.3, 43.0, 37.9, 49.5, 39.8% in order of C, B, I, O, T groups; P=0.035). In conclusion, both herbal extract (tannin) and prebiotics (inulin) can be useful as natural additives in antibiotic-free rabbit diets because growth rate improved while health status was not adversely affected. The impact of probiotics (*B. subtilis* and *B. licheniformis*) is ambiguous. The supplemental blend of organic acids (formic, acetic, propionic) was not effective since it increased the health risk and reduced 63 d body weight.

EFFECT OF DIETARY PHOSPHORUS REDUCTION AND PHYTASE SUPPLEMENTATION ON GROWTH OF RABBITS

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New Zealand White rabbits were used to study the influence of exogenous phytase enzyme additions at the expense of supplemental inorganic phytate on growth performance. A total of 300 rabbits aged 35 d were weaned and with respect to body weight half were housed individually or in pairs up to 77 d of age. Within each housing system, rabbits were assigned randomly to three dietary groups in a balanced manner according to body weight at weaning. A basal diet with 0.24% added inorganic monocalcium phosphate (MCP) was used in the control group (C). This feed was supplemented with 1000 FTU/kg of *Aspergillus niger* phytase (Natuphos®5000) at the expense of MCP that was reduced by 50% of the control level (i.e. from 0.24 to 0.10%) in the Phytase group (P) or not used (0% MCP) in the Phytase-MCP group (P-MCP). Total phosphorus contents of the diets were 0.58, 0.45 and 0.35%, respectively. The 35-77 d feed intake was 136, 138 and 131 g/d in the C, P and P-MCP groups, respectively (P=0.112). Feed conversion was not affected (3.30, 3.32, 3.27; P=0.592). Differences in 35-77 d weight gain (40.3, 40.2, 39.6 g/d; P=0.556) and 77-d body weight (2706, 2699, 2677 g; P=0.651) were not significant. Health risk was comparable during the fattening period (30, 26, 28%; P>0.05). Supposing lower faecal phosphorus excretions with Phytase or Phytase-MCP feeds than with the control diet (under evaluation), both enzyme supplemented diets can be promising since using them resulted in production similar to that of conventional rabbit feed.

INFLUENCE OF DIETARY PROTEIN REDUCTION AND ENZYME AND/OR AMINO ACID SUPPLEMENTATION ON FATTENING PERFORMANCE OF RABBITS

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The effect of dietary protein reduction combined with protease enzyme and/or addition of synthetic limiting amino acids was studied on growth performance of 240 single-housed (n=48/group) and 240 group-housed (3 rabbits/cage, n=48/group) NZW rabbits fed five diets from 35 to 63 d of age. The control diet (C) with 16.7% crude protein (CP) content was supplemented with 0.4% DL-methionine and 0.1% HCl-lysine and contained 0.34% Met and 0.75% Lys. Dietary CP was reduced to 14.2% (three diets) or 13.1% (one diet). The first 14.2% CP diet was supplemented with 1 g/kg protease (Avizyme®1500) but contained 15% less Met and Lys than the control diet (P group). The second 14.2% CP feed was supplemented with more DL-methionine (0.7%) and HCl-lysine (0.3%) than the control to reach the same essential amino acid (AA) dietary levels but without added enzyme (AA group). The third 14.2% CP diet was supplemented with 1 g/kg enzyme plus more AA's (0.7% and 0.3%) than the control (PAA-I group). Compared to the control feed, CP was lowered to 13.1% but the highest level of synthetic Met (0.8%) and Lys (0.4%) plus 1 g/kg enzyme was used in the 13.1% CP diet (PAA-II group). Diets were formulated to have similar DE, EE and CF contents. Growth rates between 35 and 49 d and 49 d body weights were higher (P=0.007) in the C and PAA-I groups (39.0, 1540 and 38.2 g/d, 1528 g), intermediate in PAA-II (37.4 g/d, 1518 g) and lower in the P and AA groups (34.9, 1482 and 34.5 g/d, 1477 g). Between 49 and 63 d, weight gain did not differ but over the whole fattening period it was better (P=0.033) in the PAA-I and C, moderate in PAA-II and poorer in the P and AA groups. Between 35 and 63 d, differences on feed intake and feed conversion among groups were not significant. The 63 d body weight tended to be higher (P=0.063) in the PAA-I, C and PAA-II groups (1995, 1993, 1991 g), intermediate in AA (1933 g) and lower in the P group (1903 g). Health risk was not significantly affected (47.8, 45.3, 52.2, 46.7, 41.6% in order of the C, P, AA, PAA-I, PAA-II groups; P>0.05). In conclusion, reducing the dietary CP content to 14.2% combined with either added protease enzyme or amino acids was not effective because growth rate was significantly decreased. Growth performance was maintained without any increase of health risk even with a 20% lowering of CP from 16.7% to 13.1% if the diet was supplemented with protease plus higher doses of essential amino acids.

IN VITRO CAECAL GAS PRODUCTION AND DRY MATTER DEGRADABILITY OF SOME BROWSE LEAVES IN PRESENCE OF ENZYMES FROM ANAEROBIC BACTERIUM IN NZW RABBITS

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This work was conducted to assess the nutritive value of four browse leaves (*Acacia saligna*, *Atriplex halimus*, *Lucena leucocephala*, and *Eucalyptus camaldulensis*) grown in the northern region of Egypt using the caecum content obtained directly after slaughtering of 12 New Zealand White rabbits. ZADO[®], enzymic preparation containing cellulases, xylanases, α -amylase and proteases from an anaerobic bacterium (patent No. 22155 of Egypt) was added at 0, 2 or 5 mg/ml of buffered caecal content. *In vitro* caecal gas production after 24 h of incubations (IVGP₂₄) and dry matter degradability (IVDMD) were determined. Crude protein content of browse leaves ranged from 105 g/kg DM (*A. halimus*) to 221 g/kg DM (*L. leucocephala*). The highest ether extract (68 g/kg) was in *L. leucocephala*, while this species had the lowest neutral detergent fibre (NDF), acid detergent fibre (ADF), and acid detergent lignin content. *E. camaldulensis* had the highest NDF, ADF and lignin. Total extractable phenolics (TEP), saponins (SAP) and alkaloids (ALK) contents were low in *L. leucocephala* and high in *A. halimus* and *E. camaldulensis*. The IVGP₂₄, and IVDMD varied ($P < 0.001$) among leaves, with the highest ($P < 0.001$) value in *A. halimus*, intermediate in *A. saligna* and *E. camaldulensis*, and the lowest ($P < 0.001$) in *L. leucocephala*. When comparing all leaves species, addition of ZADO[®] improved ($P < 0.05$) caecal IVGP₂₄, and DMD in all leaves without a significant difference ($P > 0.05$) between the two ZADO[®] levels (2 or 5 mg ZADO[®]/ml). ZADO[®] increased the rate of gas production during the first 6 h of incubations. Our results suggest a positive effect of ZADO[®] addition on browse leaves degradation in rabbits. ZADO[®] could have a positive influence on the caecal microbial activity and nutrient digestion, as well as the degradation of secondary compounds of the browse leaves.

STUDY OF THE EFFECT OF ROSEMARY (*ROSMARINUS OFFICINALIS*) AND GARLIC (*ALLIUM SATIVUM*) ESSENTIAL OILS ON THE PERFORMANCE OF RABBIT

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This study aimed to evaluate the efficiency of rosemary or garlic essential oils or their combination on the daily weight gain, feed intake and feed conversion ratio of young grower rabbits weaned at 23 days of age. Two hundred Pannon White rabbits were used and divided into 4 groups: 1 control and 3 treated groups. Control rabbits were fed standard medication-free growers diet supplemented with 1% sunflower oil containing or not the essential oils. Feeds of treatment groups were supplied with 0.025% garlic essential oil (group 2), with 0.15% rosemary essential oil (group 3), and combination of 0.025% garlic essential oil+0.15% rosemary essential oil (group 4), respectively. Essential oils were blended in 1% sunflower oil to ensure their homogeneity in feed. Mortality was recorded all over the experimental period. Average body weight and feed intake were measured at 3, 5, 7, 9, 11 weeks of age and used to calculate daily weight gain and feed conversion ratio. Mortality was very high between the 5th and 7th weeks of age in all groups (Group 1: 32%, Group 2: 30%, Group 3: 24%, Group 4: 38%). Rosemary oil supplementation showed some beneficial, but not statically significant, effects, as death rate was the lowest in Group 3. Combined essential oils supplementation had aggravating effect on digestive disorders as compared to the 2 single essential oils supplementation. Feed intake was only slightly and not significantly modified by essential oils. Average body weight and daily weight gain were also similar among groups. The lowest daily weight gain was found in the control group (35.9 g/day), while group 2, 3 and 4 showed somewhat higher gain (36.1, 37.8 and 37.1 g/day, respectively), but differences were not significant. Feed conversion ratio (FCR) was modified in the first four weeks of the growing period. Each treatment group (3.95, 3.93, 3.65 kg/kg, respectively) showed reduced FCR compared to the control (4.10 kg/kg). The effect was significant only in the combined treatment and between 5 and 7 weeks of age, which coincided with digestive problems. Altogether, no beneficial effect on the studied performance traits was found due to the essential oils supplementation. Digestive disorders of early weaned rabbits were not prevented by the garlic and rosemary essential oils, moreover severity of diarrhoea was increased when these essential oils were combined.

IN VITRO GAS PRODUCTION OF DIETS WITH INCLUSION OF SEAWEED (*LITHOTHAMNIUM* SP.) FLOUR FOR WHITE NEW ZEALAND RABBITS

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Thirty White New Zealand rabbits were fed a standard fattening diet with the following composition: crude protein 16.5%, ADF 17.5%, and digestible energy 2568 kcal/kg. This diet was fed *ad libitum* since weaning. The reference diet was used as a control for comparison of diets with increasing levels (0.25, 0.50, 0.75 and 1.0%) of seaweed (*Lithothamnium* sp.) flour. The seaweed flour replaced an equivalent amount of inert aluminium veterinary phyllosilicate in the reference diet. The aim of the present work was to investigate the relationship between increasing levels of seaweed flour, degradability of dry and organic matter, and fermentation gas production using the caecal content from rabbits as a source of inoculum. The completely randomized design was used with five treatments and six repetitions. The means were compared by Scott-Knott test at a probability level of 5%. Caecal inocula from rabbits fed with higher level of seaweed flour produced more gas. This result shows that seaweed flour could influence caecal microbiota, but this was not confirmed by degradability of dietary organic matter and dry matter.

IMPROVING THE NUTRITIVE VALUE OF LUPIN SEED FOR GROWING RABBITS: α -GALACTOSIDASE ENZYMES VS. WASHING

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To improve the nutritive value of lupinus seed for growing rabbits, four diets containing 30% of lupin (*Lupinus albus*) and similar levels of NDF and CP were formulated. In diet TS, lupin was not supplemented with enzymes or washed. Diets TE₁ and TE₂ were supplemented with 0.1 and 0.25% of a α -galactosidase preparation (500 GALU/g), respectively. Diet TM contained lupins which had been previously washed during 5 days (tenfold volume of water, stirred frequently, and changed daily), then dried. Each diet was fed *ad libitum* to 12 rabbits from 27 to 63 days of age. Intake was measured three times a week, live weight weekly. Caecotrophs were collected at the ninth day of the trial, faeces during the fourth week, and the caecal contents after the rabbits were slaughtered. Caecotrophs were tested for DM and fibre-degrading activity, caecal contents for DM, fibre-degrading activity, pH, and VFA. Although feed intake was lower ($P < 0.05$) with diet TM (130.1 g/d) than with diets TS (141.3 g/d), TE₁ (141.0 g/d) and TE₂ (137.7 g/d), both the ADG (about 43 g/d) and the FCR (about 3.2) were not statistically different across all four treatments. Diet TM had generally higher digestibility of nutrients than the other three diets: higher digestibility of DM, OM, energy (about 59% vs. about 56% in the other three), NDF (27.7 vs. 23.6, 20.4 and 20.2% for TS, TE₁ and TE₂ respectively) and hemicelluloses (estimated as NDF-ADF) (41.3 vs. 31.6, 29.1 and 26.4% for TS, TE₁ and TE₂, respectively). CP digestibility (about 80%) did not vary across diets. On the other hand, both the fibre-degrading activities of the caecotrophs and caecal contents and the VFA levels of the latter were higher ($P < 0.05$) in both enzyme-supplemented diets. We can conclude that both α -galactosidase and washing had some statistically significant effects on nutrients digestibility and caecal enzyme activities, however neither of the two treatments had any important practical effect on the rabbit performance.

EFFECT OF FEED RESTRICTION WITH OR WITHOUT FREE ACCESS TO DRINKING WATER ON PERFORMANCE OF GROWING RABBITS IN HEALTHY OR EPIZOOTIC RABBIT ENTEROPATHY CONDITIONS

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The aim of the present study was to check the interest of forcing the water to feed ratio at 1.7 when rationing rabbits in good sanitary conditions (without experimental reproduction of Epizootic Rabbit Enteropathy) and in Epizootic Rabbit Enteropathy conditions. Three hundred thirty six rabbits, housed in cages each containing 8 rabbits, were divided into 7 groups controlled from weaning at 32 days of age to 67 days. In the control group, rabbits were given feed and water *ad libitum*. In the second group, feed was restricted, but not water (water/feed ratio >3), and in the last group, feed and water were restricted (water/feed ratio=1.7). In good sanitary conditions, the slaughtering weight was higher for the control group as the compensatory growth was not sufficient to compensate the body weight difference due to restrictions. There was no difference between the two ways of restriction during the fattening period. Except for the 1st week (adaptation to restriction) the feed conversion ratio was improved for groups restricted in feed and/or water. Quantitative feed restriction is interesting in Epizootic Rabbit Enteropathy conditions as it leads to less mortality and morbidity compared to the control group (*ad libitum*). There was no significant effect of the type of quantitative feed restriction (with or without water restriction) on mortality, but feed restriction with water *ad libitum* tended to be more efficient to reduce mortality (from 29.2 to 16.7). Moreover, this restriction did not generate a difference in slaughtering weight compared to the control group and produced more meat (+18%).

EFFECT OF YEAST β -GLUCANS ON RABBIT PERFORMANCES AND MORTALITY FROM 35 TO 63 DAYS OF AGE

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The aim of this work was to study the effect of increasing levels of β -glucans (Fibosel®) in rabbit feed by measuring rabbit performances and mortality from 35 to 63 days of age. Six experimental diets were formulated with increasing levels of β -glucans (0, 50, 100, 150, 200 and 250 ppm of Fibosel®). The trial was performed at the Fattening Rabbit Units from the Nutreco Poultry and Rabbit Research Center facilities. To avoid any disturbance or interference due to mortality on rabbit performance analyses, the effect of increasing levels of β -glucans on rabbit performances was studied in individually housed rabbits, while their effect on mortality was studied in collectively housed rabbits. On individually caged rabbits 7, 5, 5, 4, 4 and 4 rabbits died, respectively, for the control diet and diets containing 50, 100, 150, 200 and 250 ppm of Fibosel®. Performances of individual housed rabbits showed that animals fed diets containing 100 and 150 ppm of Fibosel® reached 9.4, 13.5 and 14.3% higher body weight at 63 days, weight gain and feed intake, as average, respectively, than rabbits fed the control diet. In the group-housed rabbits, a significant effect of Fibosel® on body weight and weight gain was also detected. In this group of animals, rabbits fed diets containing 50 and 150 ppm of Fibosel® showed a 5.5 and 9.8% higher body weight and weight gain, as average, respectively, than rabbits fed the control diet, while rabbits fed the diet of 100 ppm of Fibosel® showed an intermediate value of both parameters among these diet. Although significant differences were not detected, lower mortality rate was obtained in rabbits fed diet-containing 150 ppm of Fibosel® than rabbits fed the control diet (11 vs. 20 dead rabbits, respectively). Therefore, the use of Fibosel® at 150ppm in rabbit diets could be recommended as it improved rabbit growth performances and seems to decrease mortality rates. Further research with Fibosel® will be carried out to corroborate these findings.

NUTRITIVE VALUE OF CARROT (WHOLE PLANT), DRIED AT LOW TEMPERATURE, FOR THE GROWING RABBIT

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The nutritive value of carrot (whole plant) dried at low temperature (35°C max) was studied in the growing rabbit, by comparing 4 diets (regression method) containing an increasing incorporation rate of carrot: 10-20-30% in substitution to the control diet (0%). The faecal digestibility of the diets were measured between 42 and 46 days of age on four groups of 12 young rabbits fed *ad libitum* since weaning (35 d). The ash level of the carrot, analysed here, was high (21%), probably originating from soil residues on the root or top of the plant. The crude protein level reached almost 13%, while ADF was almost 14%. This data classed the whole carrot as a balanced source of protein and fibres. The digestible energy (DE) concentration of the carrot was estimated to 2160 kcal/kg (± 82.7 kcal, as fed basis) for an incorporation rate up to 20%. The mean digestibility of crude protein was estimated to 66.5%, corresponding to a digestible crude protein concentration of 64 g/kg (± 2 g, as fed basis). Even at a high incorporation rate in the feed (30%) carrot did not produce adverse effects on the animal growth (mean 39.0 g/d), intake (mean 155 g/d) or health. However, further experiment is necessary to compare growth performances on more numerous rabbits fed with balanced diets containing an increasing level of whole carrot.

SUPPLEMENTATION OF RABBIT DIET WITH CHESTNUT WOOD EXTRACT: EFFECT ON *IN VITRO* GAS PRODUCTION FROM THREE SOURCES OF CARBOHYDRATES

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In vitro gas production kinetics of barley grain (BG), sugar beet pulp (SBP) and wheat bran (WB) were determined using three different inocula prepared from caecum contents of 78 day old rabbits. The first inoculum was prepared from caecum contents of rabbits fed diet supplemented with 0.5% of chestnut wood extract (CWE, 75% of tannins) in the form of powder (POWD), the second one from caecum contents of rabbits which diet were supplemented with 0.6% of coated CWE (COAT), while the third one from caecum contents of rabbits not supplemented with CWE (CONT). The cumulated gas productions over 60 h of incubation were modelled with Gompertz model. The total potential gas productions (parameter “B” of the Gompertz model) were higher when BG and SBP were incubated in CONT (300 and 288 ml for BG and SBP, respectively) than in COAT (270 and 264 ml for BG and SBP, respectively) and POWD (278 and 264 ml for BG and SBP, respectively). For WB the highest parameter “B” was obtained when incubated in POWD; however, the differences between inocula were not statistically significant ($P>0.05$). Maximum fermentation rates (MFR) of BG (21.1 ml/h) and WB (9.7 ml/h) incubated in CONT were greater than those incubated in POWD (16.3 and 8.7 ml/h for BG and WB) or COAT (18.5 and 8.1 ml/h for BG and WB). However, for SBP the greatest MFR was obtained when incubated in POWD (15.1 ml/h), followed by CONT (13.3 ml/h) and COAT (11.8 ml/h). The times of maximum fermentation rate (TMFR) were generally shorter for substrates incubated in POWD (12.6, 7.4 and 8.4 h for BG, SBP and WB, respectively) than those incubated in CONT and COAT (12.9 and 12.9 h for BG, 9.5 and 9.4 ml for SBP and 9.5 and 8.4 ml for WB, respectively). In the first 8 h of fermentation (Gas8), higher ($P<0.05$) amounts of gas were produced from substrates incubated in POWD (106, 60 and 35 ml for SBP, WB and BG, respectively) than in CONT (88, 46 and 23 ml for SBP, WB and BG, respectively) and COAT (78, 48 and 23 ml for SBP, WB and BG, respectively). In conclusion, the use of CWE as powder increases the microbial activity of caecal microorganisms and that the effect is greater with pectinolytic than with amylolytic or hemicellulolytic microbes and/or enzymes. The similar TMFRs and Gas8s of substrates incubated in COAT and CONT suggest that the process of coating was not very efficient as the CWE was not released at the beginning (in first hours) of incubation in the rabbit caecum contents.

SUPPLEMENTATION OF RABBIT DIET WITH CHESTNUT WOOD EXTRACT: EFFECT ON *IN VITRO* GAS PRODUCTION FROM TWO SOURCES OF PROTEIN

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In vitro gas production kinetics of soybean meal (SBM) and sunflower meal (SFM) were determined using three different inocula prepared from caecum contents of 78-day old rabbits of Slovenian meat line SIKA. The first inoculum was prepared from caecum contents of rabbits fed diet supplemented with 0.5% of chestnut wood extract (CWE) in the form of powder (POWD), the second one from caecum contents of rabbits fed diet supplemented with 0.6% of CWE coated with plant oil (COAT), while the third inoculum was prepared from caecum contents of rabbits not supplemented with CWE (CONT). Gas productions were measured over 60 h of incubation and obtained gas volumes were modelled with Gompertz model. The total potential gas productions (parameter "B" of the Gompertz model) were higher ($P < 0.05$) when SBM and SFM were incubated in POWD (147 and 108 ml/g DM for SBM and SFM) than in CONT (137 and 97 ml/g DM for SBM and SFM). The highest parameter B was obtained for SBM when COAT was used (170 ml/g DM), while for SFM the highest parameter B was obtained when POWD (108 ml/g DM) was used. When both substrates were incubated in POWD they had shorter time of maximum fermentation rate (TMFR: 10.0 and 7.4 h for SBM and SFM) and higher maximum fermentation rate (MFR: 4.73 and 6.06 ml/h for SBM and SFM) than when incubated in CONT (TMFRs of 16.0 and 12.0 h for SBM and SFM, respectively, and MFRs of 3.58 and 4.07 ml/h for SBM and SFM, respectively) or COAT (TMFRs of 18.4 and 12.2 h and MFRs of 2.96 and 4.39 ml/h for SBM and SFM, respectively). In the first 8 h of fermentation, higher ($P < 0.05$) amounts of gas were produced from substrates incubated in POWD (44 ml for both SBM and SFM) than in CONT (24 and 20 ml for SBM and SFM, respectively) and COAT (34 and 27 ml for SBM and SFM, respectively). These results suggest that CWE fed as the powder reaches the caecum increasing the caecal microbial fermentation, while CWE coated with plant oils (COAT) decreases the activity of CWE in the caecum, especially in the first 8 hours of fermentation. The caecal microorganisms are assumed to degrade the coating around CWE only when the incubation in the caecum is long enough, allowing to CWE to increase the fermentation. This effect was more evident in the fermentation of SBM, suggesting that the crude protein (CP) content of substrates (515 vs. 388 g/kg DM for SBM and SFM, respectively) could affect the kinetics of *in vitro* fermentation.

LIVE YEAST STABILITY IN THE DIGESTIVE TRACT OF THE RABBIT: RELATIONSHIP WITH DIGESTION, GROWTH AND DIGESTIVE HEALTH

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Live yeast *Saccharomyces Cerevisiae* NCYC Sc 47 (Biosaf®) was added to the diet of weaning rabbits to evaluate the effects on growth, feed efficiency, health status, digestibility and caecal parameters. Two Biosaf® levels (1 and 10 g Biosaf®/kg feed, group C1 and C10 corresponding to 10⁶ and 10⁷ CFU/g of DM in diet) were compared to a control group (C0) without yeast addition. Thirty rabbits (3 groups of 10) were used to measure the digestibility and caecal parameters and to calculate the yeast survival rate in the digestive tract. Growth performances and health status were studied on 120 rabbits (3 groups of 40 cages individually). In diet, live yeast concentration fell slightly (-0.2 to 0.5 log CFU/g DM) after pelleting, although the pelleting temperature was high (70-80°C). The survival rate of yeast in digestive tract was high and increased from 90 to 97% with yeast supplementation, but this did not affect the faecal digestibility or the caecal pH. However, redox potential (Eh) of the caecal content increased significantly with yeast addition. Mortality was 50% lower with the highest yeast addition (C10) compared to C0 and C1 groups, while growth, intake or feed efficiency remained similar.

EFFECT OF DIFFERENT WEANING AGE (DAYS 21, 28 AND 35) ON CAECAL MICROFLORA AND FERMENTATION IN RABBITS

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One-day-old Pannon White rabbits of average birth weight were distributed into litters of eight, and these litters were randomly divided into three groups (21-22 litters/group) according to the weaning age: 21 (G21), 28 (G28) and 35 (G35) days. Milk consumption, feed intake and body weight were measured weekly. On day 14, 22, 29, 36 and 42 subsequent to birth 6 healthy animals from each group were examined for some digestive physiological parameters: pH and volatile fatty acid content (VFA) of the caecal content, and composition of the caecal microflora. There was no significant difference between groups in body weight till the 28th day of age. At day 35 and 42 d, G35 rabbits had a significantly higher body weight compared to rabbits of the other two groups. Milk consumption increased from day 21 to day 28. Thereafter G35 rabbits had lower milk consumption, presumably due to the decrease in the mothers milk production and the increase in the solid feed consumption. There was a sudden increase in the solid feed consumption after weaning. In group G35 the feed intake was higher already on day 28, related to the decreasing milk production of the doe. Anaerobic bacteria growing on the Schaedler agar were in a high amount (10⁸/g) already on the 14th day of age. On day 42 their number was significantly higher in G35 and G28 than in G21 rabbits. Counts of coliforms and bacteria growing in the presence of air were in accordance with the literature and decreased with age and decreasing milk intake. Concentration of total VFA was significantly higher in G21 than in G28 and G35. The percentage of acetic acid (C2) was above 70% after weaning in G21. In the other two groups rabbits consumed more milk and less solid feed, so the concentration of C2 was significantly lower. Because of the early solid feed consumption of G21, the concentration of propionic acid (C3) was significantly lower, while that of butyric acid (C4) was significantly higher on day 29 than in G28. The highest C4 content was found in G21 during the whole experimental period. In G35 milk consumption and the simultaneous increasing solid feed intake resulted in better growth. The composition of the caecal microflora was mainly influenced by age, effect of weaning was less pronounced. On the other hand milk and/or feed consumption, i.e. age of weaning, had major effect on the VFA composition. In summary, early weaning (age 21 d) did not result in detrimental changes in the digestive physiological parameters examined, but resulted in lower production in rabbits.

EFFECT OF ESSENTIAL UNSATURATED FATTY ACIDS IN FISH OIL ON LITTERS AND COMPOSITION OF MILK OF RABBIT DOES

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The aim of the study was to determine the degree to which a 3% fish oil supplementation in female rabbit diets affected the quantitative composition of fatty acids in milk, litter size, litter weight, and birth-to-weaning mortality of young rabbits in three successive litters. Twenty New Zealand White female rabbits of the foundation stock and all their offspring were investigated. Animals were assigned to two feeding groups: group I, fed a pelleted basal diet with standard components; group II, fed a pelleted diet with 3% fish oil. The fish oil was a byproduct of fish (herring, sprat, silver carp, mackerel, cod) meal production and contained 2.9% of C18:3n-3, 7.4% of C20:5n-3 (EPA), 12.6% of C22:6n-3 (DHA) and 0.8% of C22:5n-3 (DPA). Fat milk increased from 18.4 to 22.2% in female rabbits receiving 3% fish oil compared to the control group. In the milk fat of female rabbits, there were highly significant differences between the control and experimental groups in the level of polyunsaturated fatty acids of the n-3 series (n-3 PUFA) and saturated fatty acids (SFA). The fish oil supplementation reduced the level of short-chain fatty acids in milk (C8:0-C10) and increased the level of long-chain fatty acids (C16:1–C22:6). Milk was characterized by a higher concentration of EPA and DHA. Fat supplemented diets increased kit weight at birth, 21 days and 35 days of age ($P < 0.01$) in the three reproductive cycles. In the experimental group, there was a higher percentage of reared rabbits in all the litters analysed. The high mortality of young rabbits among primiparous rabbits was due to relatively low milk production, which increased with parity. The bacteriological tests of the rabbits with diarrhoea symptoms showed that the main causes of mortality were the proliferation of conditionally pathogenic *Escherichia coli*, responsible for enteritis and the presence of *Streptococci*, which are relatively or conditionally pathogenic bacteria. There were no significant differences between the milk production of female rabbits from groups I and II, which shows that the fish oil supplementation had no effect on this parameter. Milk yield was significantly affected by litter size. For this reason, the lower mortality of young rabbits may result from the higher fat content of milk rather than from the higher milk yield.

EFFECTS OF MOLASSES INCORPORATION IN RABBIT FATTENING DIET ON GROWTH PERFORMANCES

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The effect of molasses incorporation in rabbit fattening diet on growth performances was studied on 48 local rabbits, initially 35-37 days old. They were distributed at random among two groups (experimental and control) at the rate of 24 young rabbits per group. The experimental group received a pellet diet which contained 5% of molasses (MPD) and the second group received a control pellet diet without molasses (CPD). Diets contained about 18% crude protein and 11.5% crude fibre on dry matter basis. The animals were weighed once a week during 8 weeks, corresponding to the fattening period. During the trial, the mortalities of the young rabbits were recorded but were not different between treatments: 4/24 and 5/24 for the MPD and the CPD diets respectively. At the start of this trial, no significant difference was recorded between the young rabbit weight of the experimental group (454 g) and that of the control group (431 g). At the end of the trial, the average live weight of the young rabbits feed with the MPD was still similar (1718 g) to that of the control group (1693 g). The feed conversion ratio during the trial period was 3.59:1 g DM/g LWG for the experimental group and 3.39:1 g DM/g LWG for the control group. In conclusion, the incorporation of molasses at 5% in the rabbits diet did not affect average daily gain, feed conversion ratio, feed consumption or mortality rate of the young rabbits. The opportunity of the introduction of molasses in rabbit diets depends mainly of their relative price compared to that of other ingredients.

**EFFECT OF INCLUSION OF PROBIOTICS ON MICRO-ORGANISMS
CONTENT, HEALTH AND PERFORMANCE OF FATTENING RABBITS:
1. STUDY IN A COMMERCIAL FARM WITH
INTERMEDIATE HEALTH STATUS**

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The objective of the present trial was to determine the effect of a thermostable probiotic containing *Bacillus licheniformis* and *B. subtilis* on health and production parameters of fattening rabbits from weaning until slaughter. In a rabbitry with average post-weaning mortality of 5-9%, 1680 rabbits in 2 treatment groups (4 weaning batches, 105 cages per treatment, each cage of 8 rabbits) were fed with: a) a basic feeder; b) the same basic feed supplemented with probiotic from the 4th day postweaning (41th days of age) up to 88th day of age. The rabbits were slaughtered on an average of 93 days. Clinical signs, microbiological status (enumeration of *E. coli* and *C. perfringens* and presence of *P. multocida*) and growth performance were recorded for two distinct fattening periods, growing and finishing. A significant decrease in mortality of probiotic-treated rabbits when compared to the control ones was observed during growing and entire fattening periods. Within these periods, *E. coli* and *C. perfringens* – but not *P. multocida* – were isolated at a lower frequency from probiotic treated rabbits ($P < 0.05$). Compared to the control animals, probiotic-treated rabbits were 54 g and 123 g heavier at the end of growing and finishing phases, respectively, and had significantly higher average daily gain and better feed conversion ratio ($P < 0.05$).

FIELD BEANS AND BREWER'S GRAINS AS PROTEIN SOURCE FOR GROWING RABBITS IN ALGERIA: FIRST RESULTS ON GROWTH AND CARCASS QUALITY

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Three isonitrogenous (crude protein: 16.9% as fed) and isofibrous diets (crude fibre: 9.7%) were manufactured in Algeria: one control diet containing 10% soya meal (SM10diet) and two experimental diets based on 30% field beans, supplied with *dl*-methionin (FB30 diet) or 30% brewer's grains (BG30 diet), as main and alternative protein source, in complement to dehydrated alfalfa and wheat bran. Diets were distributed *ad libitum* to 3×17 commercial growing rabbits from weaning (35 days, 565 g) until 84 days of age (slaughter) in order to study growth and slaughter performances. All rabbits were caged individually. The protein source had a significant effect on average growth rate, which was significantly higher for SM10 and FB30 than for BG30 animals (31.5 on average vs. 27.1 g/d), but not on feed conversion ratio (3.2 on average). The slaughter weight at 84 days of age was correlated with the protein source: the consumption of SM10 and FB30 diets permitted rabbits to reach a higher slaughter live weight (2080 g on average) than the BG30 group (1877 g). Nevertheless, the dressing percentage (67.4% for the cold carcass on average) and the muscle/bone ratio (6.3) were similar for the three groups of rabbits. In conclusion, brewer's grains, and especially field beans, seem to be suitable alternative source of proteins (in total substitution to soya meal) in Algerian conditions of production of balanced diets for growing rabbits.

VERY LOW PROTEIN, AMINOACID-SUPPLIED DIET FOR HEAVY BROILER RABBITS: EFFECTS ON NITROGEN METABOLISM, AND DIGITAL EVALUATION OF EXCRETA AND PRODUCTS

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A very low protein diet (A, 15.9% DM) for the fattening of heavy broiler rabbits was supplied by the four available synthetic amino acid and compared to a control feed (C, 19.7% DM) in a study carried in metabolism cages with 12 (6+6) rabbits controlled at 81-84 days, then slaughtered at 87 days. The diets were anisoproteic, but isoenergetic and isofibrous. The low protein supply produced strong reduction in Blood Urea (-32%) and in Urea Urine (-37%) thus in the N-urinary emission (-29%; P<0.12). In the gastro-intestinal trait, however, N-feed was on average less utilized by the A group and refused (N faeces +20%; P<0.2). This findings appear to be biased because an exceptionally late growth in the Control group (50 vs. 40 g/d) provided by a push in glucose, unreal with respect to the growth trial (30.1 vs. 36.8 g/d) corroborated by favorable environmental conditions, resulting in a 29% higher N-retention for C a value clearly and strongly overestimated. Digital evaluation of fresh urine and faeces were able to discriminate the groups, while less or nothing the live, or the carcass or meat.

JOINT VIS-NIRS EVALUATION OF FEEDS AND DRIED FECES TO ESTIMATE INGESTION AND DIGESTIBILITY IN GROWING RABBITS

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A group of 51 feed samples taken from 12 experimental diets and of 66 dried grouped feces belonging to four nutrition experiments were submitted to a Uv-VIS-NIRS scan (350-2500 nm) in order to calibrate the chemical composition and nutritional parameters, that is namely the ingestion aptitude and digestibility. A new chemometric system has made it possible to contemporary use the spectrum of the diet together with the spectra of the relative pool of dried feces. The daily measured ingestion, in absolute as the intake and in relative as the palatability per unit of metabolic weight, obtained a good resolution for the spectra of the feeds ($R^2_{cv}=0.80$ and 0.75 , respectively), for the feces (0.81 and 0.80) and for the joint evaluation (0.87 and 0.81). The intake was positively correlated to the mineral, insoluble ash, protein, gross energy, crude fiber and acid detergent fiber (ADF) in the feeds, and negatively correlated to N-free extract, lignocellulose and to all the digestibility coefficients except crude fiber. Very significant improvements, on the average of some 0.20 absolute R^2 points, corresponding to 87% as relative, were also provided to the digestibility coefficients by the joint method; in decreasing order: neutral detergent fiber ($R^2_{cv}=0.00$; 0.18 and 0.50 , respectively, for the feeds, feces and joint: $+41\%$ vs. the average of feeds and feces), ADF (0.00 ; 0.45 and 0.62 : $+39\%$), ether extract (0.53 ; 0.52 ; and 0.86 : $+34\%$), crude protein (0.53 ; 0.53 and 0.75 : $+22\%$), gross energy (0.61 ; 0.74 and 0.83 : $+15\%$), crude fiber (0.61 ; 0.82 and 0.82 : $+11\%$), dry matter (0.60 ; 0.79 and 0.76 : $+7\%$), organic matter (0.65 ; 0.80 and 0.79 : $+6\%$) and the N-free extract (0.86 ; 0.82 and 0.87 : $+3\%$). The results corroborate previous knowledge and show the possibilities of using NIRS fecal profiling in rabbit nutrition, which joined together with NIRS of the feeds, could optimize nitrogen monitoring.

EFFECT OF RESTRICTED FEEDING AFTER WEANING ON THE PRODUCTIVE AND CARCASS TRAITS OF GROWING RABBITS

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The aim of the experiment was to study the influence of feeding medicated or non-medicated diets followed by a feed restriction after weaning on the productive and carcass traits of growing rabbits. Half of the rabbits was fed non-medicated (NOMED: 10.65 DE/kg, CP: 16.9%, CF: 17.3%; n=106) while the other half was fed medicated diet (MED: the same diet was supplemented with 500 mg/kg Oxitetracycline and 50 mg/kg Tiamuline; n=108) between 4 and 9 weeks of age, then they consumed non-medicated diet. Within both diet groups half of the rabbits was fed *ad libitum* (ADLIB) while the other half restricted (RESTR). In the latter group rabbits were allowed to consume for 9, 10, 12 or 14 hours between 4-5, 6-7, 7-8 or 8-9 weeks of age, respectively, then they were fed *ad libitum*. The feed intake was 26.7, 18.3 (P<0.001) and 5.3% (P=0.059) lower in the RESTR group at the ages of 4-5, 5-6 and 6-7 weeks, respectively. After 7 weeks of age no difference was found between groups. Body weight gain of RESTR rabbits was 20.9 (P<0.001) and 8.5% (P=0.039) lower at the ages of 4-5 and 5-6 weeks, respectively, while between 6 and 8 weeks of age it slightly increased. Body weight of RESTR rabbits was 9.2% lower at 5 weeks of age compared to ADLIB group (871 vs. 959 g) but this was partly compensated at the end of the experiment (2737 vs. 2799 g, P=0.056). Feed conversion of RESTR group was better compared to ADLIB rabbits: 2.57 vs. 2.64 (P=0.01). No significant difference was found in the mortality (1 vs. 3.6%). Dressing out percentage (59.4 vs. 58.6%, P<0.001) and the proportions of hind part (19.2 vs. 18.8%), hind legs (18.3 vs. 18.0%) and that of the *m. Longissimus dorsi* (7.1 vs. 6.8%) to the body weight were higher in ADLIB rabbits. No difference was found in the proportion of perirenal fat. Production and mortality of MED and NOMED groups were similar however, the feed intake and the body weight gain of MED rabbits decreased during the transition from MED to NOMED diet at 9 weeks of age. Significantly higher dressing out percentage (59.5 vs. 58.5%, P<0.001) and proportion of fore and hind parts of carcass as well as that of the hind legs and *m. Longissimus dorsi* to the body weight were found in MED rabbits.

EFFECT OF ADDING DIETARY CAPRYLIC ACID ON THE BACTERIAL POPULATION IN THE RABBIT CAECUM AND STOMACH

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The effect of dietary supplementation with caprylic acid on the bacterial population of the rabbit caecum and stomach was investigated using two different PCR-based methods: Denaturing Gradient Gel Electrophoresis (DGGE) and Terminal Restriction Fragment Length Polymorphisms (TRFLP). Caprylic acid was added to the diet either in the form of 5 g/kg of the pure acid, or within Akomed R at 10 g/kg (with or without lipase at 10 g/kg). Rabbits fed *ad libitum* on their respective diets for 27 days before being euthanized and the digestive contents of the caecum and stomach removed for DNA analysis.

Neither analytical method suggested a change in the bacterial populations in the stomach dependent on the feeding group of the animal (control, caprylic acid, Akomed R or Akomed R and lipase supplementation). However, the use of DGGE suggested that the caecal samples could be split into two groups; those with no additional fatty acid supplementation of the diet, or where lipase was added along with the fatty acids; and the other where there was caprylic acid added either in its pure form, or in conjunction with other fatty acids in the form of Akomed R supplementation. Furthermore, there was an apparent increase in the detectable biodiversity following supplementation with either caprylic acid or Akomed R. No such segregation between dietary regimes was detected by TRFLP analysis. This is suggestive of there being a shift in relative numbers of specific organisms (DGGE data) without this effect being restricted specifically to one large-scale taxonomic group of organisms (TRFLP data). Thus dietary supplementation with caprylic acid, either in a pure form, or in conjunction with other medium-chain fatty acids, has the ability to target specific groups of microbes, whilst allowing a relatively large-scale bacterial diversity to persist.

PERFORMANCE OF GROWING RABBITS FED *LABLAB PURPUREUS* FORAGE WITH MOLASSES MINI-BLOCKS AND RESTRICTED COMMERCIAL PELLETS

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This study aimed to evaluate the forage legume, *Lablab purpureus*, with or without molasses mini-blocks (MMB) as an energy supplement in Altex, New Zealand White, and crossbred rabbits (n=78) from 13 litters (initial age 49 to 55 d, mean body weight 1,088 g) during a 42-d feeding experiment. Rabbits fed commercial pellets *ad libitum* served as the positive control diet, while rabbits fed 24-h sun-dried lablab leaves *ad libitum* with MMB (without commercial pellets), served as the negative control diet. Diets also included lablab fed *ad libitum* with restricted amount of pellets (25, 50 or 75%) with or without MMB for a total of eight diets. Each diet had three pen replicates, except for positive and negative control groups which had four pen replications. All pens contained three rabbits. Commercial pellets had higher DM, NDF, ADF, and ADL (93.7, 39.5, 18.2, and 6.2%) compared to lablab forage (59.2, 29.8, 18.0, and 4.8%) and MMB (90.9, 20.5, 7.7, and 4.0%). Lablab had higher CP and EE than commercial pellets or MMB (16.7 vs. 16.1 and 8.8%; 4.9 vs. 4.0 and 3.5%). Final weights and daily gains were lowest for negative controls (1643 vs. 2336 to 2539 g and 11.2 vs. 25.2 to 27.5 g/d; P<0.05), but not significantly different among the 7 others diets. Total DM intake was different (P<0.05) for all diets, except for positive controls and the 25% pellets without MMB group. Lablab forage intake was similar (P>0.05) between pens receiving 50 or 75% pellets (77 to 80 g DM/d), whereas pens receiving 25% pellets consumed more forage (91 d DM/d) without influence of the presence of MMB. The highest forage intake was observed for the negative controls (101 g DM/d). DM intake of MMB was lowest (P<0.05) for the 75% pellets diet (19 g DM/d), all other means were higher but statistically similar (24, 22 and 25 g DM/d for 50, 25 or 0% pellets). Feed conversion ratio was poorest for negative controls, whereas the 50 and 75% pellets without MMB diets were intermediate and similar, and all 5 remaining diets had the better mean conversion rates. Final weights closely paralleled results for live pre-slaughter and hot carcass weights. Negative controls clearly had the poorer mean dressing percentage (62.3%; P<0.05). Abdominal fat percentage was the lowest for negative controls (P<0.05) compared to positive controls and 25% pellets with or without MMB and 50% pellets with MMB diets. In conclusion, poor performances were observed for rabbits fed only lablab with MMB. There were no benefits of feeding MMB with lablab forage. In lesser developed countries, the limit feeding (25%) of a concentrate supplement of similar nutritive value (but ideally using local feedstuffs from on-farm forage plots) to commercial pellets may produce more acceptable results.

EFFECT OF *PSOPHOCARPUS SCANDENS* REPLACING PARA GRASS IN THE DIETS ON FEED UTILIZATION, GROWTH RATE AND ECONOMIC RETURN OF GROWING CROSSBRED RABBITS IN THE MEKONG DELTA IN VIETNAM

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One experiment was carried out to evaluate the effects of levels of fresh *Psophocarpus scandens* (PS) replacing fresh para grass (*Brachiaria mutica*) in the diets on feed and nutrient utilization, growth performance and economic return of crossbred rabbits. Rabbits received in addition 15 g/d of concentrate during the experiment. A complete randomized design with five treatments and three replicates was used. Two female rabbits at 60 days of age (799 g on average) were allocated in one experimental unit for 70 days. The treatments were levels of 0, 15, 30, 45 and 60% (DM basis) of *Psophocarpus scandens* replacing para grass in the diets corresponding to the treatments named PS0, PS15, PS30, PS45 and PS60, respectively. The PS protein content was higher than that of PG (23.1 vs. 9.9% DM) and NDF content was lower (41.8 vs. 61.6% DM). The results showed that dry matter intake was significantly reduced by the highest PS proportions ($P < 0.01$) with 93.5, 93.0, 92.7, 85.0 and 84.5 g/d for the PS0 to PS60 diets, respectively. Crude protein intake of rabbits significantly ($P < 0.01$) increased with increasing PS proportion. The daily weight gains of the rabbits were higher in the diets with PS replacement (15.1, 17.3, 18.2, 16.3 and 17.5 g/d for diets PS0 to PS60, respectively). However, only the daily weight gain of the PS30 treatment was significantly higher than that of the PS0 one. The profit was improved with the increased replacement of PS. The nutrient digestibility (dry matter and crude protein) and nitrogen retention of rabbits were also significantly ($P < 0.01$) improved with increasing PS levels in the diets. The conclusion of the study was that using *Psophocarpus scandens* to replace para grass improved nutrient utilization, daily weight gain and profits. Replacing para grass by *Psophocarpus scandens* at a level of 30% could be practiced by farmers.

EFFECT OF WATER SPINACH AND SWEET POTATO VINE ASSOCIATED WITH 2 OTHER NATURAL PLANTS ON GROWTH PERFORMANCE, CARCASS VALUES AND ECONOMIC RETURN OF GROWING CROSSBRED RABBITS IN THE MEKONG DELTA OF VIETNAM

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This study aimed to evaluate the effects of fresh Sweet Potato Vine (*Ipomoea batatas*) and Water Spinach (*Ipomoea aquatica*) associated with Mom grass (*Hymenachne acutigluma*) and Cuc (*Wedelia spp*) in the diets on feed and nutrient utilization, growth performance and economic return of crossbred rabbits. A complete randomized design experiment with six treatments and three replicates was used. Two male rabbits at 60 days of age were allocated in one experimental unit. The treatments were six diets including Water Spinach (WS), Sweet Potato Vine (SP), WS+Mom grass (WS+M), WS+Cuc (WS+C), SP+Mom grass (SP+M) and SP+Cuc (SP+C). The combination of two kinds of forages in the diets was at a ratio of 1:1 (DM basis). In addition 15 g/head of paddy rice were distributed daily to rabbits throughout the 70 days of experiment. Chemical composition of forages was given. The two types of *Ipomoea* were richer in protein (18.1-18.8 %DM) than the two other forages (12.4% DM for *H. acutigluma* and 10.0% for *Wedelia spp*). On the contrary their proportion of fiber was lower. Paddy rice used as concentrate had a protein content of 6.68% DM and NDF of 29.1%. There was no significant difference for intakes of DM (P=0.22) and crude protein (P=0.07) among the diets, but a significant difference (P<0.001) of NDF intake was found. The daily weight gain was significantly higher in the WS diet: values for the 6 diets in the above order were 17.7, 15.8, 15.0, 15.2, 14.2 and 14.4 g/d. The feed conversion ratios (DM basis) were in the same order 3.35, 3.91, 4.34, 4.19, 4.50 and 4.39. Values of feed conversion ratio observed for the two single forage diets (WS and SP) were significantly (P<0.05) lower than those observed for the four other diets involving two forages. However, the higher financial benefits were found for SP+M and SP+C diets, *i.e.* for water spinach associated with one other forage. The carcass values and meat composition were not significantly different (P>0.20) among the treatments. The conclusion of the study was that Water Spinach, Sweet Potato Vine, Mom grass and Cuc could be used for feeding the growing rabbits for an utilization of local available feeds. Water spinach and sweet potato vine associated Mom grass or Cuc at ratio of 1:1 would be economically used and increase economic return.

**EVALUATION OF THE NUTRITIVE POTENTIAL OF CASSAVA
(*MANIHOT ESCULENTA* CRANTZ) PEELS-BLOOD MEAL MIXTURE ON
THE PERFORMANCE CHARACTERISTICS OF FEMALE GROWING
RABBITS IN THE DERIVED SAVANNAH ZONE OF NIGERIA**

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A feeding trail using sixty crossbred 7-9 weeks old female rabbits was conducted to investigate the effect of pre-treatment methods as well as the inclusion levels of sun dried cassava peel/blood meal mixture (3:2) on their performance characteristics. Three types of cassava peels treatment were compared. Ten experimental diets were formulated with diet 1 serving as the control without cassava peel/blood meal mixture. Diets 2, 3, and 4 had 10, 20 and 30% inclusion of ash treated cassava peels/blood meal mixture. Diets 5, 6 and 7 had 10, 20 and 30% parboiled cassava peel/blood meal mixture while diets 8, 9 and 10 had 10, 20 and 30% simply sun dried cassava peels/blood meal mixture. The rabbits were divided into 10 treatments (after weight balancing) groups of six rabbits each and assigned randomly to the ten diets with each rabbit serving as a replicate in a randomized complete block design experiment. The average initial weights were 610, 611 and 613 g for the control, ash treated, parboiled and sun dried cassava/blood meal diets as well as the 10, 20 and 30% inclusion levels. Rabbits received feed and water *ad libitum* during the 12 weeks of the experiment. The pre-treatment methods of cassava peels had no significant ($P>0.05$) effect on the average daily gain which was 8.94 g/d for the control, 8.13 g/d with ash treatment, 8.48 g/d for parboiled treatment and 8.92 g/d sun dried cassava peels. The rate of inclusion also had no significant ($P>0.05$) effect on the average daily gain which were 8.94, 8.92, 8.53 and 8.40 g for the control, 10, 20 and 30% levels of inclusion. The pre-treatment methods as well as the rate of inclusion did not significantly ($P>0.05$) affect the feed to gain ratio which were 6.16, 7.04, 7.27 and 7.15 (pre-treatments) for the control, ash treated and parboiled respectively and 6.16, and 7.45, 6.99 and 6.49 (rate of inclusion) for 10, 20 and 30% respectively. In conclusion, growing rabbits can tolerate up to 30% of either treated, parboiled or sun dried cassava peels/blood meal mixture (3:2) without negative effect on performance.

USE OF SIMPLIFIED DIET WITH CASSAVA BY-PRODUCTS FOR RABBITS

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The performance and the carcass quantitative characteristics of 144 New Zealand White rabbits were evaluated from weaning to slaughtering (31 to 70 d of age) whose does were fed with control or experimental cassava diets. This latter diet was prepared with cassava meal from peeling roots obtained from floor wastes in the industrial processing line and hay made with the upper-third of the plant foliage, representing in total 80% of the diet volume. At weaning the rabbits were allocated in the iron cages, in a factorial arrangement 2 x 3 (two diets for mothers and litters before the weaning combined with three diets from weaning to slaughter) with 12 replications of two animals (one female and one male) per experimental unit. The two experimental (simplified) diets were formulated with the upper third of cassava foliage hay (41.6%) and cassava meal wastes ensiled or not (40%), representing a total of more than 80% of the diet. Rabbits from mothers which were fed with the maternity cassava diet weighed less at weaning than rabbits from control does (526 vs. 758 g; $P < 0.001$). Independently of diets offered after the weaning, these animals had lower live weight at 50 and 70 days, but average daily growth was similar for the two groups: 38.7 and 38.6 g/d between 31 and 70 days for the control and the maternity cassava diet respectively. A significantly lower feed intake ($P = 0.007$) was observed for rabbits which received the maternity cassava diet until weaning (129 vs. 138 g/d) resulting in a lower feed:gain ratio (3.03 vs. 3.22; $P < 0.001$) during the fattening period. From weaning to slaughter, the use of cassava diets reduced significantly the growth performance of rabbits (36.8 and 37.3 g/d with non-ensiled and ensiled cassava vs. 41.8 g/d for the control) and feed intake (116 and 108 g/d vs. 137 g/d). Similarly carcass weight and slaughter yield were reduced with non-ensiled or ensiled cassava (52.9% and 52.6% vs. 53.8% for the control). The silage process improved significantly the feed:gain ratio (2.92 vs. 3.16 with non ensiled cassava and 3.28 for the control) in relation with of a lower feed intake, but there was non-significant effect of silage processing on average rabbits growth rate and carcass characteristics. The carcass cuts were heavier ($P < 0.01$) for animals fed on control diet. Nevertheless hindlegs and forelegs proportions were higher with the cassava diets ($P < 0.01$). The silage process of cassava meal had no specific effect ($P > 0.05$) on carcass parameters. In conclusion, the simplified diet formulated with 80% of cassava by-products decreased the performance and carcass characteristics of rabbits (final carcass weight reduced by 10%).

EFFECTS OF DIETARY INCLUSION OF TOYOCERIN® (*BACILLUS CEREUS* VAR. *TOYOI*) ON PERFORMANCE, HEALTH AND FAECAL NITROGEN EXCRETION IN GROWING RABBITS

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The effect of dietary supplementation of *Bacillus cereus* var. *toyoi* on the performance, health, caecal fermentation and microbiota, and faecal N excretion of growing rabbits in an Epizootic Rabbit Enteropathy scenario was evaluated. Two diets were manufactured including 0 (Diet C) or 1000 (Diet T) ppm of Toyocerin® (1×10^9 spores of *Bacillus cereus* var. *toyoi* per g). For the growth trial, 100 weaned rabbits (50 per diet) were randomly housed in collective cages (5 per cage) and had free access to one of the experimental diets from 28 to 58 d of age. Mortality and morbidity were recorded daily and individual live weight and average feed intake of the cage were checked at 28, 42 and 58 d. To evaluate the effect of the product on caecal microbiota and fermentative parameters, 8 animals per diet were slaughtered at 58 d of age. Another group of 19 rabbits of 42 d (8 per diet) was used to determine faecal N excretion in a digestibility trial. The dietary inclusion of the probiotic did not affect rabbit growth rate, feed intake and feed conversion rate during the experimental period. However, the inclusion of Toyocerin® significantly reduced the mortality (−6%) and sanitary risk index (−12%), confirming the general reduction of digestive problems with diets supplemented with this probiotic in previous works. As what concerns caecal fermentation, only a reduction of branched chain fatty acids (isobutyric+isovaleric) frequently related with the presence of *Clostridium* sp was observed. A trend was observed where the presence of *Clostridium* decreased and lactic acid bacteria counts increased in animals fed with diet T. The addition of Toyocerin did not change the total cellulolytic bacteria counts. Finally, the dietary supplementation of this probiotic did not affect DM and crude protein digestibility coefficients. The reduction of faecal excretion of N observed (−14%; $P < 0.05$) for the animals with diet T was probably due to the slightly lower DM intake observed with this diet (−8%; $P < 0.10$) during the digestibility trial, the faecal excretion of N being corrected by a similar feed intake for both diets. In conclusion, dietary supplementation with 1000 ppm of Toyocerin® did not affect the growth and feed conversion rate, but significantly reduced the mortality and sanitary risk index of rabbits during the fattening period. This improvement in growing rabbits health could be related to positive changes in hindgut microflora.

FIBER QUALITY TO GROWING RABBITS

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An experiment was carried out with the goal of studying the influence of fiber quality on performance and metabolic parameters of White New Zealand growing rabbits. The experiment was conducted in the the period September 24 to November 11, 2007. Twenty four animals with forty days of age were utilized and separated in three treatments: TAH, alfalfa (*Medicago sativa*) hay; TCP, citrus pulp and TLB, linseed (*Linum usitatissimum*) bran. The experimental design was completely randomized with three treatments and eight replications, where each animal was consider an experimental unit. The analyzed variables were: slaughter weight, weight gain, feed conversion, empty caecum weight, caecocolic pH and sanguine parameters, such as triglycerides, total proteins, hemoglobin, cholesterol and high density lipoproteins. The animals fed on TCP diet obtained similar results to those fed TAH about slaughter weight, weight gain, feed conversion and caecocolic pH, showing that the high quantity of soluble fiber in the citrus pulp did not affect the animal performance. This treatment also caused accentuated reduction in the circulating hemoglobin and cholesterol levels, which can probably be attributed to the pectin ability to bind and hold iron and bile salts, respectively. The performance results were lower for TLB diet than for the other diets, probably in function of the high hydration capacity of the soluble fiber found in linseed bran. The results showed that fiber quality requires more attention than its quantity about the effects over performance and metabolic responses.

THE EVOLUTION WITH AGE OF *CLOSTRIDIUM PERFRINGENS* CONCENTRATION IN SOFT FAECES IN RELATION WITH EPIZOOTIC RABBIT ENTEROPATHY SYMPTOMS

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Clostridium perfringens has been shown to play an important role in the development of the Epizootic Rabbit Enteropathy (ERE). The average count of *C. perfringens* in the caecal contents of young rabbits was found to be highly correlated with average diarrhea mortality in the fattening period, whereas high counts ($>2 \times 10^6$ cfu/g) of *C. perfringens* were related with the appearance of the clinical signs of ERE. Accordingly, the caecal enumeration of *C. perfringens* might be used as a good indicator of ERE. Furthermore, *C. perfringens* enumeration in soft faeces was found to be highly correlated with its concentration in caecum. The present work aimed to follow the evolution with age of *C. perfringens* concentration by means of the caecotrophs excreted by rabbits and to look for a correlation between these values and the weight gain of the rabbit at each age. Thirty-eight rabbits, weaned at 31 d of age, were weighed and fitted a neck collar during four hours (from 08:00 to 12:00) at 34, 38, 41, 45 and 48 d of age. They were fed a common commercial diet, not supplemented with antibiotics, and housed individually in flat-deck cages. No significant effect of age on *C. perfringens* concentration in soft faeces was detected. Mean counts were respectively: 5.30 ± 0.97 , 5.00 ± 0.99 , 4.95 ± 0.97 , 5.25 ± 0.99 and 5.13 ± 0.98 log cfu/g at 34, 38, 41, 45 and 48 d. Ten rabbits among the thirty-eight used in this trial presented clinical ERE signs, such as low body weight, aqueous diarrhoea and mucus excretion. These animals had a higher ($P < 0.001$) *C. perfringens* concentration than healthy ones: 6.50 ± 0.69 vs. 4.97 ± 0.95 log cfu/g. Values in diseased animals ranged from 5.23 to 8.45 log cfu/g. Regression procedures were used to relate daily weight gain (DWG, g/d) and body weight (W, g) with the *C. perfringens* concentration in the soft faeces (CPsf, log cfu/g). The regression equations obtained were: $DWG = 57.2 (\pm 6.20) - 2.21 (\pm 1.19) \text{ CPsf}$; $P = 0.07$ and $W = 1680 (\pm 189) - 65.1 (\pm 36.1) \text{ CPsf}$; $P = 0.08$. Even if the *C. perfringens* proliferation in soft faeces increased, ill animals were able to survive but ended the fattening period weighing less than the others, so that mortality is not the only economic loss in a ERE situation. In conclusion, caecotrophs can be used as a tool in evolutionary microbiological studies and the weight loss is both a ERE sign and an important economic loss.

THE USE OF SOFT FAECES FOR THE PREDICTION OF THE CAECAL CONTENTS CONCENTRATION OF *CLOSTRIDIUM PERFRINGENS* IN RABBITS WEANED AT TWO AGES

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Since its emergence in 1997, the Epizootic Rabbit Enteropathy (ERE) has become a severe disease affecting Europe almost as a whole. The aetiological agent remains to be identified, even if several researchers have shown the bacterium Gram-positive *Clostridium perfringens* to be involved in ERE development. The average count of *Clostridium perfringens* in the caecal contents of young rabbits was found to be highly correlated with average diarrhea mortality in the fattening period ($R^2=0.92$; $P<0.001$), whereas high counts ($>2 \times 10^6$ cfu/g) of *Clostridium perfringens* were related with the appearance of the clinical signs of ERE. Accordingly, the caecal enumeration of *Clostridium perfringens* might be used as a good indicator of ERE. However, previous slaughter of rabbits is required to make this determination. Slaughter might be avoided by using samples of caecotrophs. In this context, the main aim of this work was to develop a method leading to estimate the caecal contents counts of *Clostridium perfringens* through its concentration in the rabbit soft faeces. Forty-four rabbits weaned at 28 or 42 d of age were used. Animals were fed a common commercial diet, not supplemented with antibiotics, and housed in pairs in flat-deck cages. Nine days after weaning, rabbits were fitted neck plastic collars during four hours (from 08:00 to 12:00). Afterwards, they were slaughtered and both their soft faeces excretion and caecal contents were sampled in order to determine the concentration (cfu/g) of *Clostridium perfringens*. This concentration was determined by plate counting according to the standard ISO 7937 (1997). Values were transformed to a logarithmic scale to homogenize variances. Eight out of the 44 animals used did not excrete soft faeces; five of them showed clinical symptoms of ERE. Otherwise, concentration of *Clostridium perfringens* in the caecal contents tended to be higher in animals weaned at 28 than at 42 days of age (5.46 vs. 5.13 log cfu/g), although differences did not reach significance levels. Regression procedures were used to relate *Clostridium perfringens* quantification in the soft faeces (CPsf, log cfu/g) with that of the caecal contents (CPc, log cfu/g) for each rabbit. The regression equation obtained was: $CPc=0.622+0.858 CPsf$ ($r=+0.885$, $RSD=0.529$; $P<0.001$). In conclusion, collecting and analyzing soft faeces could be used as an alternative method to predict the content of *Clostridium perfringens* in the caecal contents of rabbits before presenting symptoms of ERE.

RESPONSE OF GROWING RABBITS TO DIETARY ANTIOXIDANT VITAMINS E AND C. 1. EFFECT ON PERFORMANCE

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A seven-treatment experiment was carried out to study the response of seventy 6-week old New Zealand White (NZW) rabbits to dietary supplementation with the antioxidant vitamins, α -tocopheroyl acetate (vitamin E) and ascorbic acid (vitamin C), provided individually or in a combination on some performance traits. Rabbits were equally allocated to one of the following supra-nutritional levels of the two vitamins per kg diet up to the 13th week of age: 1, control with no extra vitamin supplement (40 ppm vitamin E provided through the vitamin A and mineral premix; 2, supplemented with 40 ppm vitamin E (E40); 3, supplemented with 80 ppm vitamin E (E80); 4, supplemented with 200 ppm vitamin C (C200); 5, supplemented with 400 ppm vitamin C (C400); 6, supplemented with 40 ppm vitamin E and 200 ppm vitamin C (E40C200); and 7, supplemented with 80 ppm vitamin E and 400 ppm vitamin C (E80C400).

The C200 group recorded the highest live weight gain and best feed conversion ratio (1312 g and 2.68 vs. 943 g and 3.68 in control group; $P < 0.01$); E80 group had the highest dressing percentage (65.9% vs. 62.5 in control group; $P < 0.05$) and C400 group showed the highest total antioxidant capacity values and lymphocytes percentage (2.4 mmol/l and 63% vs. 1.89 mmol/l and 58% in control group; $P < 0.01$). In conclusion, the vitamin E and/or vitamin C successfully enhanced the growth performance, anti-oxidant status and immunity traits of growing rabbits.

THE USE OF A GINSENG EXTRACT IN RABBITS

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In recent years, essential oils and other extracts of plants have evoked interest as sources of natural substances. They have been screened for their potential uses as alternative remedies for the treatment of many infectious diseases, improvement in animal welfare and hygiene, food production techniques as well as food safety. The aim of this work was to study the efficacy of *Eleutherococcus senticosus* dry extract on the zootechnical, microbiological (pathogenic and harmful bacteria), immunological and biochemical blood parameters, occurrence of *Eimeria* sp. oocysts in rabbits as well as the quality of rabbit meat. Forty eight rabbits (weaned at 35 days) were divided into experimental (EG) and control (CG) groups. Rabbits in EG received dry extract of *Eleutherococcus senticosus* (15 g/100 kg feed) for 21 days; the experiment lasted for 42 days. All animals were found in good health conditions throughout the trial. The application of *Eleutherococcus* extract had a beneficial effect on the mortality (EG: 8% vs. CG: 17%) and feed conversion ratio (EG: 3.55 vs. CG: 3.63); average daily weight gain significantly increased in EG (35.5 g vs. 29.5 g; $P < 0.001$). The antimicrobial effect of *Eleutherococcus* extract was observed against coagulase-positive staphylococci (CPS), *Staphylococcus aureus*, *Clostridium*-like species and *E. coli* in faeces ($P < 0.01$). Haemolytic *E. coli* were absent in faecal samples. Caecal microbial profile was slightly influenced during the extract application. Reduced counts of *Eimeria* sp. oocysts was observed through the whole experiment ($P < 0.001$). The values of blood parameters were changed in the framework of physiological level; increased levels of serum total proteins, total lipids, cholesterol were observed in EG at day 21. Phagocytic activity was not influenced during the *Eleutherococcus* addition and the measurement of the activity of glutathione-peroxidase in rabbits indicated that oxidative stress was not evoked during the experiment. Concerning the quality of rabbit meat, in rabbits receiving *Eleutherococcus* extract, increased contents of fat (EG: 2.33 ± 1.16 g/100 g vs. CG: 1.40 ± 0.20 g/100 g) and energy value (EG: 448.6 ± 3.5 kJ/100 g vs. CG: 415.1 ± 6.2 kJ/100 g) were observed.

COMBINED EFFECT OF ENTEROCIN CCM4231 AND SAGE IN RABBITS

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Enterocin CCM4213 produced by bacteriocinogenic and probiotic *Enterococcus faecium* CCM4231 strain and sage plant extract were applied to rabbits separately and in the combination. A total of 96 rabbits (5-weeks old, male sex, HyPlus breed) were divided into three experimental (EG1, EG2, EG3) groups and one control group (CG). Animals of EG1 received *Salvia officinalis* plant extract (10 µl/animal/day into water), rabbits of EG2 received partially purified bacteriocin (PPB)–enterocin CCM4231 (50 µl/animal/day into water) produced by *E. faecium* CCM4231 strain, animals of EG3 received the combination of PPB CCM4231 (50 µl/animal/day into water) with sage plant extract (10 µl/animal/day into water) for 21 days. The experiment lasted 42 days. The animals were fed by the complete granulated mixture. Faeces were sampled at the start of the experiment, at day 7 (1 week of administration), at day 21 (3 weeks of administration) and 28 (1 week after cessation of application) and at day 42 (3 weeks after cessation of application) to count rabbit intestinal microflora as well as the occurrence of *Eimeria* sp. oocysts. Samples of caecal contents (3 slaughtered animals of each group) were collected at days 21 and 42 to determine caecal bacterial counts. To check phagocytic activity, the animals were sampled at the start of the experiment, at days 21 and 42. The reduction of *E. coli* in EG2 (difference 1.3 log₁₀ CFU/g) was noted at day 7 as well as the reduction of *Clostridium*-like sp. (difference 1.57 log₁₀ CFU/g) at day 42 in comparison to CG. Reductive effect against *Pseudomonas*-like sp. (P<0.001) was also noted in EG3 at day 42 in comparison to CG. The other bacteria were not influenced by additives. The bacterial counts in the caecum were lower than in the faeces and no significant changes were noted. In the experimental groups prolonged immunostimulative effect (P<0.001) was observed in comparison to CG at day 42. The most pronounced anticoccidial effect was recorded in EG3 at day 7 compared to CG (in CG 65 OPG, in EG3 10 OPG). The oocysts shedding were also reduced in EG2 (20 OPG) compared to CG (65 OPG). At day 21, oocysts were also reduced in EG1 (30 OPG to CG - 1184 OPG); in EG2 and EG3 were found only rarely. Results of our experiment suggest possible commercial utilization of natural additives tested.

COMBINED EFFECT OF BACTERIOCIN-PRODUCING *ENTEROCOCCUS FAECIUM* CCM4231 STRAIN AND SAGE IN RABBITS

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The aim of this experiment was to study the combined effect of biological active matters – natural substances like a bacteriocin-producing strain with probiotic properties *Enterococcus faecium* CCM4231 and sage plant extract. Ninety six weaned rabbits (age of 35 days) were divided into three experimental (EG1, EG2, EG3) groups and one control (CG) group. Animals in EG1 received *E. faecium* CCM4231 strain (10^9 cfu/animal/day into water); for rabbits in EG2 the sage plant extract was applied at doses 10 µl/animal/day into water; rabbits in EG3 received both *E. faecium* CCM4231 strain and sage plant extract at the same doses as in the groups EG1 and EG2. The experiment lasted 42 days; natural substances were applied for 21 days. Faeces were sampled at the start of the experiment, at day 7 (1 week of administration), at day 21 (3 weeks of *E. faecium* administration) and at day 42 (3 weeks after cessation of application) to measure the counts of rabbit intestinal microflora as well as the occurrence of *Eimeria* sp. oocysts. Samples of caecal contents (3 animals in each group) were collected at days 21 and 42 to determine bacterial counts. The immunological parameter - phagocytic activity was examined at days 1, 21, 42. The inhibitory effect of *E. faecium* CCM4231 strain (EG1) was observed at day 21 (3 weeks of CCM4231 application) by decrease of *E. coli* (difference of 1.84 log₁₀ CFU/g) as well as *Clostridium*-like sp. (difference of 1.1 log₁₀ CFU/g) in comparison to CG. Inhibitory effect against coagulase-negative staphylococci (P<0.01) was also noted at day 42 (3 weeks of in EG1 (*E. faecium* CCM4231) and in EG3 (the combination of *E. faecium* CCM4231 with sage) in comparison with CG. No changes in the other bacteria were noted. The bacterial counts in caecum were lower than those in faeces and no significant changes were found. At days 21 and 42, immunostimulative effect of the natural substances (P<0.001) was noted in the experimental groups (EG1, EG2, EG3) in comparison to CG. The reduction of *Eimeria* sp. oocysts was demonstrated after application of each natural substance in the EG1, EG2 and EG3 compared to CG at day 21. Thus, bioadditives represent promising alternatives to synthetic preparations.

PRODUCTIVE PERFORMANCE OF RABBITS FED WITH DIETS CONTAINING RAMIE (*BOEHMERIA NIVEA*) HAY IN SUBSTITUTION TO ALFALFA (*MEDICAGO SATIVA*) HAY

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An experiment was carried out to evaluate the performance of growing rabbits fed with non pelleted coarsely ground diets, containing, besides maize (*Zea mays*) and soybean (*Glycine max*) meal, alfalfa (*Medicago sativa*) and ramie (*Boehmeria nivea*) hays. The experiment was conducted from August 18 to October 6, 2006. Thirty nine animals of 40 days of age were utilized and assigned to three treatments: Alfalfa 15% (control), Ramie 15% and Alfalfa 7.5%+Ramie 7.5%, corresponding to the forage inclusion level in each diet. The experimental design was completely randomized with three treatments and thirteen replications, where each animal was considered an experimental unit. The evaluated parameters were: average daily weight gain, average feed intake, feed conversion, carcass yield, hot carcass weight, cold carcass weight, drip loss percentage, pH 1 hour and pH 24 hours after slaughtering. Data were analyzed by analysis of variance and the averages compared by Duncan test ($P < 0.05$). The treatment Alfalfa 7.5%+Ramie 7.5% showed higher average daily gain (28.0 g/d) against 26.4 and 25.7 g/d than the treatments Ramie 15% and Alfalfa 15%, respectively. The average feed intake was higher with the treatments with ramie inclusion, 93.7 g and 87.1 g for the treatments Alfalfa 7.5%+Ramie 7.5% and Ramie 15%, respectively, than with 15% alfalfa (83.8 g/d). Although, with no differences in the feed conversion, which stayed around 3.30 on average. For the main slaughter parameters, effects of type of forage were not significant. The results indicate that the combination of ramie+alfalfa hays, as main fiber source ingredient in the diets, caused a positive synergic effect. Apparently, this combination increased the palatability, but this hypothesis must be tested in further studies, because the preliminary analyses of the formulations did not show nutritional data that can subsidize this explanation. The authors conclude that ramie could replace 15% of alfalfa of the control diet and its association with alfalfa may improve growth performance.

WHITE LUPIN (CV. AMIGA) SEEDS AS A PROTEIN SOURCE IN DIET FOR GROWING RABBITS: EFFECT ON GROWTH PERFORMANCE, DIGESTIBILITY OF NUTRIENTS AND CARCASS TRAITS

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This study aimed to evaluate whole white lupin (cv. Amiga) seeds as an alternative dietary crude protein source to soybean and sunflower meal. Diets contained soybean meal (10%, SBM diet), sunflower meal (17%, SFM diet) or white lupin seeds (15%, WL diet) as the main protein source. The experimental diets were similar in the level of crude protein (16.2%), starch (15.0%), fat (4.6%) and in the limiting amino acids. In comparison with the SBM diet, the diet based on sunflower meal contained slightly more cellulose and lignin, and less hemicelluloses and digestible energy. Diet based on white lupin seeds contained a higher level of raffinose-series oligosaccharides than other diets. Ninety Hyplus® rabbits (30 rabbits per group; 1058±72 g), weaned at 37 d of age, were used for growth performance evaluation (from 37 to 79 days of age). At the end of the growth trial, 18 rabbits of average body weight were slaughtered without fasting in each group, and used for the evaluation of carcass traits. Other 39 Hyplus® rabbits (13 rabbits per group; 950±92 g), weaned at 33 d of age, was used in a digestibility trial to determine digestibility coefficient of diets. No significant effects was detected on the weight gain (38.4, 40.1, and 40.0 g/d in rabbits fed the SBM, SFM and WL diet, respectively; $P>0.05$). The feed intake was higher in rabbits fed the sunflower meal-based diet than in other rabbits (135.0, 122.9 and 128.7 g/d in rabbits fed the SFM, SBM and WL diet, respectively; $P=0.02$). Similarly, feed conversion tended to be higher in rabbits fed the diet based on sunflower meal (3.40, 3.22 and 3.24 kg/kg in rabbits fed the SFM, SBM and WL diet, respectively; $P=0.12$). Dressing out percentage was significantly higher in rabbits fed the lupin-diet (58.7, 57.4 and 57.3% in rabbits fed the WL, SFM and SBM diet, respectively; $P=0.01$). There were no significant differences in digestibility of organic matter, crude protein, starch or fat. Digestibility of energy tended to be lower in rabbits fed the diet based on sunflower meal (66.5, 69.5 and 68.2% in rabbits fed the SFM, SBM and WL diet, respectively; $P=0.06$). Digestibility of NDF (35.7, 41.1 and 40.9% in rabbits fed the SFM, SBM and WL diet, respectively; $P=0.03$) and ADF was lower in rabbits fed the sunflower meal-based diet (26.8, 31.9 and 31.9%; $P=0.09$). In conclusion, white lupin seeds (cv. Amiga) are suitable dietary protein source for growing rabbits which can fully replace the traditionally used sources of protein, without adverse effect on the parameters evaluated in this study.

EFFECTS OF PROBIOTICS AND NANOMETER IMPLEMENT ON GROWTH PERFORMANCE OF REX RABBIT

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To study the effects of Probiotics and Nanometer Implement on performance of Rex Rabbit, eighty 30-day old rabbits were selected and randomly divided into 4 groups: control group (group C), Nanometer Implement processing water group (group N), Probiotics processing water group (group P) and Probiotics and Nanometer Implement processing water group (group PN). The average daily weight gain of groups P, N and PN were respectively higher 15% ($P<0.05$), 6% ($P<0.05$) and 23.9% ($P<0.05$) than the group C. The feed conversion ratio of groups P, N and PN were respectively lower 12.7% ($P<0.05$), 12.7% ($P<0.05$) and 18.1% ($P<0.05$) than that of group C. At 1 month of age, digestibility of crude protein, ether extract and crude fibre were higher in group PN, respectively 9% ($P<0.05$), 5.8% ($P<0.05$) and 25.3% ($P<0.05$) than that of group C. While at 3 months of age no differences were found between groups on digestibility of crude protein, ether extract and crude fibre. This study shows both probiotics and Nanometer Implement improved the average daily gain and remarkably enhance the digestibility. The use of both Probiotics and Nanometer Implement together can have better effect.

EFFECT OF DIET METHIONINE RATE ON PERFORMANCES AND BLOOD PROTEIN LEVELS OF FATTENING RABBITS

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Nutrition is known to have an effect on immune system. Amino acids, such as methionine, must be used by animals to build their antibodies or gamma globulins. It was suggested that more methionine is needed to provide the greatest immune protection than is needed for maximum growth in broilers. This trial aimed at defining more precisely the effect of methionine addition in fattening rabbit diet on both production performances and on blood immune system indicators. The trial compared four diets (9,65 MJ/kg) with different methionine incorporation rates: 0.26%, 0.36%, 0.46% and 0.56%. The trial was made on 120 Hyplus rabbits from weaning (35 days of age) to 70 days of age. Live weight (LW) and feed intake (FI) were registered at 49 and 70 days of age. Mortality and morbidity were controlled to determine the Sanitary Risk Index (SRI). In order to measure the effect of the methionine rate in feed on immunity, blood protein concentrations (total protein, albumin, alpha-1, alpha-2, beta-1, beta-2 and gamma globulins) were measured by electrophoresis in six animals per treatment. The ratio between each protein and the total protein concentration was compared between diets, as well as the total protein concentration. No significant effect of methionine rate was observed, neither on mortality, morbidity and SRI, nor on zootechnical performances. Total blood protein concentration did not change significantly from a diet to another one. The gamma globulin rate in blood protein tended to change with methionine rate in feed ($P=0.0528$). The diet containing 0.56% of methionine led to the highest Gamma/Total Protein ratio. The Gamma/Total Protein ratio increased linearly with methionine rate in feed. Since blood was taken from animals showing no sign of morbidity and no growth problem, this trial brings to the conclusion that immune defences could be improved by a methionine rate of 0.56% in feed.

DIGESTIBLE FIBRE TO STARCH RATIO AND ANTIBIOTIC TREATMENT TIME IN GROWING RABBITS AFFECTED BY EPIZOOTIC RABBIT ENTEROPATHY

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The study aimed to evaluate if the digestible fibre (DF, hemicelluloses+pectins) to starch ratio of the diet and the time of antibiotic treatment after epizootic rabbit enteropathy (ERE) outbreak affect health status, digestive physiology, growth performance, and carcass traits of early weaned rabbits. Two hundred forty Grimaud hybrid rabbits were put in individual cages and controlled from 25 d of age to slaughter (70 d). The rabbits were assigned to four groups according to a 2x2 factorial arrangement (2 DF to starch ratios by 2 antibiotic treatment times). Half of the rabbits fed *ad libitum* diet L, with low (1.0) DF to starch ratio (19.1% DF and 18.9% starch as-fed) and half fed diet H with high (2.5) DF to starch ratio (23.9% DF and 9.6% starch). The diets were characterized by similar protein (16.8%), ADF (19.8%), and ADL (3.9%) concentrations. After the ERE appearance, half of the rabbits within diet were submitted to an early antibiotic treatment (from 38 to 42 d of age), while half of the rabbits to a late treatment (from 45 to 49 d). The antibiotic treatment was realized by an association of oxytetracycline hydrochloride (100 g/100 l) and colistin (24 g/100 l) administered in water. At 43 d of age, 36 rabbits (6 per group) were sacrificed to collect caecal content and ileum mucosa samples. Increasing DF to starch ratio of the diet improved fibre fraction digestibility ($P<0.001$) therefore maintaining a similar nutritive value (digestible energy=10.7 MJ/kg) among diets. Mortality (31.7% vs. 11.5%) and morbidity (38.5% vs. 18.5%) significantly decreased ($P<0.001$) by increasing DF to starch ratio, thus reducing sanitary risk by two thirds. These results were apparently associated to a higher caecal fermentation activity (volatile fatty acids: 49.6 vs. 60.7 mmol/l; $P=0.03$) in rabbits fed the high DF to starch diet, while ileal mucosa morphometry was unaffected. Once the health status was recovered, growth performance and slaughter results were scarcely affected by the dietary treatments. An early antibiotic treatment, administered within one week from the first ERE symptoms, reduced mortality (17.3 vs. 26.0%; $P=0.07$) and improved growth performance and slaughter results in comparison with a later treatment. Even the early antibiotherapy did not avoid that rabbit mortality reached a level unacceptable for a commercial farm, however. The association of a diet with a high DF to starch ratio and an early antibiotic treatment permitted to maintain the mortality at a basic level (5.8%) and provided the best growth performance and carcass quality.

EFFECT OF MINERAL BLOCKS ADDITION ON FATTENING RABBIT PERFORMANCE

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One hundred twenty rabbits of the Algerian locally called «white population» were distributed at weaning (35 d) between two equivalent groups according to weaning weight (590 g on average) and litter origin. Rabbits were placed in collective cages of six. The control group received *ad libitum* during the 5 weeks of the study a commercial pelleted diet (36% alfalfa, 25% barley, 26% hard wheat bran, 12% soybean meal, 1% of minerals and vitamins), and fresh water through automatic nipple drinkers. The experimental group was caged and fed in the same conditions, but in addition a commercial mineral block (Blocs Harmony, Inzo, France) was fixed along a wall in each cage. New blocks were placed every week even if blocks were “consumed” within a shorter time. Mineral blocks (225 g, 47% total ash) contained mainly calcium (12%), trace elements (Cu, Mn, Zn and Se) and few total phosphorus (1.5%) and sodium (2%). An average mortality of 17.5% was observed during the experiment (the first 3 weeks) but without difference between treatments. In presence of mineral blocks, average pelleted feed intake was increased by 16% (68.3 vs. 59.0 g/d.; P=0.008) as was the growth rate (33.6 vs. 28.3 g/d; P=0.001). Feed conversion ratio was not significantly modified (2.84 with blocks and 2.92 for the control; P>0.05). At 10 weeks of age, the final live weight of rabbits with mineral block was 188 g higher than that of the control rabbits (1767 vs. 1579 g; P=0.02). Despite the high inclusion level of alfalfa in the commercial diet, its analytical calcium content was lower (0.6%) than recommended for fattening rabbits. The positive effect of the mineral blocks addition was most probably a consequence of the calcium deficiency alleviation: consumption of blocks trebles the average calcium intake of rabbits. New experiments are necessary to test the effects of mineral blocks utilisation in addition to pelleted feeds correctly balanced for the calcium content. This experiment also demonstrate that for rabbit feeding mineral supply can be separate from the main feed providing fibre, proteins and energy.

EFFECT OF SUBSTITUTION ROCKET SEED MEAL AS A SOURCE OF PROTEIN FOR SOYBEAN MEAL IN DIETS OF NEW ZEALAND WHITE RABBITS

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The present study aimed to evaluate the effect of replacing three levels from rocket seed meal (RSM) as a partial or complete replacement of soybean meal (SBM) protein of the control diet on growth performance, digestibility and blood characteristics in growing New Zealand White (NZW) rabbits. RSM contributed 0, 5, 10.5 and 21 % of the diet. Thirty-six, 8 weeks old growing NZW rabbits were distributed randomly and equally into four experimental groups, each of 9 animals. Each group of rabbits was fed one of four experimental diets (16% crude protein and 12% crude fiber on average). The study lasted for a period of 6 weeks. The results showed that feeding rabbits 10.5% RSM in their diet compared to the control group during the whole experimental period resulted in significant ($P<0.01$) improvement in total weight gain by 15.1% (1042 vs. 905 g), feed conversion ratio by 12.3% (3.84 vs. 4.38), total feed consumption increased by 1.3% (4004 vs. 3953 g). The group fed 21% RSM-diet showed body weight gain and feed conversion ratio not significantly different from those of the control. Viability was similar in all the experimental groups (7 to 8 out of 9). Digestibility coefficients of most nutrients and the nutritive values were significantly ($P<0.01$) improved in the group fed 10.5% RSM-diet as compared to the control (e.g. 77.5 vs. 74.2% for dry matter, 69.1 vs. 66.2% for N and 53.4 vs. 38.4% for crude fiber digestibility). Serum total protein, albumin and cholesterol were significantly affected by different treatments; however, serum urea-N and GPT were not affected. It can be concluded that RSM at 10.5% level of the diet in NZW rabbits had the best results without adverse effects on growth performance, kidney or liver function.

EVALUATION OF SUBSTITUTING NIGELLA SEED MEAL AS A SOURCE OF PROTEIN FOR SOYBEAN MEAL IN DIETS OF NEW ZEALAND WHITE RABBITS

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The present study aimed to evaluate the effects of a partial or complete replacement of soybean meal (SBM) of the control diet by nigella (*Nigella sativa* L) seed meal (NSM) on growth performance, diet digestibility and blood characteristics in growing New Zealand White (NZW) rabbits. NSM was used at levels 0, 6, 12 and 24% in the diets. Thirty-six 8-weeks old growing NZW rabbits were distributed randomly and equally into four treatments groups, each of 9 animals. Each group of rabbits was fed one of four experimental diets (16.4% crude protein and 12.7% crude fiber on average). The study lasted 6 weeks. The results showed that the group of rabbits fed 12% NSM had significantly ($P<0.05$) better total weight gain than the control (1046 vs. 971 g) Feed conversion ratio improved by 3.7%, but difference was not significant. Digestibility of dry matter (DM), organic matter, crude protein (CP), crude fiber (CF) and nitrogen free extract and metabolizable energy were also better with 12% NSM ($P<0.05$) than with the control diet (DM 78.4 vs. 76.6%; CP 86.6 vs. 84.6% and CF 58.7 vs. 54.5%). Serum total protein and albumin of rabbits fed 12% NSM were significantly ($P<0.05$) higher than those fed the control diet, while serum globulin, total lipids and serum glutamic pyrovic transaminase (GPT) were not significantly affected by the treatments. The lowest ($P<0.05$) values in serum cholesterol were recorded in the control group and followed by those of 6, 12 and 24% NSM in an ascending order (69.6, 74.6, 76.9 and 78.4 mg/dl, respectively). Rabbits fed 24% NSM resulted in a significant deterioration of most traits when compared to the control. It could be recommended to use NSM diets for NZW rabbits as a non-traditional source of plant protein up to 12% without harmful effects on the growth performance, kidney or liver function.

LIPASE ACTIVITY TILL 35 DAYS OF AGE IN BROILER RABBITS

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The developmental changes of lipase in stomach, small intestine, caecum and blood till 35 days of age were followed in broiler rabbits. Two experiments were carried out on Hyplus® rabbits. Digesta and blood were sampled every 2 or 4 days in both experiments. In the experiment 1, the lipase activity in stomach, small intestine and caecum was determined from 14 to 35 days of age in 5 suckling rabbits at seven different periods. In the experiment 2, the lipase activity was analysed in blood by colorimetric method by Randox Laboratories Ltd. From 21 to 35 days of age in six groups of 5 suckling and weaned rabbits at each age. Gastric and small intestinal lipolytic activity, in the experiment 1, was the highest around 25 days of age (2.572 and 1.720 mmol/g/h, respectively). Caecum activity of lipase was the highest at the beginning of the experiment at 14 days of age (1.086 mmol/g/h) and at 32 days of age (1.098 mmol/g/h). In the experiment 2, the activity of lipase in blood tended to show a non-significant curvilinear response from weaning onwards.

EFFECT OF EXTRACT OF CHESTNUT WOOD INCLUSION (ENC[®]) IN NORMAL AND LOW PROTEIN AMMINOACID SUPPLEMENTED DIETS ON HEAVY BROILER RABBITS

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A chestnut (*Castanea sativa*) extract (ENC), rich in hydrolysable tannins, was evaluated in rabbit diets without antibiotics. A total of 72 five week-weaned rabbits were fed as follows: 1) control diet (C); 2) Aminoacid supplemented low protein diet (A); 3) diet A+0.45% ENC (TA); 4) diet C+0.45% ENC (TC). Feeding was modulated in two phases characterised by different levels of CP (DM basis): diet C first period 175 g/kg, second period 197 g/kg vs. diet A first period 145 g/kg, second period 159 g/kg.

The ENC was extracted from chestnut wood by a heat and low-pressure treatment, and only the water-soluble fraction was kept and subsequently dehydrated. The product is commercially available as fine brown powder (92 to 95% dry matter) with a pure tannin content of 77% on a DM basis. The trial was carried out in full summer conditions. The productive traits were recorded each 14 days. ENC inclusion did not have any negative effects on the productive parameters, while low protein AA supplemented diets enhanced significantly ($P<0.01$) protein utilization. Over the whole period, ENC inclusion did not influence the daily feed intake, daily weight gain, feed conversion ratio and protein efficiency ratio, but reduced significantly ($P<0.01$) the mortality from 44% to 11% in group C and group TC fed high protein diets; while mortality was exactly the same (11%) in groups A and TA. The high level of protein can be suspected as being the cause of mortality.

PATHOLOGY AND HYGIENE

VIRAL INFECTION OF RABBITS

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ABSTRACT

The three most important viruses of rabbits include: Myxoma virus (MV), the poxvirus that causes Myxomatosis, the calicivirus (*genus* Lagovirus) of Rabbit Haemorrhagic Disease (RHDV), and Lapine Rotavirus (LRV), which is an enteric agent. There are some other viral agents in rabbits (parvovirus, coronavirus, adenovirus, calicivirus (*genus* Vesivirus), enterovirus-like, reovirus, herpesvirus and coronavirus) but both their occurrence and their pathological value are negligible.

Myxomatosis was firstly introduced in Europe more than 50 years ago and still represents a current and real problem. Recent studies have been focused to determine the role and function of the over 100 genes of MV encoding structural and essential proteins; in particular the immunomodulatory MV (im-MV) proteins (virokines and viroreceptors, immune modulators and anti-apoptotic factor), involved in contrasting the host immune system response towards the MV infection. One of the main tools to control myxomatosis in endemic areas is use of the live attenuated vaccines that are able to induce traceable humoral immunity for a variable time even if the protection of rabbits from the infection is not fully guarantee. Therefore, thanks also to the knowledge on the im-MV proteins, a new family of biotechnology deleted vaccines will soon be produced and made available in a few years. These should be more safe and able to induce a wider immunity and permit to apply the DIVA strategy i.e. to use serology to ascertain if the anti-MV antibodies detected in a rabbits originate by an infection or a vaccination.

RHDV is a non-cultivable calicivirus that infects rabbits and causes an acute fatal hepatitis, firstly described in China in 1984. The first consistent antigenic variant called RHDVa, was identified in Italy and Germany in 1997. Nowadays it is present in most parts of Italy and its field prevalence has reached value over 50%. In Europe it has been reported between 1997 and 2004 in Germany, France, Malta and Hungary. Outside Europe, it was reported in Reunion Islands, USA and South America and, taking account of the RHDV genetic sequences deposited at the NCBI databank, its presence in China is also evident from 1985. More recently some other RHDV isolates presenting peculiar characteristics were identified. Based on their reactivity with MAbs these strains could be considered as further and separate steps of variation of the RHDVa, possibly classified as sub-variants.

The detection of seropositivity for RHDV in the sera of healthy farmed and laboratory

rabbits taken between 1975 and 1985, which is approximately ten years before the occurrence of the disease in Europe, has suggested the hypothesis of the existence of one or more “non-pathogenic” viral strains antigenically related to pathogenic RHDV. Rabbit Calicivirus (RCV), the first of these non-pathogenic RHDV-like viruses identified in healthy rabbits, was detected in Italy in 1996. It is avirulent, replicates in the intestine at a low titre and presents a 92% genomic identity with RHDV. The diffusion of RCV in different areas of Italy has been evaluated in five consecutive serological surveys during the period 1999-2008, throughout the determination at slaughtering of anti-RHD antibodies in non-vaccinated meat rabbits from RHDV-free commercial rabbitries. The results clearly show that antibodies reactive with RHDV are present in several rabbit populations: almost 30% of controlled farms and over 80% of animals. The definitive proofs that an active infection had occurred came from the detection of IgA and IgM as well as the identification of viral strains by using PCR on faeces. In addition, the existence of other non-pathogenic caliciviruses in wild rabbits was suggested by the serological surveys of rabbit populations in European countries (UK and France), Australia and New Zealand. Either the identification of RNA particles related to RHDV in rabbit sera collected since 1955 in Britain and the very recent isolation and identification of one of these viruses in Australia finally confirmed such hypothesis.

Enteric diseases have an important role in the rabbit industry since they produce severe economic losses due to mortality, growth depression and worsening of conversion index. Among the different pathogens that could be found in rabbits suffering from enteropathy, viruses seem to have an important but not definitive role. Viruses and among others Lapine Rotavirus (LRV) particularly, should not be able to induce primary episodes of high gravity but, acting as mild pathogens, they have the capacity of became endemic. The role and importance of viruses as primary aetiological agent of rabbit enteritis are here discussed, by both reviewing the available literature and presenting the results of studies of prevalence of the viruses identified in rabbits with enteritis. That is to recognise the main features and pathogenic abilities of different viral agents and to try to attribute them an etiological role in enteric syndromes, relating their presence with pathologic lesions.

INTRODUCTION

The three most important viruses of rabbits include: Myxoma virus (MV), the poxvirus that causes Myxomatosis, the calicivirus (*genus* Lagovirus) of Rabbit Haemorrhagic Disease (RHDV), and Lapine Rotavirus (LRV), which is an enteric agent. In particular, MV and RHDV can cause severe losses and a huge economic impact due to high level of morbidity and mortality, and their occurrence in most countries is followed by the application of strict measures of health policy. The impact of LRV is lower but indeed it should be considered an important aetiological agent of the so-called “enteritis complex”. These viral infections can be efficiently controlled and limited by a correct management plan through the use of hygienic measures of direct prophylaxis together with the application of specific vaccination programs. There are other viral agents in rabbits, but both their occurrence and their pathological

value are negligible. Most of them have been detected in rabbits with enteritis, *i.e.* parvovirus, coronavirus, adenovirus, calicivirus (*genus* Vesivirus), enterovirus-like, reovirus, and are generally not considered as primary agents of disease. Herpesvirus and coronavirus (the agent of pleural effusion disease) can cause a systemic disease but they have been very rarely reported.

MYXOMATOSIS: STILL ONE OF THE MAJOR THREATS FOR RABBIT BREEDING

From a virological point of view, Rabbit Haemorrhagic Disease virus (RHDV) and Myxoma Virus (MV) are the main health and economical problems for rabbit farmers because both virus infections cause rapid, systemic and lethal diseases with a mortality rate often over 80%. Differently, while MV, illegally introduced into Europe more than 50 years ago from South America (Fenner, 1994; Fenner and Fantini, 1999), still represent a current and real problem, RHDV became a solved problem with the introduction of a reliable and efficient vaccine after its sudden and dramatic appearance. The main reason of this major difference is because RHDV and MV belong to two very distant virus families, characterized by peculiar strategies used to survive in the host over time.

MV belongs to the *Poxviridae* family, genus *Leporipoxvirus* with a very large linear double stranded DNA encoding 171 unique genes (twenty times more than RHDV!). The entire genomes of the South American strain, Lausanne (Cameron *et al.*, 1999) and the North American strain MSW (Labudovic *et al.*, 2004) have been sequenced. While the central part of the genome includes approximately 100 gene encoding structural and essential proteins, the extreme parts of the genome include many immunomodulatory genes involved in contrasting the host immune system response towards MV infection. Actually, successful MV replication and the consequent degree of disease induction are related to its ability to avoid recognition and clearance by the innate host and acquired immune system of the infected rabbits (Kerr and McFadden, 2002; Jeklova *et al.*, 2007; Stanford *et al.*, 2007). Immunomodulatory MV proteins (im-MV proteins) are included in three main categories in relation to the target specific pathways: 1) virokines and viroreceptors, 2) immune modulators and 3) anti-apoptotic factor (Stanford *et al.*, 2007).

Most of the im-MV proteins interfere in specific host pathways “miming” one of the host proteins involved in the transmission of the signal throughout the pathways (*i.e.*, they have a similar structure that allows them to compete with the normal proteins but they have a reduced capacity, if any, to transmit the signal). The final result is that the specific pathway is partially or totally blocked and as a consequence MV replicate more easily.

Whereas the first two categories of im-MV proteins target both the innate and the specific immunosystem, the proteins included in the third category act inside the apoptotic pathways. In response to virus infection, cells switch on a complex pathway of programmed cellular death (apoptosis) and elimination, with no or limited consequence for the surrounding tissues.

Importantly, most of these im-MV proteins have been experimentally demonstrated to function as specific and critical virulence factors indispensable for the infection of MV, leading to the development of myxomatosis in European rabbits that, in fact, represents a devastating state of immune suppression of the host that usually dies for supervening bacterial infection.

The main ways to control myxomatosis in areas where MV is endemic are a combination of direct and indirect measures of prophylaxis. Basically, they include the application of biosecurity measures, in order to avoid the introduction of the infection by infected animals or by contacts with arthropod vectors, and the use of the vaccine (Stanford *et al.*, 2007).

The commercially available vaccines belong to the category of the live attenuated ones and are obtained by serial passage of the virus on tissue culture or in a heterologous host. Albeit they are able to induce immunity to MV for a variable time (even 9 to 10 months) that could be easily traced by using serological methods for detecting antibodies, the protection of rabbits from the infection is not fully guaranteed.

However, because of knowledge gained in the two last decades from research on MV (in particular on the im-MV proteins), a new family of vaccines will soon be produced and made available in a few years. Biotechnology deleted vaccines will have at least two advantages: first, to be more safe and able to induce a wider immunity since it will be well known which im-MV protein(s) have been deleted. Secondly, it will be possible to apply the DIVA strategy that is based on use of a “marker vaccine”. This will allow the use of serology to ascertain if the anti-MV antibodies detected in a rabbit originated by an infection or a vaccination.

In this view, it will be necessary to develop serological assays that are able to detect specific antibodies for the single most important MV proteins. One example of these assays was developed at our laboratory where the MV serology is based on ELISA's that specifically detect the antibodies produced against the m71L protein (Cristoni *et al.*, 2007). The ELISA used in routine assays is a competitive type one (Botti *et al.*, 2007). A monoclonal antibodies (MAb) specific for the m71L is adsorbed at the solid phase. Sera are diluted in the microplate wells starting from the dilution 1/10 and the antigen, which are easily obtained from cells infected with MV. The competition for the binding of the antigen is between the MAb adsorbed onto the solid phase and the serum antibodies. Finally, the MAb anti m71L conjugated to the peroxidase enzyme is used to measure how much antigen is linked to the solid phase.

The test has been used since 2000 in different epidemiological situations and it has been shown to be reliable and sensitive (Lavazza *et al.*, 2004a; Ferrazzi *et al.*, 2007).

Presently, more studies are in progress in order to identify the level of antibody production with respect to the main MV proteins, included the im-MV ones.

CALICIVIRUS IN RABBITS: A REVIEW ON RHDV AND CORRELATED VIRUSES

Rabbit haemorrhagic disease (RHD) is a highly contagious and fatal acute hepatitis of wild and domestic European rabbits (*Oryctolagus cuniculus*), which was first

reported in 1984 in China (Liu *et al.*, 1984). It appeared in Europe in late 1986-87 causing enormous devastation to the rabbit industry, at least until the development of an inactivated vaccine and introduction of its use in prophylactic programs. RHD has been reported in over 40 countries and is presently endemic in Asia, Europe, Central America. Outbreaks have also been recorded in Saudi Arabia and West and North Africa. RHD has been intentionally introduced in Australia and New Zealand (Cooke and Saunders, 2002), where rabbits are considered a pest, as biological control in order to keep as low as possible the level of rabbit reproduction. In 2000 and 2001, three independent outbreaks were recorded in the United States of America and more recently from 2004-2005 again in USA and in South America (Uruguay).

The European rabbit is the only species affected by RHD and no other American lagomorphs (*i.e.*, *Romerolagus diazzi*, *Lepus californicus*, *Sylvilagus floridanus*) have been shown to be susceptible (Gregg *et al.*, 1991). As a general rule, the presence of RHD as an endemic disease is merely the consequence of the presence of steady wild and domestic European rabbit populations that makes almost impossible the goal of eradication of RHD in spite of the availability of an effective vaccine.

A similar disease, termed European brown hare syndrome (EBHS), has been described in the hare (*Lepus europaeus*) in the early 1980's in Northern Europe (Gavier-Widén and Mörner, 1991). Due to the existence of many similarities with regards to aetiology, epidemiological data and clinical-pathological features, at least initially, EBHS and RHD were considered as the same disease caused by a single agent.

The causative agent of RHD and EBHS

For some years (1984-1990), the identification and classification of RHDV have been debated and various hypotheses were put forward (*i.e.*, parvovirus, picornavirus, calicivirus). The definitive classification of RHD (and EBHSV) as calicivirus and the subsequent definition of the new genus Lagovirus inside the *Caliciviridae* family (Figure 1) was achieved between 1991-1992, when various authors purified the non-cultivable virus from liver organ homogenates, amplified and sequenced the capsid protein, and studied its antigenic properties.

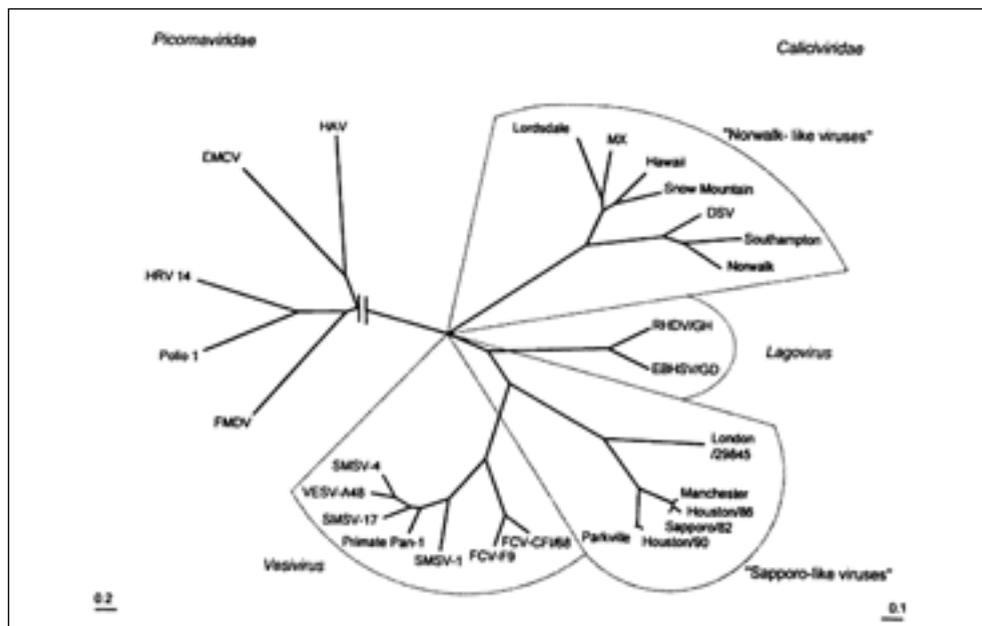


Figure 1: Classification of Caliciviridae through phylogenetic analysis of sequences of both the capsid protein and RNA polymerase.

RHDV is 32 to 35 nm in diameter, has a single major capsid polypeptide (60 kDa), a positively stranded RNA genome of 7437 kb and a sub-genomic RNA of 2.2 (Capucci *et al.*, 1990, 1991; Ohlinger *et al.*, 1990; Parra and Prieto, 1990; Meyers *et al.*, 1991a, 1991b). The RHDV VP60 capsid protein folds in two distinct domains held together by a hinge region: the N-terminal 1 – 234 residues constitute the inner domain and the C-terminal residues beyond 235–579 constitute the protruding domain. In the overall picture of the capsid, these domains form the inner shell and the outer shell, respectively, which is characterised by arch-like structures (Barcena *et al.*, 2004). This structure also correlates with the antigenic characteristics of RHDV. In fact, the main antigenic determinants are located on the C-terminal end of the VP60 (Wirblich *et al.*, 1994; Capucci *et al.*, 1995a, 1998; Schirraier *et al.*, 1999).

Presently, it has become clear that EBHSV is not the same disease. In fact, the aetiology of EBHS remained unclear for many years until it was shown by animal experiments and electron microscopy EM analysis (Eskens and Volmer, 1989; Lavazza and Vecchi, 1989) that it was caused by a virus showing morphological characteristics indistinguishable from those of the rabbit haemorrhagic disease virus (RHDV) with biochemical features typical of the Caliciviridae family. However, significant antigenic, structural and molecular differences between the two viruses were found using RHDV-monoclonal antibodies (MAbs) (Capucci *et al.*, 1991, 1995a), and cross-hybridisation and genomic sequence analysis (Wirblich *et al.*, 1994). Alignment of the RNA sequences of the EBHSV and RHDV genomes reveals 71% nucleotide identity, and amino acid alignment shows 78% identity and 87%

similarity (Le Gall *et al.*, 1998). Indeed, cross-infection did not occur by experimental infection of rabbits with EBHSV and hares with RHDV (Lavazza *et al.*, 1996).

A second type of virus particle (s-RHDV) is commonly found as the main component in approximately 5% of RHDV-positive specimens (i.e., those taken from rabbits showing a protracted course of the disease) (Capucci *et al.*, 1991; Granzow *et al.*, 1996; Barbieri *et al.*, 1997). The main characteristics of this particle, called “smooth RHDV” (s-RHDV) are shown in Table 1. It corresponds to the inner shell of RHDV with large amounts detected, especially from 3 to 4 days post-infection, when specific anti-RHDV IgM are appearing, but only in the liver and spleen, not the bloodstream.

Table 1: Main characteristics of smooth RHDV (sRHDV) in comparison with “full” mature RHD virions (RHDV).

	RHDV	sRHDV
Diameter (nm)	32-35	25-30
Sedimentation (S)	170	145
Structural Protein (Kd)	60	28-30
HA (extract 10%)	4-8x10 ³	neg
Infectivity (LD ₅₀) (1 ml extract 10%)	105-107	neg?
Antigenicity:		
RHDV MAbs (ext. epitopes)	pos	neg
RHDV MAbs (int. epitopes)	pos	pos
EBHS MAbs (ext. epitopes)	neg	pos
αRHDV serum	pos	pos
αEBHSV serum	neg	pos

These data, in association with the finding of fragments of the VP60 having different molecular weight (41–30 kDa), during transition from RHDV to s-RHDV, led Barbieri *et al.* (1997) to conclude that the genesis of the particle is due to a degradative process that is probably the consequence of the physiological clearance of the RHDV-IgM immuno-complex formed in large amounts at the beginning of the humoral response. Therefore, the identification of this second particle in the liver of a rabbit can be considered to be a marker of the subacute/chronic form of RHD that usually evolves between 4 and 8 days post-infection, and is followed either by the death of the rabbit or, more often, by its recovery (Barbieri *et al.*, 1997).

Virus properties and resistance

RHDV is very stable and resistant in the environment; viral infectivity is not reduced by treatment with ether, chloroform or trypsin, by exposure to pH 3.0, or by heating to 50°C for 1 hour (Capucci, unpublished data). The virus survives for at least 225 days in an organ suspension kept at 4°C, 105 days in the dried state on cloth at room temperature, and 2 days at 60°C, both in organ suspension and in the dried state (Smid *et al.*, 1991). Other studies indicate that RHDV can survive in rabbit carcasses for at least 3 months, while viral particles exposed directly to environmental conditions are viable for a period less than one month (Henning *et al.*, 2005). It also retains

its infectivity at low temperatures, and remains quite stable during freezing and thawing. Treatment of RHD virions at pH 11 induces the breakdown of the virions and the production of 6S VP60 subunits (Capucci, unpublished data). RHDV is inactivated by 10% sodium hydroxide, by 1.0–1.4% formaldehyde, and by 0.2–0.5% beta-propiolactone at 4°C, but such treatments do not alter the immunogenicity of the virus (Xu and Chen, 1989; Arguello Villares, 1991).

Epidemiological surveillance and virus variability

One of the most recurrent questions among researchers that study RHD and EBHS is: What is the origin of pathogenic lagovirus? In fact, it is at least outstanding that two similar diseases in two lagomorph species appeared almost instantaneously in different parts of the world. Thus, is there any relation in the emergence at the same time of two pathogenically-related but different viruses?

Most of the epidemiological studies conducted in recent years were only focused on trying to provide an answer to these questions. The extensive use of various serological methods, some of which show high sensibility, being based on Lagovirus “genus specific” reagents, permitted to reveal the presence of positive antibodies, both in rabbits and hares sampled before the first occurrence of the two diseases. Multiple data on seropositivity were gathered from wild and domestic rabbits in Australia, New Zealand, Italy, France, and United Kingdom and from hares in South America, Africa, and Europe, leading to the hypothesis of the existence of non-pathogenic RHDV-like and EBHSV-like viruses in rabbits and hares, respectively, from which originated the “new” pathogenic viruses during 1980. The factor responsible for the pathogenic shift and the molecular determinant for pathogenicity on RHDV genome have not yet been defined, but it is accepted that the RHDV-like viruses were originally “enteric” viruses, which then acquired the capacity to pass the mucosal barrier and to infect hepatic cells. How such an event occurred is not known, but it is almost certainly the outcome of a genetic mutation considering that RHDV, like all other RNA viruses, is endowed with considerable genetic variability.

This aspect and the rapid diffusion of RHDV throughout the world should have favoured a high antigenic variability. In spite of this, since the first identification of RHDV in 1984, all known viral isolates were considered to belong to one serotype. The complete sequence of geographically different RHD strains has been reported and their comparison reveals close overall homology in terms of genome sequence with few or no predicted changes in amino acid composition (differences between 2 and 5%) (Nowotny *et al.*, 1997; Le Gall *et al.*, 1998). Nevertheless, isolates that exhibit temperature-dependent differences in haemoagglutinating characteristics were successively described (Capucci *et al.*, 1996a) and then a consistent genetic and antigenic RHDV variant, named RHDVa, was concurrently identified in Italy (Capucci *et al.*, 1998) and Germany (Schirрмаier *et al.*, 1999).

The RHDV variants (RHDVa and other subvariants)

The variant strain RHDVa presents amino acid changes in the surface-exposed region (aa 344–434) of the VP60 that contains the main antigenic epitopes of calicivirus, which are three times higher than in all previously sequenced RHDV isolates. It does

not react in ELISA tests with the monoclonal antibody (MAb) 1H8, which is able to protect *in vivo* experimentally infected rabbits from the “classical” RHD strain, and is also less reactive with rabbit sera produced from the original RHDV isolate. However, rabbits experimentally vaccinated with the currently available RHDV vaccine were protected against challenge with RHDVa, even with a lower efficiency (Capucci *et al.*, 1998).

The production of a panel of specific MAbs was the basis for the development of specific methods in order to rapidly distinguish between outbreaks caused by RHDV and RHDVa and to enlarge the capacity to detect new possible variants. Using an ELISA test, epidemiological studies were carried out to compare the rate of diffusion of RHDV and RHDVa in Italy (Grazioli *et al.*, 2000; Lavazza *et al.*, 2004b) and elsewhere. RHDVa is present in most parts of Italy and its prevalence increased from its first reporting until present reaching values over 50% (Table 2). The highest percentages were found in those Italian regions where the most intensive rabbit production is concentrated (Lombardia, Emilia Romagna, Piemonte, Veneto and Campania).

Table 2: Total number of RHD cases observed in Italy during the last four years and relative frequency of classical (RHDV) and Variant (RHDVa) strains.

Year	Tot. examined	Total RHD pos. (%)	RHDV pos. (%)	RHDVa pos. (%)
1997	n.d.	211	191 (90.5%)	20 (9.5%)
1998	n.d.	80	66 (82.5%)	14 (17.5%)
1999	n.d.	65	39 (60.0%)	26 (40.0%)
2000	252	134 (53.2%)	89 (66.4%)	45 (33.6%)
2001	136	69 (50.6%)	25 (36.2%)	44 (63.8%)
2002	203	138 (67.9%)	61 (44.2%)	77 (55.8%)
2003	226	63 (25.9%)	12 (19.0%)	51 (81.0%)
2004	209	124 (59.9%)	32 (25.8%)	92 (74.2%)
2005	192	77* (40.1%)	32 (41.6%)	40 (51.9%)
2006	171	63 (36.8%)	27 (42.8%)	36 (57.2%)
2007	406	156 (38.4%)	61 (39.1%)	95 (60.9%)

*5 (6.5%) samples not determined.

The variant has been contemporaneously identified in Germany (Schirrmair *et al.*, 1999). It has been detected in France and on the Reunion Islands during 1999-2000, and more recent data from France indicate that it represents 10% the isolates (Le Gall, personal communication). It was identified in Malta in 2004, and it has also been reported as present in Hungary since 2003 (Matiz *et al.*, 2006). All together these data suggest that RHDVa could be diffused throughout other European countries that have been experiencing the disease for many years. Outside Europe, it caused the first outbreaks of RHD in USA in spring 2000, and again it has been identified in USA on 2005. It has also caused the first outbreak in South America (Uruguay 2004-05). Taking account of the RHDV genetic sequences deposited at the NCBI databank, the presence of RHDVa in China is also evident from 1985. No data from other countries are available.

In addition to the data on the presence and diffusion of RHDVa, an epidemiological investigation led to the identification of some other RHDV isolates that presented peculiar antigenic characteristics (Capucci, unpublished data). In fact, these RHDV strains, isolated during distinct outbreaks that occurred in widely-separated areas, and at different times since 2004 to present, show different levels of reactivity using the panel of MAbs with respect to both the classical strain and the variant RHDVa. Based on their reactivity with MAbs, these strains could be considered as further and separate steps of variation of the RHDVa, possibly classified as sub-variants. Indeed the genome sequencing confirmed the existence of some genomic differences in comparison with the RHDV strains previously identified.

Epidemiological surveillance and serological surveys

As part of the epidemiological surveillance effort, serological surveys have been performed since the first occurrence of RHD in Europe in 1989 in order to determine the presence of specific anti-RHDV antibodies in wild and domestic population and to verify the efficacy of indirect prophylaxis (vaccination) in industrial rabbitries.

The standard methods employed are competition ELISA (cELISA), which is a highly specific test that measures antibodies directed against antigenic determinants on the viral external surface (binding with high avidity), and the anti-isotype ELISA (isoELISA) that enables titration of IgA, IgM and IgG (Capucci and Lavazza, 2004). The combination of the results of both methods are critical for the interpretation of field serology (Table 3) (Cooke *et al.*, 2000), considering that rabbits with titres of 1/10 in cELISA are protected from RHD and that: i) convalescent rabbits show titres of 1/640-1/20480; ii) young rabbits (<35-40 days-old) with titres 1/80-1/320; iii) vaccinated rabbits with titres of 1/160-1/320 and iv) healthy rabbits are usually negative but they can even have titres of 1/10-1/320.

It was precisely the detection of seropositivity in the sera of laboratory rabbits taken between 1975 and 1985, which is approximately ten years before the first occurrence of the disease in Europe and on farms where the disease had never been previously reported, as well as similar serological data from more laboratories (Rodak *et al.*, 1990; Capucci *et al.*, 1991; Trout *et al.*, 1997; Marchandeu *et al.*, 1998a, 1998b), that suggested the hypothesis of the existence in domestic rabbits of one or more “non-pathogenic” viral strains antigenically related to pathogenic RHDV.

Table 3: Summary of different immunological classes developed on the basis of cELISA, isotypes titre and body weights (from Cooke *et al.*, 2000).

Class	Titre*				Notes
	cELISA	IgG	IgM	IgA	
Negative	–	–	–	–	
Pre-existing antibodies	± (rare)	+	–	–	
Maternal antibodies	+	+	–	–	Rabbits < 1300 g
Previously infected rabbits					
Recent infection	++	++	++	++	IgM > 640
Past infection	+	+	±	±	
Re-infected rabbits	++	++	–	+	IgA > 160

* ++, high titre; +, low titre; ±, not always detected ;–, no antibodies.

The apathogenic calicivirus (RCV)

The first non-pathogenic virus related to RHDV identified in healthy rabbits was named Rabbit Calicivirus (RCV) (Capucci *et al.*, 1996b, 1997). Its existence was first suspected when we observed the spontaneous seroconversion of rabbits reared in the animal facility of our institute in the absence of signs of the disease and mortality; we then succeeded in reproducing the phenomenon under controlled conditions (Capucci *et al.*, 1995b).

RCV may be considered an “enteric”, non-pathogenic virus highly correlated to RHDV, perhaps its progenitor. It is able to “persistently infect” commercial rabbit farms and it works as a natural vaccine. RCV significantly differs from the previously characterised RHDV isolates because it is avirulent, replicates in the intestine at a low titre, and presents a genomic identity with RHDV of around 92%. The RCV amino acid sequence of the main capsid protein (VP60) between aa 300 and 311 is the more divergent among all the known sequences of lagovirus. Therefore, this area of the VP60, which is known to be highly variable also among different feline calicivirus isolates showing different degree of pathogenicity (Seal, 1994), could hypothetically influence the degree of pathogenicity of the caliciviruses. Experimental infection of hares with RCV failed and the antigenic data and sequence comparisons demonstrated that RCV is much more closely related to RHDV than to the EBHSV (Capucci *et al.*, 1996b).

The diffusion of RCV in different commercial rabbitries from different areas of Italy has been evaluated during five separate serological surveys by checking at slaughtering the presence of anti-RHD antibodies in non-vaccinated meat rabbits from farms with no evidence of overt RHD clinical disease. These surveys were conducted during 1999 in North Italy (Veneto and Lombardia regions), during 2002-2003 in Central and South Italy (Lazio, Campania and Basilicata regions), in 2004 and 2006-2007 in Central Italy (Marche region) and in 2007-2008 again in North Italy (Lombardia and Veneto regions). The results (Capucci *et al.*, 2004; Lavazza *et al.*, 2007; Capucci and Lavazza, unpublished data) clearly show that antibodies reactive with RHDV are present in several rabbit populations (Table 3). In particular, by using standard cELISA, we found anti-RHDV antibodies in almost 30% of controlled farms with

over 80% of animals having titres $\geq 1/20$ (up to 1/1280). In addition, by using anti-isotype ELISAs, clearly shown was the presence of IgA (and sometimes IgM), proving that an active infection had occurred. In addition, during the last survey it was possible to identify the viral strains by using PCR methods on faeces (Capucci *et al.*, unpublished data).

The RHDV-like viruses

The existence of non-pathogenic caliciviruses in wild rabbits was suggested (Trout *et al.*, 1997; Marchandeanu *et al.*, 1998a, 1998b; O’Keefe *et al.*, 1999; Robinson *et al.*, 2001; Cooke, 2002; Cooke *et al.*, 2002) to explain the early discrepancies found in serological surveys of rabbit populations in European countries, Australia and New Zealand.

RNA particles related to RHDV were shown in rabbit sera collected since 1955 in Britain, confirming that RHDV-like viruses were present in Europe for many years before the first evidence of RHD (Moss *et al.*, 2002). High antibody levels detected after RHD first began to spread through Europe but in areas where RHD had never been recorded nor suspected, provided further serological evidence that non-pathogenic strains might be present in wild European rabbit populations (Trout *et al.*, 1997; Marchandeanu *et al.*, 1998a, 1998b). In addition, more recent data (Marchandeanu *et al.*, 2005) provide evidence for the existence of non-protective antibodies raised against a putative RHDV-like virus.

The serological data obtained on rabbit sera in Australia and New Zealand (O’Keefe *et al.*, 1999; Cooke *et al.*, 2000, 2002; Nagesha *et al.*, 2000; Robinson *et al.*, 2001; Cooke, 2002) clearly show that antibodies reactive with RHDV were present in feral rabbit populations before the introduction of RHDV. Furthermore, the comparison of the results obtained using different ELISA systems, providing different levels of specificity but higher sensitivity indicated that the major part of these antibodies was cross-reactive antibodies, recognizing antigenic determinants buried inside the structure of the RHDV capsid (i.e., “common” epitopes considered as “group specific” in all the calicivirus of lagomorphs) (Capucci and Horner, unpublished data). Therefore, the presence and circulation of a putative non-pathogenic RHDV-like virus in feral rabbit populations being able to induce antibodies partially cross-reactive with RHDV was postulated. In addition, the serological data (average of titres found in Europe by testing with cELISA the sera of animals infected with RCV were 8 to 16 times higher than the titres found among the feral rabbit sera from New Zealand and Australia) indicated that such a putative RHDV-like virus is characterized, differently than RCV, by a consistent genetic and antigenic difference from RHDV, estimable in more than 40% of amino acid substitutions in the outer part of the VP60 (Capucci, unpublished data). The very recent isolation and identification of one of these viruses in Australia (T. Strive, CSIRO Entomology Division - Canberra, personal communication) finally confirmed this hypothesis.

The possible role of these non-pathogenic RHDV-like viruses in reducing the impact of RHD by conferring a cross-protection was also discussed (Cooke, 2002; Cooke *et al.*, 2002; Marchandeanu *et al.*, 2005). The main question was whether these antibodies interfere with RHDV infection and the course of the disease, but the data

obtained suggest that was not the case. In fact, there is a strong correlation between the titre in cELISA and the state of protection (i.e., animals with titres $\geq 1/10$ are immune, but only when antibodies are specifically induced by RHDV or RCV). To the contrary, the antibodies directed towards internal determinants (cross-reactive antibodies) have little or no importance from a protective point of view; they are not neutralising and do not interfere with the RHDV infection (Cooke *et al.*, 2002; Marchandeau *et al.*, 2005).

In conclusion, even if the existence of non-pathogenic RHDV-like viruses in wild and domestic rabbit populations have been proven by valid serological and virological data, their epidemiological importance remains largely unknown.

Moreover some questions still need to be answered from further scientific studies. For example, are rabbits quickly building resistance to RHDV infection? Are changes in viral RNA sequences associated with virulence changes? Therefore, it will be therefore very interesting to follow the evolution in Oceania of the relationship between rabbits and the virus that cause RHD, in comparison with the previous experience of the deliberate introduction of the Myxomavirus (i.e., if a small round RNA virus, such as RHDV will evolve in less virulent strains and will select in resistant populations of rabbits as occurred for Myxomatosis virus, a large DNA virus).

VIRAL ENTERITIS OF RABBITS

Enteric diseases have an important role in the rabbit industry since they can produce severe economic losses due to mortality, growth depression and worsening of conversion index. The “multifactorial enteropathy”, known also as “enteric syndrome”, is the most important “conditioned” diseases, especially in relation to its productive and economic impact. It is a pathologic complex of growing rabbits characterized by a great number of stressors and pathogens acting in synergy with a different degree of virulence (i.e., various aetiological agents (viruses, bacteria and protozoa) that can act together to cause tissue damage at the gut level, thus determining severe diarrhoea and malabsorption).

Among the different pathogens that could be found in rabbits suffering from enteropathy, viruses seem to have an important but not definitive role. Viruses should not be able to induce primary episodes of high gravity but, acting as mild pathogens, they could have the capacity of becoming endemic.

In intensive rabbit breeding systems, this condition is characterised by intense genetic selection, exasperated by high productive performances, and sometimes by overpopulation and consequently high environmental load with facultative pathogens. Therefore, viruses and other low pathogenic agents (es. flagellata protozoa), can implicate a more important role for the occurrence of severe enteritis in rabbits, predisposing and aggravating secondary microbial infections. One possibility, already proposed by others, is that viruses can primarily cause damage to the intestinal mucosa, thus predisposing the attachment and replication of bacteria. In such cases it is not excluded as a dose dependant effect, as well as a transient infection and a short period of excretion, thus making possible the detection of viruses in association with the presence of *E. coli*, *Clostridium* spp, *Coccidia* and other protozoa.

On the other hand, we can not exclude that the change in physiologic and metabolic conditions, induced to enteric level by various factors, both alimentary or not, can enhance the replication of viruses normally present at a lower concentration, permitting them to trigger a pathogenic reaction.

Rotavirus

The Group A rotavirus, a member of the *Reoviridae* family, is considered to be the main cause of acute viral gastroenteritis in different animals including rabbits (Schoeb *et al.*, 1986; Thouless *et al.*, 1996). Lapine Rotavirus (LRV) is considered only mildly pathogenic (Thouless *et al.*, 1988), but it can primarily cause enteric disease in post-weaning rabbits and be involved in the aetiology of more severe enteritis outbreaks in association with other viruses, bacteria (*E. coli*, *Clostridium* spp) and parasites. Rabbits become infected by the oro-fecal route, and the extension and the severity of the lesions are dose dependent (i.e., the consequences of the infection (microvillus degeneration, malabsorption and diarrhoea) are higher when the infectious dose is also high).

The persistence of maternal antibodies until 30 to 45 days can reduce the symptoms of the disease. Thus, until 4 to 5 weeks of age, rabbits mostly became sub-clinically infected with particle excretion for only 3 days. The LRV infection is more frequent in growing rabbits (35 to 50 days old) and is characterised by a high rate of morbidity, with non-specific clinical signs (i.e., diarrhoea, anorexia, and depression). Diarrhoeic symptoms appear at the beginning of viral excretion that lasts for 6 to 8 days, and are generally followed by constipation. Lesions observed at necropsy are not constant: catarrhal, haemorrhagic or necrotic entero-tiflitis and caecal impaction. Meat rabbits suffering from enteritis can die due to dehydration and secondary bacterial infections. In rabbits that recover from the infection, a decrease in productivity is commonly observed due to reduced absorption capacity.

A virological diagnosis can be achieved by testing faeces and intestinal contents by ELISA, including negative staining by electron microscopy (nsEM) and PCR. LRV was detected in 16.4% (Nieddu *et al.*, 2000) and 23% of post-weaning rabbits with enteric signs (Cerioli *et al.*, 2004); however, sero-epidemiological surveys have shown that most adult rabbits are seropositive for rotavirus, thus indicating that there is normally a constant circulation of low amounts of rotavirus in industrial rabbit farms (Peeters *et al.*, 1984; Di Giacomo and Thouless, 1986). The introduction of breeders of unknown origin, without application of a quarantine period, is an important risk factor. Thus, a reduction in biosecurity and hygienic activities (e.g., cleaning, disinfection, removal of litters) can lead to a dramatic increase of the environmental contamination with rotavirus.

The classification of rotavirus strains is based on the characterization of two outer capsid proteins, VP4 and VP7, the main antigenic determinants that independently elicit neutralising antibodies and induce a protective immunity response. Based on both antigenic or genetic characterization, 15 VP7 types (G types) and 26 VP4 types (P genotypes) have been recognized (Estes, 2001). A few LRV strains have been analysed in detail in early investigations. Analyses of the few strains identified in various parts of the world (Canada, USA, Japan, Italy) have revealed a substantial

antigenic/genetic homogeneity of LRV's, as all the viruses analyzed so far belong to the VP7 serotype G3 (Petric *et al.*, 1978; Sato *et al.*, 1982; Conner *et al.*, 1988; Thouless *et al.*, 1988; Ciarlet *et al.*, 1997) and to the VP4 serotype P11[14] (Ciarlet *et al.*, 1997; Hoshino *et al.*, 2002). The epidemiological surveys carried out to investigate the distribution of the VP7 and VP4 antigenic specificities of LRVs in Italy are fully reported by Martella *et al.* (2003, 2004, 2005). Almost all the strains were characterized as P[22],G3 (Martella *et al.*, 2005), confirming the presence of the newly-recognized rotavirus P[22] VP4 allele in Italian rabbits. Only one P[14],G3 LRV strain was identified and two samples contained a mixed (P[14] + [22],G3) rotavirus infection. All the LRV strains analysed exhibited genogroup I VP6 specificity and a long dsRNA electropherotype. However, one of the P[14],G3 strains possessed a super-short pattern. Overall, these data highlight the epidemiological relevance of the P[22] LRV's in Italian rabbitries.

Coronavirus

Rabbit Coronavirus (RbCoV) is an unassigned member in the *Coronaviridae* family. It has been described as an agent of two different pathologic forms in the rabbit: a systemic disease (known as pleural effusion disease or cardiomyopathy of rabbit) and an enteric disease (Lapierre *et al.*, 1980; Osterhaus *et al.*, 1982). The systemic disease is characterized by fever, anorexia, leucocytosis, lymphocytopenia, anaemia, hypergammaglobulinemia, iridocyclitis, which is often followed by death. The lesions are localized to the myocardial and pleuric levels. The enteric disease shows the lesions and symptoms typical of enteritis caused by coronavirus in other species. The RbCoV replicates in small intestine with necrosis of apical villi and is followed by diarrhoea (Descoteaux *et al.*, 1985; Descoteaux and Lussier, 1990). A high prevalence has been found in seroepidemiological surveys (Deeb *et al.*, 1993), indicating a wide diffusion of the RbCoV in rabbitries.

Diagnosis of coronavirus could be achieved by using negative staining electron EM. The important increase of coronavirus-like positivity from our previous surveys (Nieddu *et al.*, 2000; Cerioli *et al.*, 2004) suggests the need for further improvements for studies of this agent; however, which role as either an enteric and/or systemic pathogen has not yet been completely determined. Serological surveys performed in three rabbitries, using an indirect ELISA based on the use of cross-reactive reagents for Bovine Coronavirus (BCV), indicated a widespread seroprevalence. However, by testing with a sandwich ELISA for BCV 16, samples resulted in positive cases by EM for coronavirus-like particles; we only detected a faint positivity in 6 of samples. We also tried to isolate *in vitro* the virus and to define its haemagglutination properties: RbCoV, similarly to bovine BCV, seems to grow in the HRT18 cell line, and it haemagglutinates mouse red blood cells but not those of rabbit. In these surveys coronavirus was frequently associated with other viruses (mainly with rotavirus), accounting for 80% of its association during the period 2002-2004, so it could be possible that it can act together with viral and bacterial agents to determine post-weaning enteritis. Therefore, the pathogenic role as a cause of primary enteric disease was not evident, but the widespread nature of the virus and its high seroprevalence (100% farms, 3 to 40% rabbits) as observed by other authors suggest the possibility of subclinical infection and a probable role as an opportunistic pathogen.

Other viruses

The Rabbit Parvovirus, first described by Matsunaga *et al.* (1977), has very low pathogenicity and it is commonly isolated from the gut contents of healthy animals. It could cause very mild clinical signs (lethargy, disorexia, and depression) in experimentally infected animals and a mild to moderate enteritis in the small intestine (Matsunaga and Chino, 1981). Its primary pathogenic role is still unclear, but considering its frequency of identification, it could be important only in multiple infections when combined with other infectious agents (viruses, bacteria, and other parasites).

Some of the other viruses detected during diagnostic activity has only had a sporadic occurrence, thus their pathogenic role is probably negligible. Adenovirus has been previously reported only once (Bodon and Prohaszka, 1980). Reovirus and enterovirus have never been described as enteric agents of rabbits. However, we cannot exclude that enterovirus-like particles correspond to picobirnavirus (Gallimore *et al.*, 1993), stating that strict morphological similarities exist with this group of non-cultivable RNA viruses as identified in several species (humans, pigs, chickens, guinea pigs) including rabbits. Lusert *et al.* (1995) found that picobirnavirus was commonly excreted by 10% of rabbits without causing any symptoms or lesions. A cultivable calicivirus, *genus vesivirus*, has been recently identified from juvenile growing rabbits showing symptoms of diarrhoea (Martin-Alonso *et al.*, 2005) and it was shown to be neither related to Rabbit Haemorrhagic Diseases virus (RHDV) nor to Rabbit Calicivirus (RCV).

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**PREVALENCE OF *STAPHYLOCOCCUS AUREUS* BIOTYPES IN
COMMERCIAL RABBIT FARMS**

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A cross sectional study was undertaken to estimate *Staphylococcus aureus* prevalence in rabbit flocks under rational production conditions. Fifteen farms were by convenience selected and 60 does were systematically sampled. Nose, ear, abdomen and interdigital skin swabs were collected from 900 does and data about flock production and flock health condition were recorded.

Results report a mean inside flock *S. aureus* prevalence of 77.6% (CI95% 66.4-88.2) of the selected sample. Three *S. aureus* biotypes were detected: the human one and two non host specific biotypes, mixed CV-C and mixed CV-A. Most of the flocks displayed *S. aureus* strains of just one biotype. However, in four flocks two biotypes were isolated: three flocks where one biotype was predominant and one unit where mixed CV-C and human biotypes were uniformly distributed. The among flocks distribution of *S. aureus* biotypes was correlated with the commercial source of breeders. Finally, statistic analysis of productive parameters related the presence of the mixed CV-C biotype to a lower number of weaned rabbits.

VALIDATION OF A SAMPLING METHOD TO DETECT HEALTHY RABBIT *STAPHYLOCOCCUS AUREUS* CARRIERS

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Chronic staphylococcosis, mostly caused by highly virulent *S. aureus* strains classified within the mixed CV-C biotype and the 3A/3C/55/71 phagetype, offers an ideal eradication policy target because of its low diffusivity among animals, but may cause serious health impairment and it is difficult to control due to drug resistance and lack of vaccines. The main risk for staphylococcosis introduction is purchase of healthy carriers. The identification of healthy carriers of high virulence *S. aureus* is currently based on cultivation of the micro-organism and its characterization using traditional bacteriological methods. Biomolecular methods to distinguish high and low pathogenic *S. aureus* have been recently described, but they need proper in field validation and are not yet available for rabbit *S. aureus* high pathogenic screening. The standardization of sampling protocols that meet the needs of both increased analytic sensibility and technical and economic feasibility is of importance for a good screening and culling policy. This study aimed to evaluate a sampling method, especially looking for ideal body sites, for surveys based on rabbit as primary unit, and samples to be processed by traditional culture method prior to further characterisation of isolates. One hundred clinically healthy rabbits were enrolled. Each subject was sampled in four body sites (N: nose; E: outer ear; L: interdigital skin of hind legs; A: abdomen). Sterile cotton swabs scraped on skin surface were pre-incubated in Heart Infusion Broth (Oxoid) and then inoculated on a Baird-Parker Agar Base (Oxoid) which was RPF Supplement (Oxoid) enriched. Culture medium method with the biochemical identification of *S. aureus* by micromethod ID 32 STAPH (BioMérieux) was considered as the gold standard implying 100% specificity. Any rabbit with at least one positive sampling was considered a “true positive”, rabbits with all four samplings negative were considered “true negatives”. 70% of analysed subjects hosted *S. aureus*. Sensibility was calculated for each body site and different combinations of two or more sample sites provided the following results: E=72.9%; A=52.9%; L=51.4%; N=64.3%; E+A=81.4%; E+L=81.4%; E+N=88.6%; E+A+L=88.6%; E+A+N=92.8%. The sampling and analysis procedure adopted resulted in a high percentage of *S. aureus* rabbit isolation. High sensibility of the E+A+L sample combination (88.6% sensibility; 95%CI: 81.1-96%) and the ease of sampling from the cutaneous areas while excluding the nose make this method of interest for detecting healthy rabbit *S. aureus* carriers.

CLOSTRIDIUM SPIROFORME DRUG SUSCEPTIBILITY

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Clostridium spiroforme causes severe rabbit gastroenteritis with subsequently important production losses. Outbreaks are controlled with therapy but field data report frequent failures and suggest *C. spiroforme* being of high drug resistance. In order to establish whether therapy failure is due to intrinsic or acquired drug resistance, 30 field strains of *C. spiroforme* were tested against spiramycin, amoxicillin, doxycycline, sulfadimethoxine, norfloxacin, and tiamulin, respectively representative of the following classes of antimicrobials: macrolides, β -lactams antibiotics, second-generation tetracyclines, sulfonamides, quinolones and diterpenes. The minimum inhibitory concentration (MIC) was determined with the agar diffusion method (NCCLS M11-A6 manual, 2004). Resulted MICs were: sulfadimethoxine MIC₅₀: 256 μ g/ml and MIC₉₀: 256 μ g/ml; spiramycin MIC₅₀: 256 μ g/ml and MIC₉₀: 256 μ g/ml; tiamulin MIC₅₀: 64 μ g/ml and MIC₉₀: 128 μ g/ml; norfloxacin MIC₅₀: 32 μ g/ml and MIC₉₀: 32 μ g/ml; amoxicillin MIC₅₀: 0.063 μ g/ml and MIC₉₀: 0.063 μ g/ml; doxycycline MIC₅₀: 8 μ g/ml and MIC₉₀: 16 μ g/ml. Among all antimicrobials tested, only doxycycline showed MICs supposed to be of therapeutic efficacy. Results support the field hypothesis of an extensive acquired resistance of *C. spiroforme* to antimicrobials and the presence of intrinsic resistances of importance. Both findings suggest the necessity to give greater priority to prophylaxis of enteritis caused by *C. spiroforme* by attempting to reduce risk factors rather than controlling outbreaks by therapy. The ambitious and complex objective can be pursued by optimising technical management and by a prudent use of antimicrobials.

PREVALENCE AND MOLECULAR CHARACTERIZATION OF *CLOSTRIDIUM DIFFICILE* ISOLATED FROM RABBITS AND DETECTION OF ITS MAIN TOXINS

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This study investigated the prevalence of *Clostridium difficile* and its toxins in 132 Italian rabbit farms. Intestinal contents were collected from the small intestine and the caecum of 317 rabbits affected by enteric diseases and 80 control rabbits. Samples were stratified by age (<35 days, 35-55, >55, breeders) and enteric lesions (caecal constipation or fluid-filled caecum). The intestinal contents were cultured on selective media and screened for *C. difficile* toxins A and B production using a commercial ELISA. *C. difficile* isolates were tested by multiplex PCR to confirm the biochemical identification and to assess the presence of *tcdA*, *tcdB*, *cdtA*, *cdtB* genes encoding for toxin A, toxin B, and the two components of the binary-toxin respectively. The isolates were analyzed by PCR-ribotyping to investigate their genetic relatedness. *C. difficile* was recovered from the intestinal content of 10 rabbits older than 35 days with enteric disorders in 7 farms (prevalence=5.1%). *C. difficile* was not isolated from control rabbits. Eight strains resulted positive for both *tcdA* and *tcdB* genes. One strain was *tcdA/tcdB*, whereas another resulted *tcdA/tcdB*⁺ and was also positive for both genes encoding for the binary-toxin. Toxin A and B were detected in four of the 10 samples positive for *C. difficile*. Typing analysis demonstrated that 5 strains isolated in two different farms belonged to the same clonal group that is also responsible for enteric disease in humans. The results show that *C. difficile* is occasionally involved in outbreaks of enteric disease in rabbits.

SEARCH FOR KEY HEALTH AND WELFARE INDICATORS FOR MEAT RABBIT PRODUCTION AND DEFINITION OF A SCORE METHOD OF EVALUATION

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The aim of this study is to define parameters related to health and welfare of animals even in industrial farms with intensive husbandry. Therefore, we tried to increase the knowledge on rabbit welfare by the use of correct tools for monitoring the different aspects of rabbit industrial farming, in order to improve the efficiency of farms and the productive performances of animals. Our study is based firstly on the characterization of health, management, environmental and physiological parameters and, secondly, on the definition of protocols to assess welfare of rabbits. All the entered values are then utilized for a score system to establish health and welfare conditions in industrial farms.

TOXIN-GENOTYPING OF *CLOSTRIDIUM PERFRINGENS* STRAINS ISOLATED FROM RABBITS WITH ENTERIC DISEASE

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In commercial rabbitries, enteritis are considered a major cause of economic loss. Among the infectious agents responsible for enteritis/enterotoxaemia, *Clostridium perfringens*, *C. difficile* and *C. spiroforme* are the most common isolates even if the role played by *C. perfringens* in rabbit enteritis is still under debate. In one commonly-used toxin typing scheme, *C. perfringens* isolates are assigned to one of five types (A-E), depending on the production of the following major toxins: α , β , ϵ , and ι . Other toxins, such as enterotoxin, traditionally associated with type A, and toxin β_2 , are involved in the pathogenesis of enterotoxaemia in animals. Type A *C. perfringens* strains, for example, are commonly found in the intestinal tract of both humans and animals. In the past, type E *C. perfringens* has been considered the etiological agent of rabbit enteritis, even if various authors failed to isolate it from the caecum of diseased rabbits. The pathogenic role of *C. spiroforme*, mediated by the production of an iota-like toxin, was demonstrated. This binary toxin has a functional and antigenic resemblance with the binary iota toxin, typical of type E *C. perfringens*. Enteritis caused by clostridia are conditioned pathologies. Errors in diet, stress, and drug administration, in fact, can all cause alteration of bowel microflora that leads to an overgrowth of *Clostridium spp.* The aim of this study was to investigate the distribution of toxin types in *C. perfringens* field strains, isolated in rabbit enteritis. One hundred and fifty colonies of *C. perfringens*, each randomly chosen from a culture obtained by streaking the caecal content of diseased rabbits, were initially subjected to PCR for toxin type identification. In order to verify whether one or more *C. perfringens* toxin types was present in the rabbit gut at the same time, 14 animals positive for *C. perfringens* were studied and 5 different *C. perfringens* strains collected from each one were toxin-genotyped for a total of 70 strains. The results showed type A to be the type most commonly recovered and the analysis of the distribution of the toxin types recovered from a culture confirmed the uniform distribution of toxin types; then one colony seemed to be representative of all the types recovered in the culture. This might prove useful in clostridiosis diagnostic procedure. A high percentage of the new toxin type A+ β_2 was observed to be present among toxin type A; the gene codifying for β_2 toxin is revealed to be present in association with type E, too. The new toxin type E+ β_2 was therefore discovered in diseased rabbits. Moreover, the absence of enterotoxin poses no little amount of concern to public health, because this toxin is involved in human foodborne disease. Further studies are required to verify the exact role played in the pathogenesis of this conditioned pathology by both toxin type A and the newly discovered toxin, β_2 .

LABORATORY LIMITS ON DERMATOPHYTE DIAGNOSIS IN RABBITS WITH CLINICAL LESIONS

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Dermatophyte infection or ringworm is a superficial cutaneous infection with one or more of the fungal species of the keratinophilic genera *Microsporum*, *Trichophyton*, or *Epidermophyton* and is a zoonosis with a great impact on Public Health. Dermatophytes were identified from rabbit sample cultures submitted to mycological examination in the Laboratory of Microbiology of the University of Trás-os-Montes e Alto Douro, Vila Real, Portugal. All samples were collected from suspected clinical cases. Dermatophytes were cultured from 4 of the 55 specimens (7.3%). The dermatophytes isolated were *Trichophyton mentagrophytes* var. *mentagrophytes* (1/4) and *Microsporum gypseum* (3/4). Microscopic examination was negative in all specimens. In this work, *Scopulariopsis* spp., a contaminant mould, was identified in 13 specimens (23.6%). The proportion of positive samples in relation to the number of samples examined from cases suspected was very low. As all samples were collected from rabbits with compatible signs, we presume that the low prevalence of isolation was due to laboratory constraints on dermatophytes diagnosis.

COOLING PANEL TYPE AND LOCATION INFLUENCE NEST TEMPERATURE, GROWTH AND SANITARY STATUS OF RABBITS DURING THE COLD SEASON IN HUNGARY

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Evaporative cooling panels fitted to the wall of rabbit houses can have adverse effect on the nearby nests and newborn rabbits, especially in the cold season of continental climate as in Hungary. Changing the way of installation could prevent that. This study was performed at winter in two buildings of a commercial rabbit farm in Hungary which were different only in the way of installation of the evaporative cooling panels. In building A the panel was fitted immediately onto the wall as a single sheet, whereas in the Building B a small room was joined and the panel was fitted onto the wall of that in the form of three smaller sheets but with the same total surface. Air temperature (7.5°C vs. 14.1°C), nestbox wall temperature (14.8°C vs. 18°C), nest temperature (27.2°C vs. 28.8°C) and newborn skin temperature (35.4°C vs. 36.2°C) were significantly ($P < 0.001$) different in building A when measured at the panel or at the end of this building (30-40 m far from the panel). This difference was not present in building B. Temperature gradients between the newborn skin and the nest, as well as between the nest and the nest box wall were high, what means that the heat flow was small and the insulation provided by the nest material was good. Installation type did not affect litter performance, even if higher probability that a kit is dying before 21 days of age was found in each building in the nest boxes placed near to the panel (0.21 vs. 0.13 in A and 0.27 vs. 0.16 in B, $P < 0.005$). The frequency of the *P. multocida* carrier does at day 7 post partum was higher at the end (0.35) of the two buildings than in front of the panel (0.23), but the difference was not significant. In summary, fitting of cooling panel could decrease inside air, nest box wall, nest and newborns skin temperature during the cold season in Hungary. However, insulation provided by the nest material ensures a near normal body temperature even in front of the panel. Installation type of cooling panels thus could influence only inside air and nest box wall temperatures.

INFECTION WITH *PASTEURELLA SPP.* IN A NEW ZEALAND RABBIT COLONY

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The purpose of the research was to establish the etiology and to fight against the acute respiratory syndrome to New Zealand rabbits, of all ages to which the morbidity and mortality were high, from a farm with intensive breeding. Clinical 760 rabbits were examined, adults, young and new born raised in wire net cages in a closed stable, without draught. The necropsy examination was performed in laboratory on 57 dead rabbits with acute respiratory and also cultivations from trachea, lung, liver and spinal marrow were carried out. We used the bacterioscopic technique, cultivation on usual and selective media for *Pasteurella*, *Mycoplasma* and *Streptococcus* for isolation and identification of the micro-organisms present in the respiratory tract. Out of the 57 examined samples, 28 ones were positive and identified as *Pasteurella spp.*, of which 21 were isolated from trachea, lung and liver and 7 from spinal marrow. Seventeen samples of the 28 examined ones contained both *Pasteurella spp.* and *Mycoplasma spp.* and 11 samples *Pasteurella spp.* and *Streptococcus spp.* also isolated and identified in trachea and lung. The antibiogram test used bio-discs with penicillin, amoxicillin, gentamicin, spectinomycin, oxytetracycline, erythromycin, enrofloxacin and cefaclor. The rabbits were divided into two groups: the first group was treated with enrofloxacin for 5 days, and the second group was treated with oxytetracycline for 5 days, both administered in drinking water. One day after the beginning of the treatment the rabbits got better and the mortality stopped after the fourth day of treatment.

PCR IDENTIFICATION AND TOXIN-TYPING OF *CLOSTRIDIUM SPIROFORME* FIELD STRAINS

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Rabbit diarrhoea caused by toxigenic *Clostridium spiroforme* is responsible for significant losses in commercial rabbitries. This micro-organism usually affects rabbits during weaning and its pathogenicity is related to the production of a strong cytotoxic binary toxin that is similar in function and structure to *Clostridium perfringens* type E iota toxin. The definitive diagnosis of *C. spiroforme* enterotoxaemia is currently difficult due to the absence of both biochemical commercial panel and bio-molecular methods for specific identification.

The aim of this study was therefore initially to develop PCR protocols for the specific detection of *C. spiroforme* and its binary toxin coding genes and then apply them in a field study. A total of 80 *C. spiroforme* field strains isolated from rabbits with intestinal disorders were analysed; 30 isolates were obtained from the caecal content of rabbits with anatomopathologic evidence of enteritis caused by *C. spiroforme*, 19 from rabbits for which *C. spiroforme* was a co-agent of infection, and 31 from rabbits with intestinal diseases not caused by *C. spiroforme*. All strains were positive for species-specific identification and the presence of the genes coding for *C. spiroforme* binary toxin.

In conclusion, we developed a useful and reliable biomolecular tool for specific identification of *C. spiroforme* and detection of genes coding for its binary toxin. When subsequently applied in a field study, these PCR protocols demonstrated all the strains analysed to be potentially toxigenic.

ESTABLISHMENT AND APPLICATION OF REVERSE TRANSCRIPTASE-POLYMERASE CHAIN REACTION FOR DETECTION OF RABBIT HEMORRHAGIC DISEASE VIRUS

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A Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) method to detect *Rabbit hemorrhagic disease virus* (RHDV) was established. Total RNA was extracted from the fresh liver of 53 rabbits died of RHDV collected from different region in Jiangsu and Shandong province during spring in 2007. A pair of primers was designed according to the sequences of RHDV published in GenBank, which could be amplified a 269 bp segment. The result of sequencing showed that the homology was 95.8-98.5% compared with the reference sequence published in GenBank. The least amount of RHDV that could be detected was 3.32 ng/ μ l, which also showed its high sensitivity and, what's more, the sensitivity was 4×10^4 times higher than that of hem agglutination (HA). The detection of RHDV by RT-PCR was established successfully. Compared to HA, RT-PCR could detect RHDV in all the viscera except for in feces, and HA only in liver, while kidney, spleen, blood and lung were positive. Samples kept at -20°C for 12 months could be detected by this RT-PCR. All above results indicate that this RT-PCR method has strong specificity, high sensitivity and good repetition, and can be used in RHDV clinical diagnosis, epidemiology study and quarantine of rabbit products such as meat.

RABBIT HEALTH CONTROL BY MANAGEMENT

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Cumulative mortality by Epizootic Rabbit Enteropathy was recorded from 1999 by the fattening section of the Experimental Centre in Viterbo and by a commercial unit. Both units utilized the same alternative open air system of a group of farmers that adopted a not pharmacological control of pathologies. When the enteropathy appeared, the commercial unit had to adopt a prophylactic antibiotic treatment (Tiamuline 60 ppm and Apramycin 70 ppm) for four weeks after weaning. The rabbits were treated until 2003 when the evolution of the not pharmacological prophylactic strategy became effective. Results were compared for 5 years, showing how the mortality after weaning could be progressively reduced from 43.4% to 7.0% (no pharmacological treatment) and from 16.8% (pharmacological prophylaxis) to 8.5% when the pharmacological treatment was suspended. This was obtained, after successive trials, reducing the density in the fattening cages from 4 to 3 subjects, stamping out the rabbits at the first suspect of not perfect health and isolating the other animals of the same cage. A sequence of 88 consecutive days with no mortality was recorded in 2007 when a mean of about 150 rabbits was present in the open air fattening area of the Centre. To explain these results the mortality from the third week after weaning was compared in the cages where in the first two weeks there were cases suspected of enteropathy and in the cages where this was not observed. The results confirmed that the direct or indirect (drinkers and feeders) contact with rabbits showing the first symptoms of enteropathy is related with the spreading of the sickness (29.1% in comparison with 0.7% of the control; $P < 0.001$). The many empiric trials performed from 1999 show that the alternative open air keeping units can be maintained in a very good healthy state without any pharmacological control of pathologies, enteropathy included. Among the many attempts to get this result the most effective were the reduction of the density of the animals in the fattening cages, the immediate stamping out of each suspected rabbits and the isolation of the other subjects present in the same cage. Observing these rules it is proved that global mortality can be maintained under 10% in the growing period.

AN OUTBREAK OF MYXOMATOSIS IN GREEK RABBIT FARMS DURING 2007

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Myxomatosis is considered as one of the most frequent epizootic viral diseases of rabbit all over Europe. In Greek rabbitries, where all parent stock is exclusively imported from European countries with high myxomatosis incidence, surprisingly the presence of the disease was limited. In the present study, the diagnosis and isolation of a wild, highly pathogenic Myxoma virus is reported, causing a myxomatosis outbreak in Greece. The last myxomatosis outbreak in Greece was reported in 1973 and till February 2007 no further cases had ever been reported. No vaccination strategy against Myxoma virus was ever been applied and early in the spring 2007 the spread of the disease was inevitable, as no special preventive measures had ever been taken for the imported carrier animals. At that period, a myxomatosis outbreak was reported in two commercial rabbit farms, with a population of 500 and 800 does, situated within a 150 km distance that shared the same parent stock supplier. In both farms, morbidity and mortality reached 80-90% and myxomatosis diagnosis was based on clinical signs and laboratory confirmation that included virus isolation, histopathology profile and molecular detection by standard PCR. Samples from 15 fattening rabbits were processed for virus isolation according to OIE standard protocol using the established RK-13 cell line. Typical Myxoma virus cytopathogenic effect was always present after the second passage. Apart from the pathognomonic gross facial lesions, samples from eyelids and dermis presented histologically myxoid material mixed with collagenous tissue. Total DNA was extracted from tissues carrying lesions and from infected RK-13 cell culture supernatants and a 492bp segment of the major envelope gene was amplified. The specific PCR product was further analyzed by sequencing. Sequence alignment was performed against all available Myxoma virus genome sequences in Genbank. Compared to the major envelope gene of the Lausanne strain, the Greek isolate exhibited a 96% sequence homology and was submitted to Genbank and assigned accession number EU399816. Disease eradication was based on euthanasia that was performed to the total rabbit farm population, together with thoroughly applied hygienic measures. Preventive policy by vaccination program was not encouraged by the national veterinary authorities, in order to be maintained the myxomatosis free status of Greece. On the other hand, veterinary services were alerted and quarantine measures were strongly recommended to rabbit farmers every time that rabbit parent stock was imported. Preliminary genetic analysis of the Greek viral isolate indicates sequence identity with the Lausanne reference strain.

RATIONING OF DRINKING WATER SUPPLY IN RELATIONSHIP WITH GROWTH AND SANITARY PERFORMANCES OF GROWING RABBITS

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The aim of the research was to try to control the epizootic rabbit enteropathy and improve performances by rationing the same amount of water when a feed without antibiotics was administered. Different ways of water supply for a total of 4 hours a day were tested in an industrial rabbit farm. A total of 480 rabbits were utilized in two trials of 240 subjects each. The rabbits, weaned at the age of 35 days, were randomly distributed into 4 homogeneous groups with 10 replications of 6 subjects each. The control could drink *ad libitum* for 24 hours a day. The other three treatments received water according to different supply planning: once a day for 4 hours; twice a day for 2 hours each time and four times a day for 1 hour respectively. Indicating the time of water supply followed by the time of not supply, the treatments were indicated as 24/0 (control), 4/20, 2/10 and 1/5 respectively. Except the control, all the experimental treatments received water for 4 hours a day globally. In the first trial growth was not significantly different among the treatments. Feed conversion rate was lower than 4.5 in the 24/0 and 4/20 treatment, but rose to 5.9 and 5.1 in the 2/10 and 1/5 treatments, respectively. These results were related with the high mortality rate, since water restriction was not showing any certain positive effect. The values of mortality were 20%, 23%, 35% and 28% respectively for the four treatments. In the second trial the results were still worst. The consumption of water was 21%, 54% and 72% for the groups 4/20, 2/10 and 1/5 respectively, in comparison with the control in the first trial, and 26%, 66% and 85% in the second trial ($P < 0.001$). This shows that modulating the same total time of water supply the amount of drinking water can be controlled. According to our 2 experiments, performances remained too low and mortality too high to be considered compatible with the needs of commercial production.

MOLECULAR CHARACTERIZATION AND PHENOTYPING OF *PASTEURELLA* FROM SWISS RABBITS

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Several bacteria belonging to the family *Pasteurellaceae* might act as pathogens in rabbits. In particular, *Pasteurella multocida* is considered to be an important one and outbreaks caused by this species result in considerable economic losses. However, proper identification of *P. multocida* at the species and even more so at the subspecies level is often not very precise. Moreover, *Pasteurellaceae* species isolated from rabbits are poorly characterized. We therefore phenotypically and genotypically analyzed 228 isolates from different rabbit populations including a breeding and fattening organization with group management as well as isolates from single post-mortem cases with pasteurellosis using biochemical reactions and repetitive extragenic palindromic (REP)-PCR. Furthermore, 41 samples were selected for phylogenetic analysis of 16S rRNA gene. REP-PCR identification and phylogenetic analyses correlated well and appeared to be distinct molecular methods for identification of rabbit isolates. Phenotyping, however, diverged from molecular recognition, indicating the problematic conventional diagnosis of these strains. The fermentation of sorbitol and trehalose appeared to be imprecise indicators for *P. multocida* subspecies classification. However, according to REP-PCR and sequencing results, 82% of the isolates were characterized as *P. multocida* ssp. *multocida*, 3% as *P. multocida* ssp. *septica*, 5% could be assigned to *P. multocida* and probably represented a new subspecies within this species. Further, 5% were assigned to *Pasteurella canis*. The other 5% represented a homogeneous group of unknown species belonged to the *Pasteurellaceae*. Samples obtained from post-mortem cases demonstrated a higher phenotypic and genetic heterogeneity than samples from group management rabbits.

IS EPIZOOTIC RABBIT ENTEROPATHY (ERE) A BACTERIAL DISEASE?

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The etiology of epizootic rabbit enteropathy (ERE) is still unknown despite ten years of continuous research. A putative bacterial etiology is at the basis of current research. The fractionation of the reference inoculum (TEC4) is a major step to find the potential bacterial agent(s). In this study, TEC4 was fractionated with two techniques: centrifugation on discontinuous sucrose gradient then cell adhesion. Two selected fractions were inoculated to SPF rabbits and analyzed with classical bacteriological techniques. ERE was reproduced with both fractions. The 16S rDNA gene was amplified in all fractions and in three negative controls and subsequently analyzed with Restriction Fragment Length Polymorphism (RFLP) and Denaturing Gradient Gel Electrophoresis (DGGE). A difference in bacterial DNA composition was found between virulent and non-virulent fractions which reinforce the potential role of bacteria in the etiology of ERE.

AN ACUTE OUTBREAK OF MYXOMATOSIS IN TWO GREEK RABBITRIES

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A case of myxomatosis in vaccinated and non-vaccinated rabbits is described, and detection of *Myxoma virus* was performed in this study. In two commercial farms being 150 km distant each other, myxomatosis occurred after the import of animals from a common supplier. The disease was manifested firstly in the existing non-immune population of does and fatteners, and later in all vaccinated animals, being 2 to 3 months immune at the time of first symptoms. Morbidity was almost 100% with nasal discharge, listlessness, fever, eyelid swelling, eye and nasal purulent discharge, papules in the ears, facial oedema, and swelling of the anagenital region, and animals always died. Examination by PCR revealed the presence of a 492-bp specific product in all assays of the symptomatic animals tested from both farms, having 100% nucleotide sequence identity with the homologous major envelope gene region of the *Myxoma virus* Lausanne strain. The simultaneous occurrence of myxomatosis in the vaccinated and non-vaccinated rabbits of both farms suggests that the supplier was possibly the source of a viral isolate with increased virulence.

**INFLUENCE OF DIFFERENT STRAINS AND WAYS OF INOCULATION
ON THE RABBIT'S RESPONSE TO EXPERIMENTAL INFECTION WITH
*PASTEURELLA MULTOCIDA***

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Extensively characterized *Pasteurella multocida* strains isolated from rabbits with different signs of *pasteurellosis* were used to study the response of rabbits triggered by aerogen, conjunctival, intradermal or intramuscular experimental infection. Lethality was small (5/45) in general and somewhat higher tendency (4/17) was found after conjunctival inoculation. Number of days with appearance of illness and fever within 14 days p.i., number of rabbits with at least one day occurrence of fever, *P. multocida* reisolation rate, and average rectal temperature were influenced both by the strain inoculated and by the route of inoculation. Strain 31 presented higher ($P < 0.05$) values at more parameters (all had fever, 39.9°C average rectal temperature, 60% *P. multocida* reisolation rate) except number of days with illness and with fever. Intradermal and intramuscular inoculation caused doubled values compared to that caused by aerogen or conjunctival inoculations, except *P. multocida* reisolation rate. Feed intake and body weight gain was also different by the route of inoculation, rabbits inoculated intradermally or intramuscularly consuming less feed and losing weight instead of gaining. Serum antibody level elevated 2-3 times in rabbits infected intramuscularly, and strain 1 was found to have the higher antigenicity ($P < 0.05$).

EVALUATION OF THE PROTECTION PROVIDED BY AN INACTIVATED TRIVALENT *PASTEURELLA MULTOCIDA* VACCINE AGAINST EXPERIMENTAL *PASTEURELLA* INFECTION AND PASTEURELLOSIS IN COMMERCIAL ENVIRONMENT

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Although chronic pasteurellosis and nasal *Pasteurella multocida* carriage still causes considerable losses of breeding females, preventive vaccination is not routinely used. Unsatisfactory field results might stay behind that, but observations from controlled field trials are lacking. In this study the protective effect of an inactivated trivalent *P. multocida* vaccine was evaluated in a heterologous challenge experiment and a field trial. The experimental vaccine contained three different *P. multocida* strains specific of the farm involved in the present study. Bacteria were inactivated with thiomersal and adjuvanted with Al(OH)₃. Repeated immunization raised up the specific antibody level four times in SPF rabbits. It conferred protection against challenge with heterologous strains in commercial rabbits which was proven by lower rectal temperature, fewer days with disease signs, smaller change of body weight, and increased antibody level. In the field study a positive effect of vaccination on nasal colonization with *P. multocida* was found. However, an adverse effect was observed: there was lower number of total born, lower proportion of alive new-born rabbits, and furthermore a higher mortality of sucklings in the case of immunized females. This might be related to the date (i.e. some days before artificial insemination) chosen for immunization.

ROTAVIRUS IN DIARRHEIC RABBITS: PREVALENCE AND CHARACTERIZATION OF STRAINS IN ITALIAN FARMS

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The multifactorial rabbit enteropathy has a great importance in rabbit meat production for its economical impact. Stating the pathogenic role and importance as primary agent of rotavirus, the purposes was to estimate the prevalence of lapine rotavirus (LRV), identified by negative staining electron microscopy (nsEM), on samples from rabbits showing either a “generic” enteropathy or lesions referable to mucoid enteropathy-caecal impaction and then to relate their presence with the symptoms and lesions observed. During the 2002-2007, 243 samples taken mainly from rabbits showing catarrhal, haemorrhagic or necrotic entero-tiflitis, mucoid enteropathy and caecal impaction were examined. By nsEM, the presence of viral particles was observed in 45.3% of them; rotavirus was identified in 16.0%, coronavirus-like virus in 24.7%, parvovirus in 9.0% and enterovirus-like virus in 5.8% of the positive samples. In addition, adenovirus, calicivirus and reovirus were sporadically found and, in 29 cases, 2 or 3 different viruses were contemporarily observed in association in the same sample. Using the criteria for the classification of rotavirus strains based on the VP4 (P type) and VP7 (G type) genotyping, almost all the strains were characterized as P[22] G3 confirming the presence of the newly-recognized rotavirus P[22] VP4 allele in Italian rabbits. The availability of the results of microbiological and parasitological analysis allowed to correlate nsEM observations with the contemporary presence of others pathogenic agents such as rotavirus, enteropathogenic *Escherichia coli* (EPEC) and *Staphylococcus aureus* in suckling rabbits; EPEC, *Cl. spiroforme* and flagellate protozoa in the others. The pathogenic role and importance of rotavirus as primary aetiological agent rabbit enteritis are discussed.

EFFECT OF PREDATED VACCINATION AND CHINESE HERBAL ADJUVANT RABBIT HEMORRHAGIC DISEASE VACCINE ON IMMUNE RESPONSE IN YOUNG RABBITS

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The experiments were conducted to determine effects of precocious vaccination compared to the classical schedule of vaccination and of Chinese herbal adjuvant (CHA) on immune response to vaccine against rabbit hemorrhagic disease (RHD) in young rabbits. In experiment 1, five New Zealand rabbits at 30, 35, 40 or 45 days of age were injected with 3 ml of non-adjuvant inactivated vaccine against RHD, respectively. The results showed that the titer of maternal HI antibody on day 0 in the 35-day-old rabbits was lower than the protective level of $3 \log_2$; while on days 7 to 49 after the vaccination, the antibody titer was higher than $3 \log_2$. In experiment 2, thirty New Zealand rabbits at 35 days of age were randomly assigned to 5 treatment groups, representing inoculation with 3 ml of non-adjuvant RHD vaccine, CHA-RHD vaccine, CHA-HA (half dose antigen of RHD vaccine) RHD vaccine, aluminium adjuvant-containing RHD vaccine, or phosphate-buffered saline (PBS), respectively. The results showed that the CHA promoted ($P < 0.05$) the lymphocyte proliferation on day 7 and enhanced serum antibody titer on day 21. These findings from the two experiments suggested that vaccination could be carried out at 35 days and Chinese herbal adjuvant significantly enhanced the immune response to vaccine against RHD in comparison with aluminium adjuvant.

***IN VITRO* ACTIVITY OF ROBENIDINE HYDROCHLORIDE ON RABBIT *CLOSTRIDIUM PERFRINGENS* ISOLATES**

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Digestive disorders like coccidiosis, Epizootic Rabbit Enteropathy and colibacillosis are responsible for severe economical losses in rational rabbit production. Robenidine hydrochloride is widely used to prevent coccidiosis, a disease which has detrimental effects on zootechnical performance and which acts synergistically with Epizootic Rabbit Enteropathy. Although the exact cause of the latter disease is yet unknown, *Clostridium perfringens* is often isolated from diseased animals. The present study describes the *in-vitro* effect of robenidine hydrochloride on 39 *Clostridium perfringens* isolates from rabbits. Robenidine hydrochloride concentrations of 4 µg/ml were able to inhibit the *in-vitro* growth of all examined isolates, which might be relevant for the *in-vivo* situation.

A METHOD TO EVALUATE THE MICROBIAL AIR COMPOSITION IN RABBIT FARM

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The microbiological monitoring of air and surfaces has the purpose to evaluate the hygienic conditions of environment to be sure that the disinfecting operations are correctly performed or to decide what kind of operation would be better to assure a good environmental quality. Since environmental quality, particularly of air, is tightly correlated with animal welfare, it is very important to find methods for the evaluation of the air in animal farms. The last EU directives and EFSA opinions are quite restrictive in relation to the management and housing systems. An important topic is represented by the air pollution in relation to the microbial charge; above all it is interesting to evaluate the presence of bacteria, fungi and dermatophytes (zoonotic agents) that quickly spread from animal to animal and to human workers. In fact rabbits are particularly sensitive to environmental conditions and to stress due to changes in some parameters (e.g., temperature, relative humidity, NH₃ concentration, etc.) that can increase their susceptibility to potential pathogens.

In our experience, we have used an easy method and a working protocol in order to check and control the air contamination in an intensive rabbit farm, located in Lombardia region, analyzing the air samples inside the shed and inside the cages and nests during a period of three years (2004-2007). This method is based upon the SAS® System (PBI International, Italy), an impaction method by which the air is collected directly on the Petri plates filled with the chosen cultural medium. In our protocol we have decided to evaluate the total microbial charge and the fungal one. So we used as cultural media Tryptic Soy Agar (Oxoid, Italy) for bacterial growth, Sabouraud Dextrose Agar (Oxoid, Italy) for environmental fungi (such as *Aspergillus* spp., *Penicillium* spp., *Alternaria*, spp.) and Dermasel Agar (Oxoid, Italy) for dermatophytes (*Microsporum* and *Trichophyton*).

After many trials we have observed that this method, compared with the “opened plates” method, allows to obtain repeatable results in a brief time and to perform the tests with low cost. The results allow to evaluate the environmental status of rabbitries, to know the welfare of reared rabbits and to decide the hygienic operation to perform in the breeding farms.

BACTERIAL INFECTIONS IN RABBIT AS COMPANION ANIMAL: A SURVEY OF DIAGNOSTIC SAMPLES IN ITALY

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Rabbits are becoming more popular as pets and the number of diagnostic samples is now increasing. Rabbits make excellent pets because they are clean and docile. For housing, extreme temperatures should be avoided; in fact high temperatures may cause overheating and death, but also low temperatures are harmful. Good ventilation is essential and cages should be easily accessible for daily care. Cage material must be tough enough to support constant chewing but, to avoid boredom, some environmental enrichment (e.g., toys and chewing materials) should be provided. Most important is to guarantee a good hygiene of environment and animals because rabbits can develop a variety of clinical problems. Coccidiosis, caused by several species of *Eimeria*, may be subclinical or cause mild to severe diarrhoea; instead *Clostridium spiroforme* could be responsible for clostridial enterotoxemia that causes moderate to severe diarrhoea frequently resulting in death of weanlings. Respiratory diseases, generally caused by *Pasteurella multocida*, are the major cause of morbidity and mortality in rabbits. Infection may be subclinical or cause various combinations of clear to thick nasal discharge, clear to thick ocular discharge, anorexia, lethargy. The bacterial infection may also result in abscesses in subcutaneous tissues and other sites, torticollis and circling for infection of the inner ear and septicaemia. For all these pathologies the diagnosis is based on bacterial culture or parasite observation. However other pathogens are cited, such as *Staphylococcus* spp. and *Bordetella bronchiseptica*. As what regards the antibiotic susceptibility of bacteria involved in pet rabbits diseases, a few number of studies are available. The aim of this work was to evaluate the bacterial prevalence in samples from different ill pet rabbits during diagnostic procedures from 2004 to 2007. Moreover we evaluated the antimicrobial sensitivity/resistance of bacteria to some molecules used in rabbits. Thirty two samples were collected and *Pasteurella multocida* was the most isolated bacterium (7 positives/32) followed by *Pseudomonas aeruginosa* (5/32) and *Klebsiella pneumoniae* (3/32). Others microorganisms were isolated in lower percentage. The results of the antimicrobials susceptibility tests were variable, but some molecules were decidedly ineffective.

POSSIBLE PROTECTIVE EFFECT OF AN AUTOVACCINE AGAINST HIGH VIRULENCE *STAPHYLOCOCCUS AUREUS* IN A RABBIT SKIN INFECTION MODEL

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The spread of a highly virulent (HV) *Staphylococcus aureus* strain into a rabbit flock leads to a high percentage of rabbits suffering from subcutaneous abscesses, pododermatitis, mastitis, infertility and death with chronic poor production results for the rabbitry. In practice often all animals have to be culled and the rabbitry has to restart with a new flock. Some rabbit farmers however prefer to use an autovaccine, manufactured from the specific HV *S. aureus* strain circulating in their rabbitry, in an attempt to control the infection at flock level. Since the efficacy of such an autovaccine has not been scientifically proven, a double blinded experiment was designed to study this effect in an *in vivo* rabbit skin infection model under standardized experimental conditions. For this purpose, a culture of the HV *S. aureus* strain KH 171 was used to produce an autogenous bacterin based on a whole cell suspension of formalin killed bacteria in sterile buffered saline. On day one and fourteen of the experiment, ten twelve-week-old rabbits (group A) received subcutaneous injections with the prepared autovaccine while ten rabbits of group B were injected twice with sterile buffered saline. Two weeks after these last injections, the rabbits were briefly anaesthetised and after shaving and disinfection of the flank, an "O"-shaped tattoo pin was pressed into the skin. This dermal lesion was then inoculated with 0.1 ml of a suspension containing 10^8 cfu of the HV *S. aureus* strain and after air-drying of the inoculum, the animals were allowed to recover. Three other animals served as negative controls. During a period of two weeks after infection, the development of skin abscesses was examined and skin lesions were measured daily. Thereafter, all rabbits were euthanized. Inoculation of the skin with the typical HV strain resulted in skin abscess formation in both groups within 24 hours post-inoculation. Statistical analysis showed that there was a significant effect of the autovaccine on the maximum observed abscess diameter for each animal. Secondly, the area under the abscess diameter curve tended to be smaller for the vaccinated group. With repeated measures analysis, there was also only a tendency for the main effect of the autovaccine to be lower. Thus, a positive effect might be attributed to the administration of the bacterin. However, with the vaccination schedule applied in this study, the use of an autovaccine was not able to prevent abscess formation in infected rabbits.

**CHARACTERIZATION OF BACTERIAL COMMUNITIES IN CAECUM,
HARD AND SOFT FECES OF RABBIT USING 16S RRNA GENES
CAPILLARY ELECTROPHORESIS SINGLE-STRAND CONFORMATION
POLYMORPHISM (CE-SSCP)**

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Dynamic and individual variability of caecal bacterial community were compared to those of hard and soft feces using capillary electrophoresis single strand conformation polymorphism (CE-SSCP). Soft and hard feces of 14 adult rabbits were weekly sampled during 5 weeks while caecal content was sampled on the 3rd (after surgery) and 5th week (after sacrifice). Bacterial communities were compared for their structure (peaks of CE-SSCP profiles analysis) and their diversity (estimation of Simpson diversity index on CE-SSCP profiles, D'). Bacterial community of caecal content, soft and hard feces presented individual variability (P<0.05 for diversity and structure). Without disturbance (1st to 3rd and 4th to 5th weeks), bacterial communities of soft and hard feces remained constant in time for diversity and structure. Sampling caecal content by surgical way greatly modified all bacterial communities (P<0.05 for diversity and structure) suggesting to find an alternative sample type to realize dynamic studies of bacterial caecal community within a same individual. The bacterial communities of the 3 types of sample were different (P<0.05), but presented a similar diversity (D'=3.96±0.48; NS). However, bacterial community of caecal content was closer to that of soft feces than that of hard feces (R of ANOSIM=0.13 and 0.24, respectively) and differed only on some peaks. Therefore, we can assume that soft feces could be used to realize dynamic studies of caecal bacterial community.

**APRAMYCIN IN THE CONTROL OF ENTERITIS
ASSOCIATED WITH *ESCHERICHIA COLI* IN RABBITS**

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The digestive disease is the main cause of mortality in industrial fattening rabbit farms. The economical impact of an episode of digestive disease has been assessed to be 0.78 € by produced rabbit. For some years, apramycin has been used by rabbit producers as an option to control digestive syndrome associated with *Escherichia coli*. The results of two clinical studies confirm that the optimum dose regimen of apramycin in feed for the reduction of mortality and clinical signs in case of enterocolitis outbreak associated with *E. coli* in rabbit is 7.5 mg apramycin/kg of body weight/day (i.e. 100 ppm in feed) for 21 consecutive days.

**STANDARDIZATION OF NESTED-PCR FOR THE
DETECTION OF *PASTEURELLA MULTOCIDA*,
STAPHYLOCOCCUS AUREUS, MYXOMATOSIS VIRUS,
AND RABBIT HAEMORRHAGIC DISEASE VIRUS**

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To control semen, oviductal fluid and embryos, as any other rabbit samples, for possible contamination of some of the most important infective elements in rabbit farms, especially in reproductive farms, four independent Nested-PCR were implemented to specifically detect reduced amounts of nucleic acids of *Pasteurella multocida*, *Staphylococcus aureus*, Myxomatosis virus, and Rabbit Haemorrhagic Disease virus in biological fluids of rabbits.

DIGESTIVE MICROBIOTA STUDIES IN RABBITS BY REP-PCR METHOD

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Different systems are used to discriminate the similarity degree between strains. Some methods are based on phenotypic characteristics and some others on genotypic studies. In this paper we describe a Repetitive Extragenic Palindromic-PCR (REP-PCR) method to evaluate the similarity degree between *Bacteroides* spp. strains isolated from intestinal samples of rabbits to analyse the biodiversity of one of the most abundant bacteria in the digestive system of rabbits.

**TISSUE DEPLETION RESIDUES IN FATTENING RABBITS FED DURING
28 DAYS WITH FEED SUPPLEMENTED WITH 40 PPM OF TIAMULIN
HYDROGEN FUMARATE (TIAMUTIN® PREMIX 100)**

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Thirty rabbits were medicated with feed supplemented with 39 ppm of tiamulin, equivalent to 3.21 ± 0.50 mg of tiamulin hydrogen fumarate per kg body weight and day, during 28 days, and tiamulin-derived residues (measured as 8- α -hydroxymutilin) in the muscle and liver of rabbits were evaluated at 0, 3, 6, 12 and 24 hours after the end of the medication period. A withdrawal period of 0 days can be considered, because all the residue amounts obtained in muscle and liver were below the limit of quantification in rabbits euthanized just after the end of the medication period.

OCCURRENCE OF *CAMPYLOBACTER SPP.* IN ITALIAN RABBIT FARMS

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In order to investigate the occurrence of *Campylobacter spp.* in rabbits reared in intensive and rural farms, the caecal contents of 39 animals from 13 different farms (3 rabbits per farm) were collected from April to November 2007. The whole intestinal tract from each rabbit was obtained just after evisceration at the slaughterhouse or during necroscopy, and processed within 4 hours. Approximately 5 g of caecal contents were squeezed into 5 ml of sterile saline and shaken in order to obtain a homogenous suspension. Samples were inoculated by streaking 10 µl of each suspension directly onto four different selective fresh media: Blaser-Wang's Agar (Oxoid), Skirrow's Agar (Oxoid), Nutrient Agar N°2 (Oxoid) 5% sheep blood plus CAT Selective Supplement (CAT, Oxoid) and modified Charcoal Cefoperazone Deoxycholate Agar (mCCDA, Oxoid). In addition, samples were inoculated on a non selective medium such as Nutrient Agar N°2 (Oxoid) 5% sheep blood using a modified filter technique of Steele & McDermott. All plates were incubated in a jar at 37°C±1 under a microaerobic atmosphere with hydrogen and examined daily for growth up to 12 days. From each sample, 3 colonies showing the same morphotype referable to Gram negative, curved or spiral rod bacteria, were cloned. All the selected colonies were subjected to genus-specific PCR for *Campylobacter*. Positive isolates were submitted to the PCRs specific for *C. jejuni*, *C. coli*, *C. upsaliensis*, *C. helveticus* and *C. lari*. The isolates which resulted negative to the species-specific PCRs were subjected to *rpoB* sequence phylogenetic analysis.

A total of 36 out of 39 animals (92.3%) and all the 13 farms resulted positive for *Campylobacter*. All isolates were positive for *Campylobacter* genus PCR but negative for all the species-specific PCRs tested. Phylogenetic analysis based on the partial nucleotide *rpoB* sequences of 13 isolates (one strain per farm) randomly selected and the reference strains showed that all the rabbit isolates clustered together in a tight clade. This cluster was clearly separated from all the other *Campylobacter* species with high bootstrap values (100), indicating that these isolates may belong to a new species.

This survey allowed reporting the occurrence of a probably new *Campylobacter* species in the caecal contents of farmed rabbits in Italy. Further studies are necessary to describe it and evaluate its possible pathogenic effect on rabbit as well as the eventual zoonotic role.

DOSE DETERMINATION FOR BACITRACIN AGAINST *CLOSTRIDIUM PERFRINGENS* USING A PHARMACOKINETIC/PHARMACODYNAMIC (PK/PD) APPROACH. IMPACT ON CONSUMER SAFETY

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Bacitracin was administered continuously in the drinking water to four groups of growing rabbits at doses of 105, 210, 420 or 840 IU/kg body weight as a water-soluble veterinary medicinal product (Bacivet®-S) for seven consecutive days. Cecum samples were taken 0, 4, 8, 12 and 24 hours post-dosing on days 3 and 7 of the treatment. These samples were analysed using liquid chromatography with tandem mass spectrometry detection (LC/MS/MS). The results show that 105 IU/kg did not expose the cecum to inhibitory concentrations and 210 IU/kg failed to maintain bactericidal levels for more than a few hours. The 420 IU/mg dose maintained bactericidal concentrations for the entire 24-hour period with 2102 µg/kg as the lowest mean concentration measured over the considered period, a value close to the target Minimal Bactericidal Concentration of 1860 µg/ml estimated for bacitracin *in vitro*. Concentrations obtained at 840 IU/kg were far higher. These results justify a daily dose of 420 IU/kg/day in the treatment of intestinal infections where *Clostridium perfringens* can be isolated. In addition, after 30 days of continuous administration at 420 IU/kg body weight, blood concentrations remained below the detection limit (<37.5 µg/l). As a consequence, no residues were found (<75 µg/kg) in any edible tissue from the first day after treatment withdrawal.

**SUSCEPTIBILITY, RESISTANCE AND ANTIBIOTIC PROFILE OF
BACITRACIN AGAINST *CLOSTRIDIUM PERFRINGENS* STRAINS
ISOLATED DURING CLINICAL OUTBREAKS OF EPIZOOTIC
RABBIT ENTEROPATHY**

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Bacitracin Minimum Inhibitory Concentration against *Clostridium perfringens* strains isolated during outbreaks of Epizootic Rabbit Enteropathy was determined as an MIC₉₀ of 0.93 µg/ml. No strains were resistant to bacitracin and serial passages in the presence of sub-inhibitory concentrations failed to induce the development of resistant strains. Bacitracin could be described as a concentration-independent bactericidal antibiotic. Bactericidal activity was obtained at a concentration corresponding to twice the MIC, i.e. 1,860 µg/l and did not further increase at higher levels. This profile is in favor of continuous administration, e.g. in drinking water.

HEALTH AND BODY CONDITION OF RABBIT DOES ON COMMERCIAL FARMS

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The sanitary status (presence or absence of rhinitis, mastitis, sore hocks and mange) and body condition (BC) of 3751 lactating does was evaluated on an individual basis to: a) analyse the influence of the “sanitary status” factor in lactating does on their body condition, and b) estimate the influence of BC on the sanitary status of does. The body condition scoring (BCS) was evaluated by palpating and estimating the fullness of muscle and fat of the lumbar, sacral, coxal tuberosity and gluteal regions, in relation to the size of the animal, on a lineal scale of 1 to 9. Prevalence of the diseases was 19.48% coryza, 4.34% mastitis, 5.46% sore hocks and 4.13% mange. An ANOVA was used to evaluate the effect of sanitary status on the BCS, the following factors being significant: rhinitis, mastitis, sore hocks, number of kindling, partum-AI interval, lactation stage and farm. The presence of rhinitis, mastitis and sore hocks caused the BCS to decrease by 6%, 7% and 7%, respectively ($P < 0.001$). Therefore, all possible measures must be taken to reduce the prevalence of these diseases in farms. The method of maximum likelihood (CATMOD procedure) was used to estimate the analysis of the factors of variation affecting each disease. The factors that were significant for some of the diseases were: farm, BC, reproductive rhythm, number of kindling, phase of lactation, season and use of foot-rests. The use of foot-rests reduces the prevalence of sore hocks by over 50%. The BCS is a good indicator of health in does, and is thus a criterion in farm management.

TYLOSIN MIC DISTRIBUTION FROM CLINICAL ISOLATES OF *CLOSTRIDIUM PERFRINGENS* IN FRANCE, ITALY AND SPAIN

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Antimicrobial therapy continues to be important in reducing losses due to Epizootic Rabbit Enteropathy (ERE); this enteric syndrome mainly affects weanling and weaned rabbits with a maximum incidence rate between the 6th and 8th week of age. Symptoms include diarrhoea with mucus, abdominal distension, anorexia and an increasing mortality rate exceeding in some cases 50%. Although the ERE pathogenesis is not yet completely known, it seems definitely to be excluded the viral etiology, whereas *C. perfringens* has been reported as associated agent in most of cases. Moreover *C. perfringens* type E is considered causative agent of rabbit enterotoxaemia. Tylosin is a bacteriostatic antibiotic belonging to macrolides family, commonly used to control the diseases caused by *C. perfringens* in other animal species in which is documented the *in vitro* pharmacological activity. Although the digestive syndromes associated with *C. perfringens* are considered frequent and economically important in the rabbit breeding, there are no published reports on the antimicrobial activity of tylosin against *C. perfringens* strains isolated from rabbits. In this study, the authors report on the activity of tylosin against 89 isolates of *C. perfringens* recovered from diseased rabbits in Italy, France and Spain. These isolates represent accessions from 3 most important European countries where the rabbit breeding represent a widespread zootechnical practice. The minimum inhibitory concentration (MIC) values were determined by agar dilution according to the protocol proposed by NCCLS/CLSI (M7-A6 manual, 2003). MIC₅₀, MIC₉₀, geometric mean were calculated, and the values used for comparisons. Resulted tylosin MICs were: MIC₅₀ 0.5 µg/ml, MIC₉₀ 32 µg/ml, geometric mean 1.13 µg/ml. The results demonstrate the high *in vitro* pharmacological activity of tylosin against *C. perfringens* of rabbit origin, and corroborate its therapeutic usefulness to control ERE and clostridial enterotoxaemia in rabbit intensive farms according to principles of antibiotics judicious use guidelines and to the concept of precision therapy.

APRAMYCIN, COLISTIN, NEOMYCIN AND PARAMOMYCIN MIC DISTRIBUTION FROM CLINICAL ISOLATES OF *KLEBSIELLA PNEUMONIAE*

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Antimicrobial therapy continues to be important in reducing losses due to enteric forms of *Klebsiella pneumoniae* subsp. *pneumoniae* (*K. pneumoniae*) disease in rabbit intensive farms, in which this bacterium is frequently isolated from the gastrointestinal tract of suckling rabbits, between the 2nd and 4th week of age, showing a case history of diarrhoea. Commonly *K. pneumoniae* is characterized by a high resistance to the antimicrobials and for this reason is important to have up to date information in order to define a precision therapy according to principles of antibiotics judicious use guidelines. Although the enteric forms caused by *K. pneumoniae* diseases have been documented as frequent and economically important in France, Spain and Italy, there are no published reports on the antimicrobial activity of approved compounds against Italian strains. In this study, the authors report the activity of 4 different antimicrobials against 32 recovered isolates of *K. pneumoniae*. These isolates represent accessions from 2 geographic regions of the North-eastern Italy (Veneto and Friuli Venezia Giulia) where the rabbit breeding represent a widespread zootechnical practice. The minimum inhibitory concentration (MIC) values were determined by agar dilution according to the protocol proposed by NCCLS/CLSI (M31-A2 manual, 2004). MIC₅₀, MIC₉₀, geometric mean were calculated, and the values used for comparisons. Resulted MICs were: apramycin MIC₅₀ 2 µg/ml, MIC₉₀>256 µg/ml; colistin MIC₅₀ 32 µg/ml, MIC₉₀ 64 µg/ml; neomycin MIC₅₀ 2 µg/ml, MIC₉₀ 128 µg/ml; paramomycin MIC₅₀ 2 µg/ml, MIC₉₀>256 µg/ml. The geometric means of the MIC values obtained for apramycin and neomycin were 6.04 µg/ml and 6.58 µg/ml respectively, lower than those obtained for paramomycin (10.60 µg/ml) and colistin (24.67 µg/ml); however, among examined antimicrobials, apramycin, with the 78.1% of bacterial strains that present low MIC values (≤4 µg/ml), demonstrated the highest *in vitro* pharmacological activity against *K. pneumoniae*.

STAPHYLOCOCCUS AUREUS NASAL CARRIAGE IN RABBITS

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Although nasal carriage has been described as a risk factor for *Staphylococcus aureus* infections in humans, little information exists in rabbits, where *S. aureus* is one of the most important pathogens responsible for a number of different types of infections. This study was designed to determine the extent of staphylococcal nasal carriage in Spanish farms with chronic staphylococcosis and to establish whether a relationship existed between nasal carriage and development of lesions. One hundred and sixteen rabbits with and without signs of staphylococcosis were obtained from six industrial rabbitries. Nasal swabs for microbiological assessments were obtained. Microbiological results showed that 56% of the animals carried *S. aureus* in their nasal cavities: 84.2% of the animals with staphylococcal-related lesions and 28.8% of the apparently healthy animals. Additionally, the *S. aureus* strains isolated from the nasal cavity and from the lesions, in 91.7% of animals, were clonally related. It is suggested that nasal carriage of *S. aureus* in rabbits can play an important role in the development of clinical infections.

PHARMACODYNAMICS OF AVILAMYCIN TOWARDS STRAINS OF *CLOSTRIDIUM PERFRINGENS* ISOLATED FROM EPIZOOTIC RABBIT ENTEROPATHY

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For treatment of outbreaks of epizootic rabbit enteropathy syndrome, where *Clostridium perfringens* is frequently isolated, avilamycin should be administered for 4 weeks at the dose of 7 mg/kg of body weight. This corresponds to approximately 80 ppm in the complete feed. A total of 89 clinical isolates of *C. perfringens* were recovered from diseased rabbits in Italy, France and Spain. The MIC₉₀ values for these clinical isolates were between 0.5 and 1 µg/ml. There was no noticeable MIC variation in the *C. perfringens* recovered from the three countries. In the absence of clinical breakpoints for avilamycin against *C. perfringens* a wild-type cut-off value of ≤2µg/ml has been proposed. The minimum bactericidal concentration (MBC) of avilamycin against 10 *C. perfringens* strains was determined and ranged from 2- >256 µg/ml. The Post Antibiotic Effect (PAE) and Post Antibiotic Sub-MIC effect (PA SME) exerted by avilamycin against two *C. perfringens* strains was investigated. After exposure to avilamycin at 5×MIC, PAEs and PA SMEs were demonstrated against both *C. perfringens* strains. The magnitude increased with subsequent increasing avilamycin concentrations. Growth of each strain was also inhibited in a dose-dependent manner by exposure to sub-MIC concentrations of avilamycin without initial exposure to a higher concentration. The sub-MIC effects were also observed against each strain. Prolonged exposure to sub-MIC concentrations of avilamycin exerted a greater overall effect than short (2 h) exposure to a high concentration (5×MIC). In a series of kill kinetic studies against 2 *C. perfringens* strains, against one strain the avilamycin activity was time-dependent but was additionally concentration dependent because bactericidal activity was achieved at the highest concentration tested. In the second strain avilamycin activity at concentrations above the MIC was bacteriostatic, essentially time-dependent and influenced very little by concentration.

A NOTE ON THE EFFECT OF CAPRYLIC ACID ON SHEDDING OF *ESCHERICHIA COLI* O103 AND O128 IN WEANED RABBITS

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Enteropathogenic *Escherichia coli* (EPEC) colibacillosis represents one of the major causes of enteritis in rabbit breedings. The aim of the present study was to evaluate the effect of caprylic acid and triacylglycerols of caprylic, capric and lauric acid on health status and shedding of *E. coli* in faeces of rabbits infected with EPEC O103 or O128. Two experiments were carried out. In each experiment, forty-four Hyplus rabbits, weaned at 35 days of age were used. Rabbits were housed individually and randomly divided into four groups of 12 animals. Rabbits of the 1st and the 2nd group were fed a granulated feed, free of antimicrobials. Rabbits of the 3rd group were fed the same diet supplemented with caprylic acid at 5 g/kg. Rabbits of the 4th group received the basal diet supplemented with commercially available oil (Akomed R), containing caprylic, capric and lauric acid at 60.8, 38.7 and 0.3 g/100 g of fatty acids, respectively. Supplements were added at expense of rapeseed oil in the basal diet. In the first experiment, the feed of rabbits of the 2nd, 3rd and 4th group was contaminated with 0.5 ml of an overnight culture of *E. coli* O103. In the second experiment, *E. coli* O128 was used. Rabbits were checked daily for morbidity and mortality. Faecal shedding of *E. coli* was examined three times a week. After the incubation, 10 typical colonies were isolated from each plate and detection of *E. coli* O103 or O128 serotype was determined. Weight of rabbits and feed consumption were recorded. Survived rabbits were slaughtered on the 55th day of the experiment. Immediately after the slaughter, gastric and caecal contents were taken to determine counts of *E. coli* and total anaerobic bacteria by plating technique. Numbers of *E. coli* in faeces of non-infected rabbits varied from 3.24 to 4.10 log₁₀ cfu/g. In both experiments, caprylic acid and Akomed R significantly decreased faecal output of *E. coli* (P<0.05). Ten days after inoculation, corresponding numbers were 10.13 log₁₀ cfu/g (positive control), 5.99 log₁₀ cfu/g (caprylic acid), and 8.31 log₁₀ cfu/g (Akomed R) in the first experiment. In the second experiment, *E. coli* shedding decreased from 10.49 log₁₀ cfu/g in positive control, to 5.99 log₁₀ cfu/g (caprylic acid) and 6.90 log₁₀ cfu/g (Akomed R) on the 9th day of the experiment. In faeces of non-inoculated rabbits, bacteria belonging to the O103 or O128 serogroup were not detected. In both experiments, numbers of caecal *E. coli* were significantly reduced in rabbits fed diets supplemented with caprylic acid and Akomed R, and were not statistically different from numbers of *E. coli* in the negative control (P>0.05). It can be concluded that both caprylic acid and triacylglycerols of caprylic and capric acid are effective in the reduction of EPEC in the caecum and faeces of infected rabbits.

GENETIC CHARACTERIZATION OF RABBIT *ESCHERICHIA COLI* STRAINS WITH THE USE OF MICROARRAY TECHNOLOGY

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A DNA microarray able to detect most of the virulence and antimicrobial resistance genes in *Escherichia coli* was used to genetically characterize twenty three rabbit *E. coli* strains. These strains were isolated from enteric pathologies of rabbits observed in a problematic rabbitry during two years of health surveillance. Microarray data were analyzed with clustering software. Three different groups/clusters were identified. Common virulence, putative virulence genes or markers detected in all strains were *gad*, *ompA*, *ibeB*, *b1121*, *csgE*, *tspE4.C2*, *hlyE*, *mviN*, and *artJ* genes. Cluster 1 is composed of 10 strains included genes of the Locus of Enterocyte Effacement (LEE), like *eae*, *eae(beta)* and *espA-1*, which are typical of enteropathogenic *E. coli* (EPEC), *efa1*, *lpfA*, and the antimicrobial resistance gene *ant(3)-Ia* (*aadA1*). Other virulence genes, like *afr2* which encodes an adhesin characteristically found in rabbit *E. coli*, and *cif*, a gene encoding a toxin called cycle inhibiting factor, have been found in almost all the samples of cluster 1. Cluster 2 is composed of 12 strains, the strains prevalently have a more non EPEC gene profile with only the type 1 fimbriae encoding genes (*fimA* and *fimH*), *csgE*, *tet(A)*, *int1(2)* and presence of integron class I. Cluster 3 is composed of only one strain and has a different gene profile like non EPEC, but with more virulence and antimicrobial genes. Main anatomo-pathological characters of diseased rabbits were also grouped according to the virulence gene clusters. The result has been an individualisation of a prevalently enterotyphlitis group, corresponding to cluster 1, and a prevalently constipating, mucoid enteropathies group corresponding to cluster 2.

THE ANTICOCCIDIAL EFFICACY OF ROBENIDINE HYDROCHLORIDE IN *EIMERIA* CHALLENGED RABBITS

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Coccidiosis remains one of the most important infectious causes of digestive disorders in fattening rabbits. The importance of the disease even increased with the onset of Epizootic Rabbit Enteropathy. It is thus of utmost importance to adequately prevent this disease in rational rabbit production. One of the most applied methods of prevention is the incorporation of an anticoccidial product in rabbit feed. In the present trial, the efficacy of robenidine hydrochloride, registered under the tradename Cycostat® 66G for breeding and fattening rabbits, was compared with a diclazuril treated and a non treated control group in an *Eimeria* challenge model. The trial confirmed the beneficial effects of robenidine hydrochloride incorporation in the feed, leading to significantly better zootechnical results in animals inoculated with *Eimeria media* and *Eimeria magna*.

THE SPECTRUM OF PATHOLOGY ASSOCIATED WITH NATURAL CHRONIC STAPHYLOCOCCAL MASTITIS IN RABBITS

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Staphylococcal mastitis is the main cause for culling adult does from rabbitries. However very few scientific reports study this condition in rabbits. The objectives of this work were to improve the mammary pathological knowledge in natural cases of chronic mastitis through a histomorphological classification and to compare the differences between the lesions caused by several *S. aureus* genotypes. In order to define the spectrum of gross pathology changes, the macroscopical characteristics of the mammary glands of 130 does with chronic mastitis were recorded. The number of glands was registered, which varied between 8 and 10 mammary glands per animal. The incidence of affection of the different glands was also studied. Mastitis cases were classified according to defined histopathological criteria (abscess-type, rosette-type, sandwich-type and mixed-type). The abscess-type was the most frequently diagnosed (64.4% of the animals). This type was characterized by the presence of one (unifocal) or several (multifocal) well differentiated abscesses of variable size. The purulent material consisted mainly in heterophils of different grades of degeneration and of debris, together with large clumps of Gram-positive bacteria. The rosette-type was characterized by the presence of large non-encapsulated inflammatory areas, in which there were abundant necrosis and bacterial colonies. Other smaller non-encapsulated secondary areas existed in the periphery as satellites of the previous ones. The sandwich-type lesion was characterized by a broad band of inflammatory tissue in the periphery of the mammary gland that extended to the subcutaneous tissue and sometimes to the abdominal muscles. The mixed-type lesion had two histological characteristics, as described above. The most frequent strain to be isolated from lesions was genotype A1/III/δ. However, there was no clear relationship between different genotypes and the histopathological type of mastitis.

PHENO- AND GENOTYPIC CHARACTERIZATION OF *PASTEURELLA MULTOCIDA* STRAINS RECOVERED FROM HEALTHY AND DISEASED RABBITS

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The characterized 32 *Pasteurella multocida* isolates were collected through 2-years long period, taken from rabbits in the fattening and the breeding units of a commercial rabbit farm. Rabbits were manifesting of (n=18) or free from (n=14) signs of *pasteurellosis*. All isolates were identified with standard microbiological tests and *P. multocida* specific PCR. After 24 hours incubation at 37°C on TSA, 72% and 28% of isolates developed large or small colonies, respectively. Colony type was smooth (n=17), mucoid (n=13) and rough (n=2). Capsular typing was performed with PCR and presence of *capA* and *F* genes were detected for 53% and 28% of strains respectively. Occurrence of gene *capD* was only 9%. Based upon carbohydrate fermentation results, four distinct biochemical types could be recognized: biovars 1 (25%), 3 (6%), 6 (44%) and 9 (3%). Further 22% were non typeable isolates representing lactose positive variants of biovar 2 and 6. An association between capsular type and biovar could be supposed, as biovar 1 strains (n=8) possessed exclusively *capA* capsule gene. Biovar 6 (n=14) and unassigned (n=7) strains had any capsular types. The colony size, biovar and capsular type did not show association with the rabbit sanitary state. Similarity matrix was calculated from the fermentation results of all 50 carbohydrates included in the API CH50 test strip. One group containing five quite diverse isolates taken only from animals with pathological signs appeared on the dendrogram and another two pathologic isolates were also separated as single member clusters, each differentiated around 40% similarity level. One further cluster included 7 of 9 capsular type F strains above 90% within group similarity, which were isolated within a very short time interval, and 5 from healthy rabbits. REP-PCR genotyping revealed most strikingly more pairs of genetically identical strains, each of both isolated from diseased or from healthy rabbits, or one from diseased and another one from healthy rabbits. Clustering otherwise did not show obvious association of the genotype with biochemical or serotyping characteristics of the isolates nor with the sanitary state of the sampled rabbit. The conclusion is that high phenotypic and genotypic diversity is characteristic to the *P. multocida* strains isolated from the same herd across time. Higher similarity of biochemical traits presented by isolates collected within a short period is not inevitably associated with higher pathogenicity.

MICE PATHOGENICITY OF *PASTEURELLA MULTOCIDA* STRAINS ISOLATED FROM DISEASED AND HEALTHY RABBITS

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Pasteurella multocida strains (n=12) were isolated from rabbit carcasses from pathological lesions (n=3) and from the nasal mucosa of clinically healthy (n=5) or diseased (n=4) animals. Mice were challenged intraperitoneally with 5 series of tenfold dilutions and 50% lethal dose was estimated. One strain was highly pathogenic with LD₅₀=1000 CFU. Another seven strains showed LD₅₀ between 1.9×10⁵ CFU and 2.9×10⁶ CFU and were considered as pathogenic or slightly pathogenic. Four strains had LD50 6×10⁸ or more CFU and were not pathogenic to mice at all. A set of parameters, including health status and clinical or pathological lesions of sampled rabbit, colony and capsular types of the bacteria, occurrence of neurological signs or systemic infection in mice were evaluated statistically to find any association with the virulence. The circumstances of the isolation were found to be important, as all isolates taken from dead rabbit clustered together, and three of them were also pathogenic to mice. The remaining one was classified being non pathogenic on mice, although a relatively high number of mice were killed with the concentrated inoculum containing 10⁷ CFU (but none with the diluted ones). Differences in capsular serotype also showed some interaction with the circumstances. The only two capsular type F strains were collected from healthy rabbits and were separated below 60% similarity into one cluster each. These were also non pathogenic to mice and did not produced neurological signs. A further cluster included 4 strains taken from ill or apparently healthy rabbits, and all had *capA* genotype. The lethality of these isolates to mice was heterogenous, one non-pathogenic, two moderately pathogenic and one pathogenic were found. Considering these results it can be concluded, that ill and clinically healthy rabbits too could spread pathogenic or moderately pathogenic strains, and the proportion of the non-pathogenic strains is not different in the apparently healthy rabbits. Strains isolated from the dead rabbits are characterized by higher mouse virulence level.

DEVELOPMENT AND APPLICATION OF PCR ASSAY FOR DETECTION OF *BORDETELLA BRONCHISEPTICA* IN RABBITS

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With a pair of specific primers designed to amplify fim2 gene of *Bordetella bronchiseptica* B JL06 in rabbits, a fragment of 425 bp in length of the target gene was amplified by PCR. The sequencing result showed that the homology was 100% compared with the reference sequence published in GeneBank. Specificity assays revealed that the assay did not cross react with *Escherichia coli*, *Pasteurella multocida* and *Clostridium welchii*. The least amount of the bacteria that could be detected was 3.6 CPU, and a PCR assay was developed for quick detection of *Bordetella bronchiseptica* infection. One hundred and forty six samples of nasal mucus from rabbits from Jiangsu and Shandong province were examined by the developed PCR assay. Ninety two samples were positive and the positive rates were 63.01%.

ETHOLOGY AND WELFARE

DEVELOPMENT OF SOCIAL AND FEEDING BEHAVIOUR IN YOUNG RABBITS

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ABSTRACT

European female rabbits (*Oryctolagus cuniculus*) have evolved parsimony in the relationship towards their offspring. Lactating females nurse their litter immediately after parturition, but then leave the nest and will interact with their young only once per day, for a 5-minute nursing episode. Newborns are initially blind and deaf, and totally dependent of milk to survive. In domestic conditions as in the wild, they exhibit a typical behavioural repertoire controlled by olfaction and perioral somesthesia to locate the maternal nipples. Thus, chemosensory means of communication with the mother, based on previously learned odour cues and predisposed odour signals, promote high success in obtaining milk at least over postnatal days 10 to 15. Then, audition and vision become functional and involved in social and feeding behaviour. The present paper aims to survey the behaviour of young rabbits between birth and weaning, and to offer indications on how it is controlled in the context of mother-offspring interactions by multiple sensory and environmental factors.

INTRODUCTION

A critical need of all mammalian newborns is to rapidly and efficiently interact with the mother to locate the source of milk. Later, depending on species-specific constraints, the young also need to progressively reach autonomy from the mother, in both social and alimentary terms.

The critical mother-infant exchanges are displayed following strategies of nursing and sucking, which more or less overlap between species. Typically, mammalian females invest the greatest part of their time and energy budget caring for their offspring, staying often close to them to provide frequent opportunities to suck over the day. This is the general rule in species bearing altricial young, which are relatively immature in sensory, motor and physiologic terms. Some species, such as the European rabbit (*Oryctolagus cuniculus*), are in sharp contrast with this typical pattern of maternal care. While in rabbits, mothering begins with the construction of a safe breeding

burrow, lined with both plant materials and her own abdominal fur (Mykytowycz, 1968; Hudson and Distel, 1982; González-Mariscal *et al.*, 1994), the post-parturient female returns to the nest only once per day to nurse the litter for 3-5 minutes (Lloyd and Mc Cowan, 1968; Mykytowycz, 1968; Broekhuizen *et al.*, 1986; Hudson *et al.*, 1996a). After each nursing episode, the female leaves the nest and blocks its entrance before moving away (Tinbergen, 1970; Broekhuizen *et al.*, 1986).

This pattern of mother/nest-offspring relationship appears well conserved in domestic breeds of rabbits. In rabbitries, the females can express their propensity to dig, collect hay and pull their own hair, and deposit them in a nest box appended to their cage 2-3 days before parturition. The females deliver then in this artificial “burrow”, and after each nursing episode, jumps out to spend the remainder of the day in the cage. Of course, they cannot close the access to the nest as in the wild, but their nest closing behaviour remains part of their behavioural repertoire: it re-appears when domestic females are returned to semi-wild conditions (Deutsch, 1957; Kraft, 1979). Moreover, a number of investigators have recorded the stable, apparently undisturbed, once per day periodicity of their nest visits. These studies usually report only one, exceptionally two, nursing visits/day, especially during the first postpartum week (e.g., in continuous nest-access conditions: Venge, 1963, Zarrow *et al.*, 1965; in closed nest conditions with two periods of possible access per day: Zarrow *et al.*, 1965; Hudson and Distel, 1982). Some recent studies suggest, however, that the nursing visits may happen more frequently (Schulte and Hoy, 1997; Hoy and Selzer, 2002). Nevertheless, a nursing visit remains in all cases extremely short (< 5 min; Zarrow *et al.*, 1965; Lincoln, 1974; Hudson and Distel, 1982; González-Mariscal, 2007).

It is the aim of the present paper to offer a rapid survey of the individual development of young rabbits during the first postnatal weeks, focusing on the social and feeding challenges they have to face for both domestic and wild conditions. An emphasis will be made on sensory and behavioural adaptations rabbit females and pups have co-evolved to optimize survival and growth.

FEEDING BEHAVIOUR OF YOUNG RABBIT: FROM MILK TO SOLID FOOD

Rabbit females give birth after about 31 days of gestation. Then, young rabbits undergo a period of rapid development, ending in weaning in about one month. The pups are thus progressively shifting from an almost exclusive milk diet to a diet composed of solid food that they have to select and ingest by themselves.

Milk intake

The first episode of nursing occurs shortly after birth. The female has the complete initiative in the onset of suckling until the period when pups become motorically mature to leave the nest (cf. section 3). In the nest, the female stands motionless, with the back arched over the pups, providing no direct assistance to the pups rushing to the nipples (Hudson and Distel, 1982, 1983). Nipple location and milk intake depend then on individual pups' abilities to behave efficiently under the female. During the first days

of life, they can ingest up to 25% of their live-weight in milk (5 to 10 g at birth). Their avidity for sucking is important as they can suck twice a day if they are experimentally exposed to two females that have not nursed, with several hours between the nursing bouts (McNitt and Moody, 1988; Gyarmati *et al.*, 2000). Although it may happen that one or two pups in a litter do not succeed in obtaining milk at one nursing (around 10-15% on day 1; Coureaud *et al.*, 2007), the first sucking bouts are crucial for pup survival. Starvation is indeed a key explanation of mortality that usually peaks within the first postnatal days, among other factors such as maternal inexperience (Verga *et al.*, 1978, 1986; Coureaud *et al.*, 2000a). In fact, missing the first 2-3 nursing opportunities almost inevitably ends in the loss of the pup (Coureaud *et al.*, 2000b).

In contrast with other newborn mammals (e.g., piglets- *Sus scrofa domesticus* -, kittens - *Felis sylvestrus catus* -; McBride, 1963; Mermet *et al.*, 2007), newborn rabbits do not select one or two preferential nipples, but frequently (every 20 seconds, approximately) change among nipples within a same nursing bout. This change occurs despite the high level of competition within the litter (in domestic breeds, there are often more pups in the litter than available nipples - generally 4 pairs; Drummond *et al.*, 2000; Bautista *et al.*, 2005). The pups' scrambling under the female makes possible for all valid pups to attach to one of the usually eight nipples, in which milk yields are similar during the first postnatal days. But by the end of the first postnatal week, more milk seems to be produced on the two middle pairs (Bautista *et al.*, 2005).

The individual milk intake increases from an average of 5-10 g/d after birth to reach a peak of 30 g/d at 20-25 days of age. All over this period, the milk intake is variable between pups due to individual sensory-motor capacities, success in competition among littermates and milk availability (Fortun-Lamothe and Gidenne, 2000; Rödel *et al.*, 2008a). After day 25, the milk yield decreases progressively until weaning. Rabbit females can be fertilised soon after parturition and are able to sustain a new pregnancy throughout lactation. In this case, the milk production ceases 2-3 days before the following parturition (Lebas, 1972; Fortun-Lamothe *et al.*, 1999).

In breeding conditions, weaning occurs between days 28 and 35. In wild conditions, if food resources are sufficient and if females are not pregnant, milk production can continue up to 4-6 postpartum weeks. If females are lactating and pregnant (which is frequently the case in spring), young rabbits can be weaned at 3 weeks of age (Broekhuizen *et al.*, 1986; Gidenne and Lebas, 2006).

Solid food intake

Young domestic rabbits begin to eat significant quantities of solid food around 16 to 18 days when they are able to leave the nest-box and move to access pelleted feed and the drinker in the maternal cage. Nevertheless, the first contacts with non-milk elements occur from the first postnatal week when the pups nibble hard faeces that the female left in the nest while nursing her litter (cf. the section "Sensory development of young rabbits"; Kovacs *et al.*, 2004; Moncomble *et al.*, 2004).

At the beginning, pups eat very small quantities of solid food (<2 g/d before 20 days of age). The food intake radically increases from day 25 to reach 40-50 g/d at weaning, although variability can be high between litters (Gidenne and Fortun-Lamothe, 2002). Thus, the feeding behaviour of young rabbits undergoes a radical shift within a few

days, switching from a single meal of milk to 25-30 episodes of solid and liquid (water) intake per 24 h. The mass of ingested solid food + water exceeds that of milk during the 4th postnatal week (Scapinello *et al.*, 1999; Gidenne and Lebas, 2006).

One may note that when pre-weanling rabbits begin to leave the nest, they prefer eating at their mother's feeder rather than at a feeder introduced for them into the cage (Fortun-Lamothe and Gidenne, 2003). Even if this point remains to be investigated, it suggests that young rabbits initiating solid food ingestion may model the female's choice of feeding place or of type of food.

Finally, as ingestion of soft faeces represents an important component of rabbit nutrition, at least in adults (e.g., Belenguer *et al.*, 2005), it is interesting to better understand how it develops. A recent study suggests that caecotrophy starts around days 21-25 when the intake of dry food is sufficiently significant to lead to caecum and colon filling (Gidenne *et al.*, 2002).

Development of nutritional needs

In parallel to modifications in feeding behaviour, the nutrients ingested by young rabbits greatly change between birth and weaning (Figure 1). While rabbit milk abounds in lipids (13%) and in proteins (12%), it contains only traces of lactose (Maertens *et al.*, 2006). In contrast, pelleted food contains glucids (80%; more or less digestible, for instance starch being better digested than fibres), proteins (15-18%) and only few lipids (2-5%) all of vegetal origin. Therefore, the pups' digestive capacities must rapidly accommodate with the change of nutrient profile (Gidenne and Fortun-Lamothe, 2002). At about 25 days, the level of vegetal proteins reaches the level of milk proteins in the diet, and then it exceeds it within a few days. Otherwise, lipids come mainly from milk until weaning. While the ingestion of carbon hydrates is almost null until 17 days of age (< 0.3 g/d), it becomes significant from day 21 in the form of fibres and starch. However, milk proteins and fats remain the major source of energy until weaning.

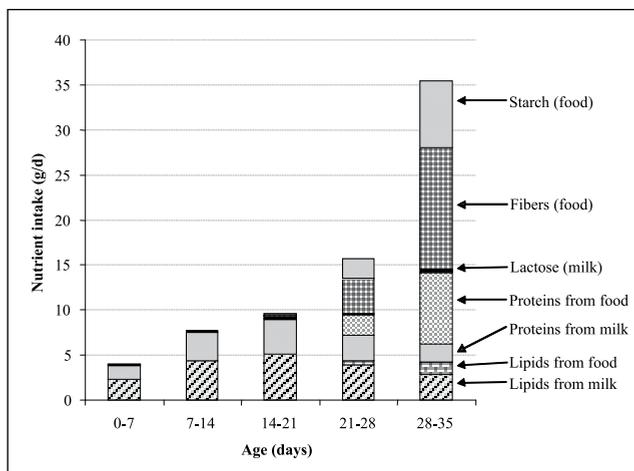


Figure 1: Evolution of the nutrient composition of food ingested by pre-weaning rabbits in breeding housing conditions.

Regulation of feeding behaviour

The individual feeding pattern of pre-weaning rabbits and its regulation are not easily measured as they take place in interaction with the littermates and the mother. Nevertheless, it is known that the availability of milk is a key regulating factor of solid food ingestion before weaning. Thus, if the size of the litter is reduced from 10 to 4 pups or if milk production increases, the beginning of solid food ingestion can be delayed by 2–4 days (Fortun-Lamothe and Gidenne, 2000) and the intake of solid feed in the whole litter is lowered (Pascual *et al.*, 2001). Similarly, offering a second opportunity to suck each day (using a second lactating female) can delay the onset of dry feed intake by a few days (Gyarmati *et al.*, 2000). In contrast, early weaning (before 25 days of age) greatly accelerates dry feed intake (Gallois *et al.*, 2005; Xiccato *et al.*, 2005).

How the nutritional composition of food impacts pre-weaning feeding behaviour largely remains a mystery. Data from Pascual and co-workers (1998, 1999) suggest that suckling rabbits regulate their non-milk food consumption according to its digestible energy content, as in weaned rabbits. But Debray and co-workers (2002) showed a greater solid feed intake when suckling rabbits received a high-energy (11.3 MJ/kg) as compared to a moderate-energy diet (10.5 MJ/kg). Finally, the mode of food presentation, the size of the pellets and their quality (hardness, durability) can also affect the feeding behaviour of the young (Maertens and Villamide, 1998).

FACTORS INFLUENCING THE DEVELOPMENT OF YOUNG RABBITS IN NATURAL OR SEMI-NATURAL CONDITIONS

Due to the difficulty to observe behaviours of young rabbits under wild conditions, their feeding development remains partially unknown. However, studies based on direct observations or video-recording showed that rabbit females usually nurse their litter once per day in the burrow (Mykytowycz and Rowley, 1958; Broekhuizen *et al.*, 1983, 1986; but cf. Hoy and Selzer, 2002). Pups are active within their breeding chamber and start to leave the nest area and explore the burrow when their eyes open (shortly after day 10). At about days 15–16, they can approach the mother outside the breeding burrow, once she has opened it, but immediately return into the nest after nursing (Broekhuizen *et al.*, 1986; Rödel, unpublished observations). Young rabbits emerge from the burrow around days 20–22, when the female starts to leave the nest open (Broekhuizen *et al.*, 1986; Rödel, unpublished observations). Weaning generally occurs before day 28 (Mykytowycz and Rowley, 1958; Stodart and Myers, 1964; Lehmann, 1991).

In line with these studies in the wild, a recent series of investigations conducted on a European rabbit population living in a field enclosure, next to the campus of the University of Bayreuth, allowed researchers to confirm and expand some of the peculiarities of the development of wild-type pups born and living in a semi-natural environment.

Milk intake

As in domestic rabbits (cf. section 1), the milk intake per nursing event of individual pups (assessed by the body mass gain of pups) increases rapidly and considerably with age (Figure 2). For the data presented here ($n = 172$ pups, from 48 litters), the pups were taken out of the natural breeding burrows to be weighed early in the morning (around 06:00), shortly before the mothers visited the nests for nursing, and again around 10:00 after nursing. Even if the data may be somewhat lower as compared to those from domestic rabbits raised in domestic breeding conditions (collected immediately before and after the controlled nursing), the milk intake appears to increase from less than 5 g/pup on postnatal day 1 to more than 15 g/pup on day 17.

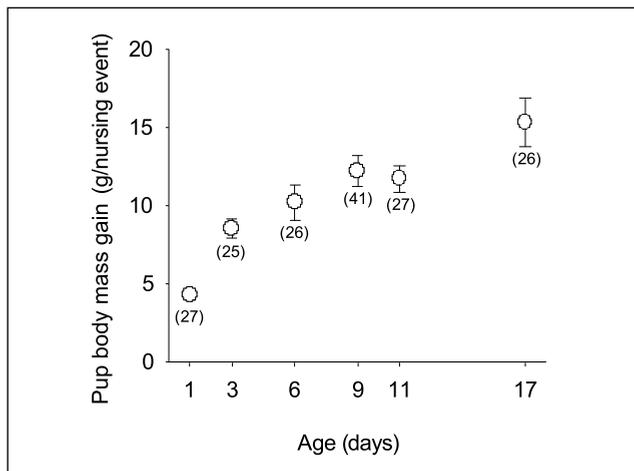


Figure 2: Body mass gain (means \pm SE) per daily nursing of wild rabbit pups living in semi-natural conditions during different days of the nest period. Sample sizes are given in brackets; pups of different ages stemmed from different, in total 48 litters (Rödel, unpublished data).

Litter size

Litter size in rabbits varies considerably, usually ranging from 6 to 12 pups in domestic rabbits (depending on the breeds; Brien, 1986) and from 1 to 9 pups in wild rabbits (on average 3.5 to 6 pups, depending on the breeding cycle; von Holst *et al.*, 2002; Rödel *et al.*, 2004, 2005). Although the milk supply of mammalian mothers is reported to be partly adjusted to variation in litter size by the adaptive stimulation of pre-partum mammogenesis (Forsyth and Hayden, 1977; Jameson, 1998), there is evidence from a wide range of species that an increase in the number of siblings reduces the share of milk obtained by individuals. This results in a negative correlation between litter size and growth rates of the dependent young (Mendl, 1988; Mock and Parker, 1997; Hudson and Trillmich, 2007). Such an effect can also be found in the rabbit: there is a clear negative relationship between sibling number and pup growth rates or weaning weight in domestic breeds (Drummond *et al.*, 2000), as well as in European rabbits living under natural breeding conditions (Rödel *et al.*, 2008a; Figure 3a).

Age and social rank of the mother

Maternal effects are also involved in shaping the postnatal growth of rabbit pups. Apart from litter size effects, the age of the mother indeed affects directly and strongly pre-weaning growth rates of the young. In the population of wild-type European rabbits from Bayreuth, Germany, offspring of middle-aged mothers showed the highest growth, while offspring growth rates of 1-year old mothers and of mothers older than 3 years were comparatively lower (Rödel *et al.*, 2008a; Figure 3b). One-year old rabbit females in natural environments usually have a comparatively lower body mass (Rödel *et al.*, 2004), and thus probably a lower food intake capacity, which might both restrict their lactational performance. In addition, young females usually occupy low social ranks and are frequently more stressed (von Holst, 1998), which might in turn reduce their milk release (Lau and Simpson, 2004). In older female rabbits, reproductive (see Rödel *et al.*, 2004) and lactational performance might be lowered due to reproductive senescence (Kirkwood and Austad, 2000; Tatar, 2001).

Maternal reproductive history

A third factor affecting pup growth in natural or semi-natural conditions is the mother's reproductive history. Due to the rabbit's post-partum oestrus, European rabbit females usually give birth to consecutive litters, starting every year in spring (between late February and late April in Bayreuth; Rödel *et al.*, 2008b). In the German population studied by Rödel and co-workers (2008a), pre-weaning offspring growth was significantly higher in the first litters of the breeding season compared to the following litters (Figure 3c), i.e. pup growth was lower in litters where the nursing females were also pregnant again.

Overall, in comparing these three effects (see the deviations of the residuals from zero; Figure 3a,b,c) it becomes obvious that litter size and maternal age show a comparatively stronger effect on pup growth rates, whereas the effects of the mother's reproductive history are rather small (Rödel *et al.*, 2008a).

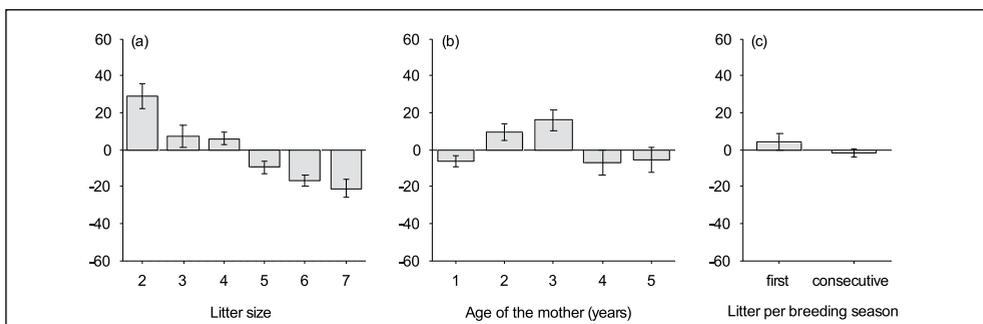


Figure 3: Comparison of residual growth rates (means \pm SE) among European rabbit pups raised in natural breeding burrows (a) from litters of different sizes, (b) from mothers of different age and (c) from mothers' first or consecutive litters per breeding season. Data represent averaged growth rates from postnatal day 1 to 12 over all pups per litter (90 litters from 69 different mothers). Residuals in each graph represent the unexplained variation of the model including the other predictors, respectively. All three effects were statistically significant (Rödel *et al.*, 2008a).

Temperature

The thermal environment is another important factor affecting the growth rates of rabbit pups. While the temperature under laboratory conditions is usually kept stable (around 20°C), wild rabbit pups have to deal with a very wide temperature range in their natural breeding burrows. Studies on the population in Bayreuth, Germany, revealed that soil temperatures in 50 cm depth varied considerably across the breeding season, ranging from 3 to 21°C (Rödel *et al.*, 2008b). Such differences can strongly affect the metabolic rates in altricial young, which are involved in maintaining stable the body temperature, and can therefore affect the growth rate (Hull, 1973; Leon, 1986). For instance, the growth rates in medium-sized litters was found to be higher than in small litters under cold environmental conditions, despite the higher number of competitors for the mother's milk supply (Rödel *et al.*, 2008b). This shift in the optimal litter size for individual pup growth in response to the ambient temperature was most probably due to the thermal benefits of a greater number of littermates (see Gilbert *et al.*, 2007).

SENSORY DEVELOPMENT OF YOUNG RABBITS BETWEEN BIRTH AND WEANING

Young rabbits develop very rapidly, despite the parsimonious pattern of maternal care. Thus, the birth weight may increase as much as 12-fold in less than 4 weeks (Hudson *et al.*, 1996a). Such rapid development ends in the weaning of the young, a mother-young separation that occurs relatively abruptly, especially when the female is pregnant again (Hudson *et al.*, 1996b; González-Mariscal and Rosenblatt, 1996). The sensory abilities of the pups, although initially limited, are highly specialised in newborns. Thereafter, sensory-motor performances are undergoing rapid changes that are causal and consequential to changes in social and feeding conditions.

Postnatal week 1: Total reliance on olfaction and somesthesia

Rabbit pups are altricial: they are born glabrous, devoid of audition and vision, and with limited motor abilities. However, they are equipped with well-developed somesthetic and chemosensory systems, which are both functional from the end of the uterine life. Thus, rabbit newborns orient efficiently following gradients of thermo-tactile stimulations (Pacheco-Cobos *et al.*, 2003) and odours emanating from the maternal body (Schley, 1976, 1979; Hudson and Distel, 1983, 1986; Coureaud and Schaal, 2000; Coureaud *et al.*, 2001). In particular, odour cues and at least one well-characterized pheromone guide them to achieve the behavioural sequence necessary to obtain milk: contacting the female's abdomen, locating a nipple by searching movements, and orally seizing it to suck.

The sensory-motor capabilities of newborn pups also support the rhythm activity that they rapidly display in the nest after nursing. In the minutes after the female leaves the nest, the pups urinate and the litter disperses by digging into the nest materials. Once covered by these insulating materials, the pups reunite again for several hours

in a quiet huddle in the depth of the nest, a collective behaviour allowing regulation of individual body temperature (Hudson and Distel, 1982; Gilbert *et al.*, 2007). Then, 1-2 hours before the next nursing episode, the pups become again restless, collectively emerging from the nest materials and remaining grouped at the surface. This vertical movement of the litter in the nest is considered to be anticipatory to the female's entry and preparatory to the immediate expression of searching and sucking behaviour (Hudson and Distel, 1982; Jilge, 1993, 1995; Bautista *et al.*, 2003). During this period of development, the pups' masticatory abilities are poor. However, from days 3-4 peaking around days 7-8, they begin to nibble nest materials and the faecal pellets that the female drops in low quantity in the nest during nursing (Mykytowycz and Ward, 1971; Hudson *et al.*, 1996a, 1996b; Kovacs *et al.*, 2004; Moncomble *et al.*, 2004). Olfaction is involved in this attraction to the faecal pellets, which constitute their first contact with non-milk compounds (Moncomble *et al.*, 2004, 2006).

Postnatal week 2: emergence of audition, vision and mastication

From days 7-8 pups begin to hear (Gottlieb, 1971; Ripisardi *et al.*, 1975). But they still remain in the nest to be nursed, under both domestic and wild conditions. Their eyes open between days 10-13, and it is approximately at the same period that the first oral manipulations of solid food and mastication can be seen (Langenbach *et al.*, 2001). In addition to the faecal pellets, pups may then bite through and ingest the nest materials, a presumable cause of decreasing amount of materials in the nest (females have also been observed eating nest materials; Hudson and Altbäcker, 1994; Coureaud, unpublished observations). By the end of the 2nd week, domestic pups begin to leave the nest and to ingest food pellets. The role of olfaction relative to vision in this initial orientation to the feeder remains to be investigated. In domestic conditions, rabbit pups also become able to solicit the mother for nursing, a solicitation that may occur independently from individual pups throughout the day, and lead to harassment of the female who finally tends to avoid the litter (Stauffacher, 1992; Mirabito *et al.*, 2004). In the wild, pups have sometimes been seen to start emerging from the nest and breeding chamber from days 12-13 (Broekhuizen *et al.*, 1986), but they rapidly returned into the burrow when the female closed it.

Postnatal week 3: sensory changes to localize, seize and ingest milk and solid food

At the end of the 3rd week, milk yield begins to rise to a maximum level, and so does milk consumption in young (in mass). In domestic conditions, pups leave the nest-box, easily locate the mother by vision, and almost systematically initiate sucking. They also progressively increase their consumption of solid food and water, supported by co-occurring sucking and fully functional chewing abilities (Langenbach *et al.*, 1992). However, milk intake remains higher than non-milk food intake until day 25 (Langenbach *et al.*, 1992; Scapinello *et al.*, 1999). In the wild, the young increasingly come up to the burrow entrance, where they are nursed. By observing this situation by video recordings in the field enclosure of a population of European rabbits from Bayreuth, Rödel (unpublished data) explained that when the pups reach an age of about 15-16 days, they approach the mother outside the breeding burrow once she

has opened the soil sealing, are nursed in front of the entrance, and then immediately return into the burrow at the end of nursing before the mother closes the entrance again with soil. Similar observations are reported by Broekhuizen and Mulder (1983) and Lehmann (1991) in other wild populations.

Postnatal week 4: weaning

By now, the sensory equipment of the pups achieves excellent functional properties, which commensurate with the challenge of reaching social and feeding autonomy. In the wild, as well as in rabbitries, the females progressively cease to nurse, enforcing the young to ingest solid foods. This process is accelerated when the female is pregnant again. Then, she may even display agonistic behaviours towards her young (Mykytowycz and Ward, 1971). As previously stated, weaning usually occurs between days 28-35 in domestic conditions, and before day 28 in the wild.

CHEMOSENSORY COMMUNICATION BETWEEN MOTHER AND YOUNG

Several studies run during the last decades have highlighted the major role of female's odour cues in the rabbit newborns' searching and oral seizing responses directed to the nipples. These studies exploited different experimental tests, such as the inactivation of the pups' olfactory system (Schley 1981; Distel and Hudson, 1985; Hudson and Distel, 1986), the deodorization by washing or selective covering of the female's mammary areas (Müller, 1978; Hudson and Distel, 1983; Coureaud *et al.*, 2001), choice tests between odours from adult conspecifics contrasted in physiological state (Hudson and Distel, 1984; González-Mariscal *et al.*, 1994; Coureaud and Schaal, 2000), and the manipulation of the fetal and/or neonatal chemosensory experience (Semke *et al.*, 1995; Coureaud *et al.*, 2002). Here, we sum up some results of these experiments and present some recent ones, emphasizing the involvement of two kinds of maternal stimuli: odour cues that depend on pre- and postnatal learning and predisposed odour signals.

Odour cues acquired before birth

As in other mammals (Schaal *et al.*, 2001; Schaal, 2005), fetal rabbits acquire odour cues *in utero*, and such stimuli are generally attractive in the postnatal environment. When simultaneously exposed to the odour of placentae and to a (non-specific) control stimulus, rabbit pups preferentially orient to the former, indicating that they prefer familiar cues from the prenatal environment. Moreover, pups born from females that had eaten cumin during pregnancy orient preferentially to placentae from cumin-eating females as compared to placentae from females eating standard food (Coureaud *et al.*, 2002). Thus, rabbit pups are born with the memory of a dominant odour encountered *in utero*, and orient to it postnatally. Similar results were obtained with other aromas introduced in pregnant females' diet, suggesting a general phenomenon (Semke *et al.*, 1995; Coureaud *et al.*, 2002). Further, pups born from females eating cumin-enriched food during pregnancy respond preferentially

to the milk from such females as compared to the milk from females eating control food (Coureaud *et al.*, 2002). Thus, there is chemical overlap between pre- and postnatal environments, and newborns are sensitive to this transnatal continuity that may canalize their orientation to the mother and her milk. When this continuity is experimentally broken by cross-fostering pups born to a cumin-eating female to a lactating female that does not ingest cumin, they present difficulties to suck during the first nursing episodes, as compared to pups that are fostered to a lactating female whose feeding regimen is similar with that of the biological mother (Coureaud *et al.*, 2002). Thus, odours cues may gain significance in the postnatal environment by their mere presence in the prenatal environment.

The odour of rabbit milk

Rabbit newborns do not respond to the whole odour of rabbit milk just because they detect odour cues prenatally learned. Indeed, they also display strong appetitive responses to the milk of their species (i.e., the milk of any rabbit female), regardless of the fact that it carries a dominant odour encountered prenatally (Coureaud *et al.*, 2002). Thus, pups are sensitive to different odour cues in rabbit milk: those related to the individual mother (odours that reflect her diet or stress level) and those that are common to all lactating *Oryctolagus* females.

After exposing pups to the outlet of a chromatograph where they could sniff all the compounds from the effluvium of fresh rabbit milk, a monomolecular volatile compound, 2-methylbut-2-enal (2MB2), has been isolated and identified as a species-specific odorant (Figure 4a; Coureaud, 2001; Schaal *et al.*, 2003). This compound is extremely efficient in releasing the typical searching-oral grasping behaviour in 2-3-day old pups (> 90% of the tested pups respond to it in a given range of concentration; Coureaud *et al.*, 2004). It has been screened for its possible pheromonal properties, according to a stringent definition of mammalian pheromones based on 5 criteria (Beauchamp *et al.*, 1976; Johnston, 2000).

A series of experiments confirmed that: (a) the behavioural activity of 2MB2 was similar to that of the whole effluvium of fresh milk, a mixture of more than 150 volatile compounds: this *chemically-simple signal* could thus by itself explain the activity of the original, entire mixture; (b) 2MB2 triggers relatively *invariant behavioural responses* (attraction, searching-oral grasping movements) *having a critical function for pups*, in that they are essential in the interaction with the female and in the achievement of milk intake; (c) the behavioural effect of 2MB2 is highly *selective* (and is not explainable in terms of a novelty effect or of a general attraction); (d) the behavioural effect of 2MB2 is *species-specific*, meaning that only pups from *O. cuniculus* respond to it without regard to their genetic origin or local feeding ecology (reciprocally milk from rats, ewes, cows, mare and women do not trigger any response in rabbit pups); (e) rabbit pups *do not learn* to respond to 2MB2 by previous exposure (*in utero* or right after birth), meaning that its activity is predisposed.

In sum, as 2MB2 satisfied these 5 criteria, it qualified as a mammalian pheromone. Moreover, as it appeared to be produced or liberated in the mammary tract (Moncomble *et al.*, 2005), 2MB2 was named “mammary pheromone” (Coureaud, 2001; Coureaud *et al.*, 2002, 2003; Schaal *et al.*, 2003).

A signal immediately active at birth and progressively controlled during the development

The behavioural activity of the mammary pheromone (MP) is extremely strong in newborn rabbits, even in normal term pups tested before any contact with the mother and milk, or in preterm pups born by caesarean delivery (Coureaud, 2001; Schaal *et al.*, 2003). As the MP releases responses that newborn pups display to locate the nipples and ingest milk, it may be suggested that its main function is to favour the success of sucking. This hypothesis has been tested in a sample of domestic rabbits housed in a rabbitry (n=293 pups from 30 litters). On day 1 (day of birth = day 0), before the daily nursing, all newborns were individually weighed and then tested for their response to the MP; these pups were weighed again after nursing to assess whether those who did not respond to the MP had difficulties in gaining milk. Moreover, mortality was checked between days 0 and 21. Results on day 1 showed that the rate of pups who did not respond to the MP was weak (8.7%). But interestingly, these non-responders ingested less milk than the responders, especially among the lightest pups (< 48g). Moreover, mortality was higher between days 1-21 among the non-responders than among the responders, and occurred mainly during the first postnatal week (Figure 4b) (Coureaud *et al.*, 2007). Thus, in this study, to respond or not respond to the MP makes a difference for pups in terms of early success in milk intake and subsequent viability (as do the birthweight and the weight gain during the first nursing bouts). This result illustrates the functional impact of the MP. The management implications may lead to the selection from among the lightest pups, which are those that cannot efficiently compete under the mother, and to reunite them to optimize their welfare and survival.

However, if the pup responsiveness to the MP is generally very high at birth, it is progressively coming under the control of at least two factors. First, the responsiveness changes as a function of the pup prandial state. During the first 3 postnatal days, rabbit pups respond to the MP at any period during the day without marked influence of their gastric filling with colostrum or milk. By day 5, the pattern slightly changes: when independent groups of pups are exposed to the MP at various periods in the 24-h cycle, their responsiveness remains high before milk intake, but it drops significantly after, with some recuperation 3h later. On day 10, the responsiveness to the MP remains high before nursing, but it decreases again, without recuperation during 12h (Montigny *et al.*, 2006). Summing up, it appears that the responsiveness of young rabbits to the MP develops in two steps during the first week. From birth to day 2 (at least), it is highly predictable and free from any feedback by post-ingestive events. Then, between days 5 and 10, the responsiveness to the MP comes under prandial regulation, remaining high only when the energetic need is high (before nursing). This fits well with the hypothesis that the MP is a signal that has its highest releasing power when pups require the milk starter to engage in survival, and when they need to refill their energy stores (as mentioned above, missing the 2-3 first nursing bouts jeopardizes survival; Coureaud *et al.*, 2000b).

Secondly, the responsiveness of young rabbits to the MP decreases with approaching weaning, even before the daily nursing. The developmental analysis of orocephalic responses (searching-oral grasping) of pups to the MP between birth and weaning

reveals that responsiveness: (1) is maximal over the first 10 postnatal days; (2) begins to decrease after the eyes open; (3) continues to decrease when pups leave the nest and ingest solid food; and finally, (4) vanishes around weaning (Coureaud *et al.*, 2008; Figure 4c). Similarly, wild-type pups (from the population studied under natural seasonal variations by the Bayreuth group) show high levels of responsiveness to the MP during the first postnatal week. But their response rate drops as soon as day 10, and becomes almost null on day 22 (Figure 4c). In other words, rabbit pups of any genotype respond maximally to the MP during the first days of life, a point that further reinforces the notion of a signal acting as a message optimizing the newborns' sucking success and fitness. Around weaning, the need for milk weakens, and a lesser dependence to the MP may favour detachment from the mother and acquisition of autonomy. Interestingly, the weakening of the releasing power of the MP goes hand in hand with its decreasing emission in milk in late lactation (Figure 4d; Coureaud *et al.*, 2006a). It is also remarkable that the responsiveness of wild-type pups to the MP vanishes earlier than in domestic pups. It may be suggested that this disparity is a consequence of domestication, domestic breeds having been selected for milk yield, body mass, and offspring growth. Their lactational period is thus longer than that of wild-type females (peaking on days 20–25, at an age when dispersion may have begun in the wild; Lebas, 1971; Broekhuizen *et al.*, 1983, 1986; Scapinello *et al.*, 1999; Coureaud *et al.*, 2008). Thus, domestic pups may remain responsive to the MP for a longer period since they are dependent on milk for approximately one week more than wild pups. Regardless, domestic pups also finally lose their sucking-related reactivity to the MP close to weaning.

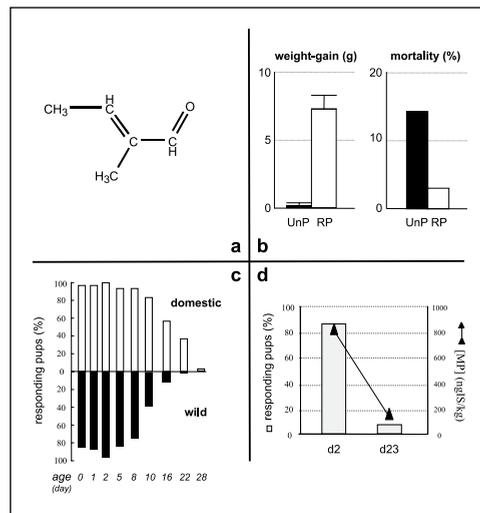


Figure 4: The mammary pheromone of the rabbit: (a) Formula of the 2MB2; (b) Weight gain during the day 1-nursing episode and mortality between day 1 and day 7 of pups unresponsive (UnP) or responsive (RP) to the MP on day 1; (c) Responsiveness of domestic (white bars) or wild-type pups (black bars) to the MP between day 0 (birth) and day 28; (d) Proportion of pups responding to the milk from females in their 2nd or 23rd day of lactation, and mean concentration of MP in the milk of these females (data in panels b, c, d are adapted from Coureaud *et al.*, 2007, 2008 and 2006a, respectively).

A signal that boosts the acquisition of new odour cues

Similar to other newborn mammals, newborn rabbits have to rapidly acquire novel information from their immediate environment. Learning is indeed a requisite to adapt one's behaviour to the unavoidable fluctuations of the environment (Gottlieb, 1976; Alberts, 1987). As mentioned above, rabbit pups start to learn in the womb, becoming familiar with odorants brought in by the maternal diet (Bilkó *et al.*, 1994; Semke *et al.*, 1995; Coureaud *et al.*, 2002). At birth, the learning abilities of pups are also strongly in demand. For instance, pups exposed to an initially neutral odour added into the nest (in absence of the mother) rapidly acquire the odour and later respond to it by preferential orientation (Hudson, 1993). Postnatal odour learning may thus guide the young to orient to safe spots in its immediate environment. Finally, the direct contact with the female during nursing may be the strongest means to learn odours associated with her. This has been shown repeatedly (Ivanitskii, 1962; Hudson, 1985; Kindermann *et al.*, 1991; Allingham *et al.*, 1999; Coureaud *et al.*, 2006b). In these conditions, it has been revealed that newborn rabbits can learn very rapidly (in only one exposure) an artificial odour painted on the mother's abdomen just before nursing: When this odorant is re-presented 24h later on the experimenter's hand, on a tanned rabbit fur, on an anaesthetized female or on a glass-rod, it releases the typical searching movement and, with the glass-rod, the complete searching-oral grasping sequence. Thus, some events during nursing act as reinforcers, transforming an initially neutral odorant into a meaningful odour cue. The candidate reinforcers are numerous in the nursing situation: they can involve tactile or thermal properties of the mother's pelt, the taste of milk, gastric filling or post-absorptive events, or even the pups' expression of searching or sucking motions. In an attempt to identify the nature of these reinforcers, Hudson *et al.* (2002) suggested that the intra-oral stimulation produced by the sucking of a nipple plays the key-role in early odour conditioning.

However, when they contact the maternal abdomen to suck, rabbit pups are also exposed to the MP carried in milk. One might therefore wonder about the capacity of the MP to influence their odour learning. When newborn rabbits are exposed for 5 min to a mixture of MP and an initially neutral artificial odorant (in absence of the mother), they exhibit a strong searching-grasping response when re-exposed to the odour alone 24 h later (Coureaud *et al.*, 2006b). In other words, they then respond to the initially neutral odour as to the MP. Thus, the MP directly contributes to an effective mechanism that facilitates the very rapid acquisition of novel odour cues, in particular odours carried on the mother's ventrum.

The adaptive consequences of such MP-induced odour learning may be numerous and some are still under investigation. For example, (1) it may expand the range of odorants that predict milk reward to pups, and hence may facilitate their orientation to the mother and improve their skill to localize the nipples (Müller, 1978; Drewett *et al.*, 1982; Hudson and Distel, 1983); (2) in a species in which newborns do not appear to olfactorily discriminate their mother from unfamiliar lactating females (at least in domestic conditions and during the first postnatal week; Patris *et al.*, in press; Val-Laillet and Nowak, in press), the MP-induced odour learning may affect early social recognition. Recent results indeed suggest that 2-3-day old rabbits having

learned an odour paired with the MP orient preferentially to their mother, or to an unfamiliar female scented with that odour (Patris *et al.*, in press); (3) finally, it may also be suggested that the MP promotes the early acquisition of odour cues that will be used later, in particular when the young have to select safe foods and optimal mates. MP-induced odour learning may thus influence feeding preferences and mate choice after weaning, as other forms of perinatal odour learning were shown to do (Bilkó *et al.*, 1994; Altbäcker *et al.*, 1995).

CONCLUSIONS

To face the multiple constraints of reproduction, mother and young European rabbits have co-evolved a set of physiological, sensory, cognitive and behavioural adaptations. These adaptations allow efficient mother-young exchanges immediately following birth, despite the brevity of their daily contacts. These exchanges are first centred on the efficient transfer and intake of milk, and on the effective transformation of this energy into pups' body mass. These mother-young exchanges are highly dynamic to optimise survival of the present litter and maximise the chances to bear the next one. One point in these dynamic exchanges is to bring the pups to weaning somatically fit and endowed with predictive information about their environment.

In the present paper, we have surveyed the important changes in feeding and social interaction that occur between birth and weaning in domestic as well as in wild rabbit pups. At first, olfaction plays a crucial role in supporting the neonates in its orientation and oral treatment of the lactating female. During this high-risk life stage, the rabbit female communicates with the young through diverse olfactory means: she promotes odour acquisition in foetuses or in pups, but also sends out unlearned odour signals. Among the latter, the mammary pheromone has an extraordinarily strong potency to release neonatal behaviour and to enforce learning. This pheromonal factor has its highest functional activity in the first postnatal days. After that early period, it becomes progressively under the control of general mechanisms that remain to be explored.

Future studies investigating how and for how long young rabbits detect, process and respond to odours acquired in the maternal environment may contribute to develop breeding procedures aiming to improve pup survival, optimal growth and deferred adaptability when they have to become autonomous. Moreover, *Oryctolagus* constitutes an excellent animal model to address interrogations shared with other mammalian species, including our own, about chemosensory processes functioning during early life transitions (birth, weaning, sexual maturity). In particular, the rabbit offers remarkable conditions to investigate the odour-based mechanisms involved in the development of mother-offspring relationships, and the consequences of early olfactory exposure on the brain and behaviour.

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SERUM CHEMISTRY AND HEMATOLOGY VALUES IN COMMERCIAL RABBITS: PRELIMINARY DATA FROM INDUSTRIAL FARMS IN NORTHERN ITALY

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The aim of this preliminary study was to establish reference normal ranges of hematological and biochemical parameters in rabbits. A total of 8 industrial farms, located in Brescia, Milano and Bergamo Provinces (Northern Italy), were monitored during a 4-year period (2004-2007). Farms showed some variability as regards size, management and production parameters. Blood samples, collected from post weaning (30-45 days old) and growing (57-65 days old) rabbits, as well as restocking females before pregnancy (nulliparous), breeders at first pregnancy (primiparous), lactating does and breeders at culling (multiparous), were delivered within three hours to the laboratory.

The following analyses were performed on serum samples: alanine aminotransferase (ALT), aspartate aminotransferase (AST), urea, Phosphorus (P_i), creatine kinase (CK), lysozyme, lactate dehydrogenase (LDH) and creatinine. Blood samples were tested for: white blood cells (WBC), neutrophils, lymphocytes, monocytes, eosinophils, basophils, red blood cells (RBC), platelets (PLT), hemoglobin (Hg), hematocrit (HCT), mean corpuscular hemoglobin (MCH), mean concentration of hemoglobin in the corpuscular volume (MCHC), mean corpuscular volume (MCV).

Results indicate that hematological and biochemical data are similar to those described in laboratory rabbits. In particular, the age-dependent trend of values was in agreement with previous observations. However, in does, a lower level of hemoglobin and lymphocyte numbers and a higher number of neutrophils and monocytes was observed, probably due to intensive production and exploitation. On the whole, this may indicate chronic stress under seemingly normal clinical conditions. LDH and CK values particularly differed from those described for laboratory rabbits. High urea and creatinine values in lactating does could be traced back to concurrent *E. cuniculi* infection.

RAISING RABBIT DOES IN PLATFORM CAGES

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Fifty eight young-female does, derived from Italian ANCI selection scheme of Macchiata italiana (Macchiata.it) and Bianca Italiana (Bianca.it) strains were raised in a standard Flat-deck (F) cage or in a enriched Platform (P: 40 cm x 60 x 44 h wired) model under a mild breeding rhythm (31 d pp; weaning: 40 d). The Platform system negatively affected conception rate in the Bianca.it (1.43 vs. 1.05 AI/conception in Flat-deck; $P < 0.05$) while the Macchiata.it was unaffected. The Platform did not significantly increase the mortality in the peripartum phase but it reduced the partial mortality in the milking period (4-19 d: 7.3% vs. 8.2%; $P = 0.58$) and in the weaning period (20-35 d: 5.8% vs. 9.5%; $P = 0.019$). The Bianca.it strain was more suitable for the Platform system (19.9% vs. 29% for Macchiata.it). When the space availability was reduced to standard flat-deck restricted conditions, the Bianca.it reacted negatively, producing stillbirths (12% vs. 7.2% for Macchiata.it). Litter size at 19 days and at weaning was substantially increased by the Platform system (11% and 15%, respectively). The individual kit weight was slightly reduced at 19 d (-13%), but the delay was compensated for at weaning. The net result was an increase of 12% and 18% in the litter weight at 19 d and at weaning, respectively. Feed intake of the family, mainly due to the mother, was greater than 10% in the Platform. This pro-Platform pattern appeared particularly confirmed in the Macchiata.it strain because on the Flat-deck the live weight of the does was reduced by around 6% due to more stressful conditions, even though milking ability was similar.

Platform cages for rabbit does may be a solution to increase welfare conditions. According to these results further studies could be envisaged to outline genotype x environment x management interactions concerning the reproductive (mating, litters) and the productive (milking) abilities of different strains of animals.

**PASTURE AVAILABILITY AND GENOTYPE EFFECTS IN RABBITS:
2. DEVELOPMENT OF GASTRO-INTESTINAL TRACT AND IMMUNE
FUNCTION OF THE VERMIPHORM APPENDIX**

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The aim of this trial was to investigate the effect of pasture availability on gastro-intestinal tract and lymphoid follicle development from the vermiphorm appendix (VA) of weaned rabbits of the Leprino of Viterbo and New Zealand White (NZW) breeds. Weaned Leprino of Viterbo and NZW weaned rabbits, (30 days old; 40 animals/breed) were divided into two groups, placed into bicellular cages (17 rabbits/m²) or wired pens (10 rabbits/m²) and provided with an external grass pasture (1 rabbit/20 m²). Rabbits were slaughtered at 90 days of age and body weights were recorded. The gastro-intestinal tracts were excised and weighed. Length, diameter and weight of VA were recorded as well as the number and area of the lymphoid follicles. Differences in pasture availability and locomotive activity were associated with different slaughter weights. Rabbits reared with pasture available exhibited a higher gastro-intestinal tract weight ($P<0.05$), in particular NZW rabbits which showed the greatest pasture intake. As expected, the highest weight of full gastro-intestinal tract ($P<0.05$) was observed in the two breeds with pasture availability. A slight increase in the number of lymphoid follicles in the VA from animals reared with pasture could explain a better health condition, even taking into account that this structure represents only a part of the total lymphoid tissue associated with the gut.

RABBITS' PREFERENCE FOR CAGES AND PENS WITH OR WITHOUT MIRROR

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Five week-old Pannon White rabbits were housed in a closed climatized rabbitry and randomly assigned to pens (56 rabbits) having a basic area of 1 m² with a stocking density of 16 and 12 rabbits/m² or to 18 individual cages (0.24 m²; 1 rabbit/cage; stocking density of 4 rabbits/m²). The pens and the cages were divided into 2 parts and animals could move freely among the 2 parts through swing doors. The vertical sides of one part of the pens and cages were completely covered with mirrors while the other part was covered with white plastic panels. A 24 hour video recording was performed twice a week using infrared cameras and the number of rabbits in each pen and cage was counted with a frequency of 15 minutes (96 times a day). The duration of the trial was 6 weeks. The lighting period was 16L/8D. Rabbits were fed *ad libitum* a standard diet and water was available *ad libitum* from nipple drinkers. Throughout the entire rearing period 67% of the individually caged rabbits showed a preference for the part of the cage enriched with mirrors (P<0.001). This preference slightly decreased with increasing age. The strong preference toward the part of the cage provided with mirror walls was independent of the time of day. That is, during the active period (11:00 pm – 05:00 am), which corresponds to the dark part of the day, rabbits still preferred the mirror-side even though they were not able to see their own reflected image at that time. Neither rearing rabbits in groups under different stocking densities (12 vs 16 rabbits/m²) nor the presence of conspecifics reduced the interest toward mirrors. 65% of animals living at the stocking density of 16 rabbits/m² and 61% of those living at the density of 12 rabbits/m² were found on the side with mirrors (P<0.001) during all the recordings. Group-penned rabbits showed a decisive preference toward mirrors during the active period (71 to 74% for stocking densities of 12 and 16 rabbits/m², respectively; P<0.001). The results suggest that the mirrors' presence offers some advantages, perhaps related to comfort and welfare that could be used as environmental enrichments for fattening rabbits. However, the installation costs should be taken into account before considering their use for long time individually caged animals and for group-penned rabbits.

EFFECT OF FLOOR TYPE ON FOOTPAD INJURIES IN DOES: A PILOT STUDY

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In the Netherlands, welfare regulations indicate that rabbits should be housed on 3.02 mm wire floors instead of the 'old' 2 mm wire floors. It was expected that does on the new 3.02 mm wire floors had less footpad injuries as compared to does housed on the old 2 mm floors. However, there were no data supporting this suggestion. In addition, data on wire floors with plastic mats were also lacking. Therefore we studied footpad injuries in does housed on 2 mm, 3.02 mm wire floors and 3.02 mm wire floors with a plastic mat. Here we show the first results measured on three different rabbit farms with 2 and 3.02 mm floors. One farm also had 3.02 mm wire floors with a plastic mat. Footpads were scored in at least 20 does per floor type per farm during seven reproduction cycles. Results indicate that there are no differences between 2 and 3.02 mm wire floors. After two reproduction cycles footpads become injured which has a negative effect on the welfare of the does. Plastic mats seem to have a positive effect on the footpads, but this should be confirmed on more farms.

EFFECT OF LIGHTING PROGRAM ON THE NURSING BEHAVIOUR OF RABBIT DOES

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The objective of the present study was to analyse the effect of the lighting regime on the nursing behaviour of rabbit does. The experiment was carried out at the University of Kaposvár using Pannon White rabbit does. Prior to the experiment does were kept using a lighting program of 16L/8D and each doe already had 2-3 parturitions. The does were randomly housed in two identical rooms which differed only in the lighting regime. In the first room a 16L/8D lighting regime was used throughout the experiment (16-16L group). In the other room a lighting system of 8L/16D was used during the days after parturition and the photoperiod was increased to 16 hours per day for 8 days prior to insemination. Insemination was performed on the 11th day after parturition in both rooms. After insemination the light period was modified to 8 hours per day (8-16L group). Luminous intensity measured in the cages at the height of rabbit does ranged between 40 and 70 lux. From kindling till 14 days postpartum 24-hour video recordings were taken with infra-red cameras (16-16L group: 16 does, 8-16L group: 18 does). The distribution of the once-a-day nursing was 62.4% and 60.4% in the 16-16L and 8-16L groups, respectively, while the twice-a-day nursing was 31.7% and 35.6% in 16-16L and the 8-16L groups during the whole period (day 1-14). Time, daily frequency of nursing and the length of stay in the nest-box were recorded for every doe. Changing the lighting (from 6:00 am-2:00 pm to 6:00 am-10:00 pm) affected the does' nursing behaviour: the daily frequency of the nursing events (nursing twice and three times a day: 37-40%) and number of nursing events per day were stable in the 16-16L group. In the 8-16L group the multiple nursing frequency increased from the 4th day postpartum (from 25.6 to 43.6%, days 1-3 and 12-14, respectively). Length of nursing was significantly different between the groups (16-16L: 202±60 sec., 8-16L: 184±38 sec., mean±SD, P<0.05). Similar differences could be observed comparing the does performing once-a-day nursing (16-16L: 195±42 sec. vs. 8-16L: 180±35 sec., P=0.001) and twice-a-day nursing, (first nursing: 16-16L: 214±80 sec. vs. 8-16L: 193±44 sec.; second nursing: 16-16L: 215±81 sec. vs. 8-16L: 189±62 sec., P<0.05). It was concluded that changing the lighting program affected the does' nursing behaviour. The frequency of multiple nursing and the number of nursing events per day increased if the dark period was shorter (8 h instead of 16 h). The lighting period modified the duration of nursing events but the difference was not relevant.

GUIDELINES FOR MINIMUM STANDARDS ON RABBIT HOUSING IN GERMANY

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The main welfare indicators to assess rabbit housing are mortality (unavoidable low), morbidity (unavoidable low), physiological parameters in the species-specific standard, species-specific behaviour and performance on a high level. The single housing of does with kits remains the main housing system in the near future which can be characterized by detailed measures and parameters. Growing rabbits are mainly kept in groups with a tendency towards pen housing with different kinds of enrichment (e.g. wooden sticks as gnawing material). In May 2007 guidelines for the housing of rabbits under the aspects of animal protection and welfare were published in Germany which are described in detail in the present paper. The minimum requirements for breeding and growing rabbits are summarized in two tables. Enriched cages should be used. The space for a breeding doe in single housing ranges between 2.000 and 4.800 cm² depending on body weight and the use of an elevated seat. If an elevated platform is used the floor space of the cage plus the space of the elevated seat plus the nest box space are added to available surface. The minimum height of a cage for the doe is 40, 60 cm respectively also depending on the use of a platform. The elevated seat in a height of 25 cm should have a space of 1.000 cm². The size of the nest box is recommended with 800 cm² and a height of 30 cm. The minimum space per growing rabbit should be 700 cm² in groups up to 5 rabbits and 600 cm² in groups of more than 5 rabbits (per rabbit) with a stocking density of 40 kg/m². The minimum height of the cage for growing rabbits is 35 cm. The diameter of the metal wire at the floor should be in minimum 3 mm. The width of slats should be in minimum 10 mm and in maximum 16 mm. The rabbits shall have permanent access to water of good quality. There are some guidelines regarding the animal-feeding place-ratio, the light program, the concentrations of ammonia and carbon dioxide in maximum and the frequency of daily controls of animal health and the technical function of feed and water supply and of microclimate.

ANALYSIS OF THE BEHAVIOUR OF GROWING RABBITS HOUSED IN DEEP LITTER AT DIFFERENT STAGES OF REARING

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The experiment was conducted with 240 Pannon white growing rabbits of both sexes, which were housed in a closed building with a temperature of 16-17 °C using a light regime of 16L/8D. The rabbits were housed in 80 cm high open top pens each having a basic area of 50x170 cm. Depending on the time of deep litter placement into the pens the following groups were formed: 1. Rabbits stayed on wire net floor throughout the experiment; 2. Rabbits stayed on wire net until the age of 7 weeks, time at which the deep litter was added; 3. Rabbits stayed on wire net until the age of 9 weeks, time at which the deep litter was added; 4. Rabbits stayed on deep litter throughout the experiment. Within all four groups 3 stocking densities (8, 12 and 16 rabbits/m²) were used. The experiment lasted between the ages of 5 and 11 weeks. A 24 hour video recording was performed once a week using infrared cameras on the same day of every week during the 6 week long experiment. Using the 24 hour video recording every 10th minute was evaluated. The activities performed at these times were recorded and their frequencies and percentages were calculated for 24 hours. The following activities were analyzed: eating, drinking, locomotion, rest, comfort, social, and agonistic behaviours, stereotypes and the frequency of deep litter consumption (for rabbits housed in deep litter). The frequency of eating was affected significantly by the type of floor. After placing deep litter on the wire net floor the frequency of eating (but not of drinking) significantly decreased. The highest locomotor activity of the rabbits was observed during the first week of the experiment and placing deep litter on the wire net floor did not change the animals' activity. Contrary to our expectations a significantly higher frequency of resting was observed on the wire net than on the presumably more comfortable deep litter. A significantly higher frequency of comfort behaviours was also found on wire net than on deep litter, which became soiled during rearing. Rabbits showed agonistic behaviours only occasionally; consequently, the effects of floor type, stocking density and age on this behaviour could not be analyzed. The occurrence of stereotypic behaviours was relatively high during the first two weeks of the experiment after which it decreased and, during the last two weeks of the experiment, it was not observed in either group. Based on the results of this study it was concluded that after placing deep litter on the wire net floor the rabbits willingly consumed the litter material which, in turn, decreased their pellet consumption. Placing deep litter on the wire net floor did not alter the frequency of other behaviours and, thus, did not improve the rabbits' well being.

SUITABILITY OF BEHAVIOURAL TESTS FOR DETERMINING THE WAYS RABBITS FUNCTION IN THE ENVIRONMENT AND THEIR RELATIONSHIP WITH SOME PRODUCTIVE TRAITS

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This study aimed to obtain by selection two lines of New Zealand white rabbits differing in total motor activity and to compare them for fertility, prolificacy, mortality and weight gains of young rabbits from birth until weaning. All females in the groups were mated for the first time. The emotional states of animals such as fear were analysed with the behavioural tests (open field, tonic immobility) and stress-induced hyperthermia (SIH). The way in which animals react to different potentially dangerous situations is stable and reflects two fundamentally different, genetically determined strategies/ways of functioning in the environment, the active and passive strategies. Active individuals strive to avoid stress stimuli or try to manipulate them, while passive individuals show reduced reactivity to environmental stimuli. These tests were the criterion for assigning animals to two experimental groups: group I, 10 males and 20 females with the highest total motor activity (A); group II, 10 males and 20 females showing little or no motor activity (N). Important differences were found in male and female fertility during the first days of mating: during the first week, 80% of males mated in group I compared to only 20% in group II. Despite their normal breeding condition, passive males were timid and ponderous during mating, unlike active males which mated willingly and rapidly. During the first week, 45% of active females and 70% of passive females were mated. Fertility was high in both groups (87.5% and 94.7% in group I and II). Unlike females from group II, females from group I showed no tendency towards nest building. 40% of females from group I gave birth in different cage areas, while 90% of females from group II gave birth in nest boxes with down and straw bedding. Active females gave birth to a high percentage (22.2) of dead rabbits. There were highly significant differences in the mean number of rabbits reared per litter (5.2 in group I and 6.8 in group II). No differences were found between the active and passive lines for body weight at birth or at 35 days of age. Passive females were better mothers and showed greater maternal care. The high mortality in the active group was due to the trampling of the young rabbits, scattered all over the cage, by overactive mothers. In conclusion, the line of active females is likely less suitable to rearing in cages of standard dimensions, which are incompatible with the "active" type of behavioural strategy. Females from this group are much worse mothers. Passive animals preferred to remain in confined space, which is considered as an adaptive mechanism to cope with environmental conditions. Active females are more confident, attain breeding maturity earlier and begin mating more quickly, which is why they are recommended for further rearing.

HALAL SLAUGHTER AND ELECTRICAL STUNNING IN RABBITS: EFFECT ON WELFARE AND MUSCLE CHARACTERISTICS

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A total of 50 recently weaned Hyla rabbits (5 weeks old) were slaughtered in a commercial abattoir located 65 km away from the farm they were raised in. Their slaughter weight was determined after 20-30 min of lairage and, afterwards, the rabbits underwent slaughter following the standard procedure (electrical stunning and exsanguination after cutting the vessels of the left side of the throat; 30 rabbits, S group) or the halal muslim procedure (ritual cut of the throat performed by an appropriate person without pre-slaughtering stunning; 20 animals, H group). Some basic animal welfare features around slaughter were evaluated. Rabbits from H group had neither vocalization, spasms or movements during the hanging phase nor after slaughtering. Their bodies remained totally relaxed and floppy on the chain from the beginning. The rabbits from the S group also had not vocalizations or movements before slaughtering and one rabbit of this group arched and flexed its back for a moment after slaughtering. No cases of haemorrhages or broken bones were observed in either group. There were significant differences between groups with regards to the blood losses (calculated by the difference between the animal's weight and the hot carcass weight plus offal), which were higher in the H than in the S group (44.86 g and 37.10 g respectively, $P < 0.01$). The external colour of carcasses, subjectively evaluated, was paler in the H than in the S group. Similar differences in colour were initially found at 0 minutes after transversal cuts of the *Longissimus dorsi* muscle: more luminous (L^*) and yellow (b^*) as well as higher h^* values in the H group, although the effect was no longer significant after 15 minutes of blooming. The lowest pH_{24} values were found in the H group on both the *Longissimus dorsi* (5.67 vs. 5.78, $P < 0.05$) and the *Biceps femoris* (5.69 vs. 5.82, $P < 0.01$) muscles. There were no differences in water holding capacity determined on the *Biceps femoris* muscle. We conclude that under both slaughtering procedures, the parameters studied had normal values for rabbit meat and correct for meat in general.

PASTURE AVAILABILITY AND GENOTYPE EFFECTS IN RABBITS: 1. HEALTH AND WELFARE

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To analyze the effect of pasture availability and genotype on health and welfare 80 growing rabbits (40 Leprino of Viterbo and 40 New Zealand White, NZW) were assigned to two groups: control, reared in bicellular standard cages or wired pen, provided of an external grass pasture. Blood samples and behaviour observations (10 rabbits per group) were performed at different ages (weaning, 49 and 89 days). Lysozyme presented at all ages and both housing systems a significant genotype effect: Leprino rabbits showed higher values than NZW rabbits. Serum Bactericidal Activity (SBA), on the contrary, was influenced by housing systems in both genotypes: Leprino showed the highest values in caged animals, while NZW in pasture ones. Haemolytic Complement Assay increased with age in Leprino, while it decreased in NZW. Plasma TBARs (Thio-Barbituric Acid Reactive substances) were always higher in Leprino rabbits whereas plasma tocopherol showed an inverse trend. Stereotyped activities were present only in caged rabbits and NZW showed the highest incidence at a young age. Eating was affected by housing system and genotype, NZW pasture rabbits showing a preference for grass. As expected, animals with pasture availability showed the highest percentage of motor activities. Regarding comfort behaviours, at a young age its percentage was higher in caged animals, while at older ages it was highest in Leprino rabbits. NZW caged rabbits showed the highest percentage of static behaviours in old age. Leprino rabbits showed more social behaviours than NZW. Mortality rate of Leprino rabbits was the higher in cage housing, while in NZW this parameter was highest in the pasture system.

MUTUAL VISUAL RELATIONSHIPS OF RABBITS RAISED IN INDIVIDUAL CAGES

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To test if rabbits located in cage rows, as in the industrial units, can manifest a kind of social relationship by looking at each other, a specific experimental set was prepared. This was formed by three contiguous cages in which three 17 weeks-old does were introduced. The behaviour of the does was video-recorded for 24 hours and a photogram was taken every 12 minutes (120 per day) for 14 consecutive days. The trial had three replications and a total of 9 does was checked. The three cages offered a different situation. The work hypothesis was that, if looking at another subject is a way of showing social interest, the does in the lateral cages should orient their head significantly more frequently towards the central cage than in the opposite direction. If the doe in the central cage looked more often at one of the two does located on either side, this should be interpreted as a social preference. Though the does manifested a clear individual behaviour, the tendency to look towards the subject nearby was highly significant ($P < 0.001$). On the contrary the differences were not significant when the doe was located in the central cage and had a conspecific animal on both sides of its cage. It is then concluded that rabbits show a tendency to look at each other also when located in different cages. In this way they can establish a kind of visual social relationship when cages are contiguous. Apparently it is sufficient to have at least another rabbit to look at. Thus the disposition of the animals in cage rows, as in the industrial systems, must be considered fit to allow the manifestation of this type of social behaviour.

EXAMINATION OF FEEDER SIZE FOR GROWING RABBITS

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In two experiments the free choice of growing rabbits among cages with different length of feeders and the effect of the length of the feeder on the rabbits' growth were examined. Experiments were carried out on the rabbit farm of the University of Kaposvár using Pannon White rabbits. The temperature of the rabbit house was 16-18°C on average and a 16L:8D lighting period was applied. Rabbits were fed *ad libitum* a commercial diet in pellet and water was available *ad libitum* from nipple drinkers. In the first experiment 24 growing rabbits were reared in cage blocks (1.5 m² floor-area, 16 rabbits/m²) from 5 to 10 weeks of age (n=144). The blocks consisted of 4 cages of the same size and the rabbits could move freely among the cages through swing doors. The cages only differed in the size of the feeder. In the various cages the lengths of the feeders were 10, 20, 30 or 40 cm, respectively. Using infrared cameras 24 hour video recordings were performed once a week and the number of rabbits in the cages was counted for 30 minutes. Feed consumption was measured weekly using the different sized feeders. The experiment was carried out in 6 repetitions. Although the choice of the cages with different sized feeders was significantly different in all weeks, only a small difference in the choice across the whole experimental period was observed: 22, 25, 26 and 27% of rabbits stayed in cages with 10, 20, 30 and 40 cm length feeders, respectively. The feed consumption also changed according to the length of the feeders: 12.6, 17.9, 24.9 and 44.6% from the feeders with a length of 10, 20, 30 and 40 cm respectively. In the second experiment 13 growing rabbits were reared in four pens (0.96 m² floor-area) from 5 to 10 weeks of age (n=156). Every pen was equipped with two nipple drinkers. The pens only differed in the size of the feeder. The lengths of the feeders were 10, 20, 30 or 40 cm in the pens. The experiment was carried out in 3 repetitions. The body weight of the rabbits and the feed consumption from the different sized feeders were measured weekly. Using infrared cameras 24 hour video recordings were performed once a week and the number of rabbits found in front of the feeders was counted for 30 minutes. The body weight of rabbits was not affected by the length of the feeders (at 10 weeks of age: 10 cm: 2298 g; 20 cm: 2329 g; 30 cm: 2320 g; 40 cm: 2363 g, P=0.425). There was no significant difference in feed intake (P=0.968) or feed conversion in pens with different sized feeders. It can be concluded that the feeders used in rabbit breeding have of an appropriate size from the viewpoint of animal welfare and rabbits' growth.

EFFECT OF GNAWING STICKS ON THE WELFARE OF GROWING RABBITS

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The objective of the experiment was to analyse the effect of the application and type (hard vs. soft) of gnawing sticks on the productive performance, gnawing stick consumption and occurrence of ear lesions related to aggressive behaviours in growing rabbits. Pannon White rabbits (n=156) weaned at the age of 5 weeks were placed in pens having a basic area of 0.86 m² (13 rabbits per pen) using a stocking density of 16 rabbits per m². The rabbits were housed in the pens until the age of 11 weeks. Three groups were formed according to the gnawing sticks: H = hard stick (White locust); S = soft stick (Little-leaf linden); C = control (no gnawing sticks were provided). Diameter and length of the gnawing sticks were 3 cm and 20 cm, respectively. Productive traits in the three groups (i.e., weight gain, body weight, feed consumption, feed conversion) were not different among the three groups. However, rabbits in the S group consumed significantly (P<0.001) more gnawing stick than those in the H group (H: 0.11 vs. S: 1.24 cm³/day/rabbit). Compared to the C group (17.3%) occurrence of ear lesions was significantly (P<0.05) lower both in the H (7.7%) and the S (1.9%) groups. From these results we conclude that, under group housing conditions, the application of gnawing sticks (especially those made from soft tree like little-leaf linden) can decrease the frequency of aggressive behaviours of rabbits without influencing their performance.

EFFECT OF THE FLOOR TYPE AND STOCKING DENSITY ON THE PRODUCTIVE PERFORMANCE OF GROWING RABBITS

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The aim of the experiment was to study the effect of the floor type and stocking density on the performance of growing rabbits. Five-week-old Pannon White rabbits (n=120) were housed in pens (0.82 m²) until 11 weeks of age. Three groups were established according to the floor type. Straw was put into the pens at different times after weaning: 6W = wire net during the whole fattening period, 4W2D = wire net between the 5th and 9th and straw between the 9th and 11th week, 2W4D = wire net between the 5th and 7th and straw between the 7th and 11th week. The number of rabbits per pen was 13, 10 or 7 using the stocking density of 16, 12 or 8 rabbits/m². Rabbits were fed *ad libitum* with a commercial diet in pellet with and without medication after weaning and during the last two weeks of fattening period, respectively. The time at which the straw was placed into the pens had a weak effect on the average daily gain, feed intake and feed conversion ratio. In groups 4W2D and 2W4D the feed intake and average daily gain decreased after placing straw into the pen. The stocking density did not affect significantly the productive performance of growing rabbits. We conclude that housing of rabbits on wire net after weaning and rearing them on a straw floor during the second part of the fattening period could be a good strategy for preventing enteritis (coccidiosis) and achieving a good production.

SOCIALIZATION OF DUTCH BELTED RABBITS FOR USE IN RESEARCH: PRELIMINARY OUTCOMES

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Dutch belted rabbits are commonly used as research animals due to their small size compared to New Zealand White rabbits. As a generalization, Dutch belted rabbits tend to be approximately 2/3 smaller than New Zealand Whites in our colonies. In the laboratory, this translates to the utilization of less test article and easier manipulations of the animals with fewer worker injuries. However, in the United States, it has been reported that commercially raised black Dutch belted rabbits are more aggressive towards their human handlers and less adaptive to environmental change than their New Zealand White counterparts.

An intensive socialization program was initiated in a colony of black Dutch belted rabbits raised exclusively for research. This program includes adaptation to common animal handling procedures, environmental enrichment devices, and other materials encountered in the laboratory. This program encompasses handling pre-and post-weaning and prepares the animals for use in research with less overall aggression to human caretakers and less stress to novel environmental stimuli. Success of the program has been measured in terms of a decrease in the number of complaints received about animal behavior once the animals have arrived at the research facility.

EFFECT OF ENVIRONMENTAL CONDITIONS ON PRODUCTIVE AND PHYSIOLOGICAL RESPONSES IN GROWING RABBITS

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Environmental conditions are important for the welfare of intensively-reared rabbits. The presence inside the cage of a piece of wood represents a form of environmental enrichment, but also an alternative type of feeding. A trial was carried out to study the effect of lighting and type of feeding on the productive performance, bone conditions and plasma profile of growing rabbits. Male rabbits (Hycole genotype) were reared from 44 to 80 days in single cages and submitted to either of two photoperiods: long 9L:15D (group LP) or short 5L:19D (group SP) and to either of two types of feeding: feed only (group F) or feed and a piece of wood (group W). The experimental factors were arranged following a factorial model 2x2 and their interactions (LPF – LPW – SPF – SPW) were also considered. Wood of *Salix alba* was used, placed on the slat of each cage.

Lighting did not affect the productive performance, whereas significant effects of feeding type were observed: the W group had a significantly higher ($P<0.05$) growth rate and feed intake than the F group. The feed:gain ratio did not change according to the feeding type.

Lighting did not affect femur and tibia length and modulus of elasticity, but SP rabbits showed higher ($P<0.05$) tibia stress than the LP group. Calcium was lower ($P<0.01$) in SP bones. The physical and chemical characteristics of the femur and tibia were unaffected by feeding type, except for calcium which was slightly lower in W tibiae. The plasma profile did not change according to experimental factors except for urea, which had a higher ($P<0.05$) level in W rabbits than in F animals.

The data obtained indicate that the lighting type does not represent an environmental factor capable of strongly affecting the productive performance of growing rabbits kept in conventional cages. However, it becomes more important for the skeletal status, especially when applied to rabbits reared in enriched cages since an interaction between photoperiod and wood activity may exist.

The results demonstrate that the use of *Salix alba* wood for growing rabbits allowed a better productive performance without notable effects on skeletal bones or plasma profile.

EVALUATION OF RABBIT WELFARE AT STUNNING AND SLAUGHTERING IN A COMMERCIAL ABATTOIR

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A total of 1020 crossbreed rabbits were individually examined to evaluate their welfare at electric stunning and slaughtering in a commercial abattoir. Stunning (position of electrodes and repetition of current applications) and sticking (position, length and depth of the cut) procedures were checked. Rabbits were behaviourally monitored from current application to death. The stunning system was incorrectly applied one hundred and ten times (10.8%). Three rabbits failed to be stunned and were still conscious at sticking. Eighteen rabbits recovered before the onset of death, as evidenced by the corneal reflex, and in a few cases vocalization (n=3) and head righting (n=1) were observed. Corneal reflex seemed to be the best indicator of recovery at the abattoir.

TEMPERATURE AND CAGE FLOOR ENRICHMENT AFFECT THE BEHAVIOR OF GROWING RABBITS

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The effects of temperature and cage floor enrichment on some behavioral activities of growing rabbits and the effect of temperature on cage floor choice were investigated. Forty-eight rabbits from the Botucatu genetic group, weaned at 35 d of age, were housed at low density (1,500 cm²), four per cage, in wire net cages up to 10 weeks of age. Cages were placed either in a natural temperature chamber or in a refrigerated one. In each chamber, half of the cages had part of the floor surface covered with a wooden board enriched with litter straw such that, in enriched cages, rabbits had free choice between the straw and the wire net floor. In all cages, rabbits had free access to water and feed on both sides of the cage. In enriched cages, the number of rabbits on the wire net floor and on the litter straw was recorded by scan sampling every 5 minutes in five 24-h observation periods carried out once a week over five weeks. The behavior of animals was evaluated in the same five 24-h observation periods. The mean daily temperature and relative humidity were higher in the natural temperature chamber than in the refrigerated one (23.6°C and 78.7% vs. 20.6°C and 71.0%, respectively). In the natural temperature chamber, rabbits preferred the wire net floor over the litter straw (77.9 vs. 22.1%, $P < 0.01$), whereas in the refrigerated chamber they did not show any preference (45.9 vs. 54.1%, $P = 0.41$). Self-grooming was the most frequent behavioral activity observed (51.9%), whereas stereotypes were the least frequent (5.2%); exploratory and ludic activities and social interaction showed intermediate frequencies (20.6, 11.7 and 10.6%, respectively). A higher number of ludic and exploratory events were recorded in the enriched cages of both chambers, which may be associated with a higher degree of welfare. Stereotypes were less frequent in the refrigerated chamber; however, a higher incidence of stereotypic behavior was observed in non-enriched cages of both chambers. Self-grooming was also more frequent in non-enriched cages, which may be interpreted as an expression of stereotypy. In the natural temperature chamber, social interaction was higher in non-enriched cages, whereas no differences between enriched and non-enriched cages were observed in the refrigerated chamber. Although environmental enrichment with straw showed a favorable impact on rabbit welfare, its positive effects seemed to be less intense at higher temperature. Furthermore, higher temperature substantially decreased the proportion of rabbits that chose to stay on the straw in enriched cages.

EFFECT OF CAGE FLOOR AND STOCKING DENSITY ON GROWTH PERFORMANCE AND WELFARE OF GROUP-HOUSED RABBITS

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Two hundred forty rabbits weaned at 36 d (LW=1103±41 g) were housed in groups to evaluate the effect of stocking density and type of cage floor on behaviour, health status, growth performance, and carcass and meat quality. The rabbits were put in groups of 6 into 40 cages of two sizes (78 x 64 cm or 58 x 64 cm) corresponding to two stocking densities (D12, 12.1 rabbits/m² vs. D16, 16.2 rabbits/m²). Within density, four types of floor were compared (steel slat, plastic slat, wire net and straw litter on wire net) according to a 2 x 4 factorial arrangement with five replications (cages). The rabbits were fed a unique diet (CP: 15.8%, ADF: 19.3%, DE: 9.8 MJ/kg). No antibiotic was added to feed or water. Rabbit reactivity was assessed by the tonic immobility and open-field tests at 70 and 74 d of age. Rabbits were slaughtered at 78 d to evaluate carcass and meat quality. Femur dimensions and resistance to fracture were measured. Sanitary status and growth performance were highly satisfying in all treatments: growth rate averaged 45.5 g/d and final weight 2967 g, with a feed conversion rate of 3.49. Stocking density did not affect growth performance, meat quality, nor animal reactivity. In contrast, the type of floor significantly affected growth performance: rabbits kept on straw bedded floor showed the lowest final weight (2865 g vs. 3000 g on average, P=0.03), weight gain and feed intake. Differences in carcass and meat quality mainly depended on the weight at slaughter: dressing percentage and separable fat of the carcass were lower (P<0.01) in the lightest rabbits kept on straw (58.6% and 2.3%, respectively) compared to the heavier animals of the other three experimental groups (average values 59.9% and 3.0%, respectively). The type of floor also affected animal reactivity: the number of attempts necessary to induce immobility, considered inversely correlated with fear towards humans, was lower in rabbits kept on straw; these rabbits also showed a more passive reaction during the open field test, spending less time exploring and more time in an immobile state (P<0.01) in comparison with the animals housed in the cages with plastic slat or wire net floors.

EFFECTS OF LOADING METHOD AND CRATE POSITION ON THE TRUCK ON SOME STRESS INDICATORS IN RABBITS TRANSPORTED TO THE SLAUGHTERHOUSE

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The aim of this study was to determine the effects of loading method and crate position on the truck on some stress indicators in commercial rabbits transported to the slaughterhouse. On two journeys in July a total of 192 animals (82 days old) were transported to the slaughterhouse during the morning (9.00 a.m.) for a mean transport time of 100 min. The transport truck was uncovered, with a total of 128 fixed plastic transport crates, for an overall capacity of 1500 subjects. To evaluate the effects of crate position, at each session, 96 animals were distributed at random in 8 crates on the same side of the truck (12 animals per cage, 57.7 kg/m²) as follows: 24 animals in 2 top front (TF) crates, 24 animals in 2 bottom front (BF) crates, 24 in 2 top rear (TR) crates and 24 in 2 bottom rear (BR) crates. In the middle of TF, BF, TR and BR crates four data-loggers were placed for temperature (T°) and relative humidity (%RH) control. To evaluate different loading methods one cage from each position was loaded in a smooth way (S: 12 rabbits from the farm crates were placed in a wide trolley and carried gently into the transport cage - loading time for 48 subjects: 12 min.) while the other was loaded in a rough way (R: rabbits from four crates were carried all together in the same trolley and loaded hurriedly - loading time for 48 subjects: 4 min.). All rabbits were individually weighed before transport and at unloading. To assess some stress indicators, blood samples were collected from 40 male rabbits (20 per journey: 5 per truck position, 10 per loading method) on the farm 2 days before loading (basal level) and at slaughter during exsanguinations. The TR crates showed the highest mean temperature and the lowest relative humidity (P<0.001) while the other cages on the truck differed only in humidity. No effects on weight losses during transport could be ascribed to loading methods or crate position in the truck. Corticosterone showed a tendency to increase from basal levels in all animals, the increase being significant only during transport using the rough loading method. Neutrophilia and lymphocytopenia were significant for all rabbits, independently of their position in the truck or the loading method. Packed cell volume never differed significantly among groups. Rabbits transported in TR crates (with higher mean temperature) showed a significant augmentation of total protein level, as a possible consequence of dehydration. A significant upsurge of aspartate amino transferase and creatine kinase activities was observed in all the animals. In conclusion, stress parameters were more influenced by transport and handling itself rather than by specific conditions related to different loading methods or crate position in the truck.

FREQUENCY OF ENGAGEMENT WITH DIFFERENT MATERIALS BY GROWING RABBITS

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The frequency of engagement with different materials as environmental enrichment by growing rabbits in cages with plastic slatted floor was studied in two experiments using a total of 108 rabbits (ZIKA hybrids). In each experiment, 54 rabbits were kept in four group-cages with different group-sizes (8, 8, 16 and 22) at the same stocking density (625 cm²/rabbit). The cages were equipped with nipple drinkers and feeders. Feed and water were available *ad libitum*. The duration of the photoperiod was 16 hours. In the first experiment (D1), pellets were fed *ad libitum* from a conventional feeder installed outside the cage. In the second experiment (D2), another feeding system which gives the rabbit the possibility to take crude-fiber *ad libitum* was additionally located in the middle of every cage. Three different occupational tools were installed in the cages: an iron-chain with a wooden gnawing stick (WGS) for the two groups with eight rabbits (A, B), one iron-chain with a plastic gnawing stick (PGS) placed in the group with 16 rabbits (C) and two iron chains connected by a horizontal iron stick (ICS) for the group with 22 rabbits (D). In both experiments, the same kinds of occupational tools were located in the same cages. The frequency of occupation was studied by 24 hours video-observation with infrared cameras at the beginning, the middle and the end of the fattening period (eight weeks). Continuous ethological analyses were performed with the program Observer (Noldus). Additionally, the time sampling method with an interval of five minutes during three periods a day (08:00-10:00; 19:00-21:00; 01:00-05:00) was used to register the number of rabbits in each group showing the following behavioural patterns: eating, drinking, lying, hocking, hopping, own body licking, erecting, engagement with the materials, miscellaneous. In D1, the frequency of occupation was the highest in cages A and B with WGS followed by cage C with PGS and cage D with ICS. In D2, there were no significant differences between the cages, but the frequency of occupation with the different tools in all cages was significantly lower than in D1. Also the results of the time sampling method showed a significant higher frequency of occupation in D1 compared with D2. In the second experiment, the rabbits of all cages were more often observed “eating total” (hopper with pellets plus feeder for crude-fiber) than in D1 (only pellets from the hopper). Experiment 2 shows that a feeding system which gives the rabbits the possibility to satisfy the demand for crude-fiber-intake is a tool in the housing system that allows the rabbits to express a species-specific behaviour.

WELFARE AND HAEMATOLOGICAL INDICES OF WEANER RABBITS AS AFFECTED BY STOCKING DENSITY

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Forty two weaner rabbits of mixed breeds and sexes with an average initial weight of 956.7 ± 45.6 g were used to evaluate the effect of stocking density on welfare and haematological characteristics. The rabbits were allotted to four stocking densities of 10, 14.3, 20 and 25 rabbits/m². This corresponded to 2, 3, 4 and 5 rabbits per cage (50 x 40 x 30 cm) in a completely randomized design. Each condition was replicated three times. Animals were fed *Centrosema pubescens* supplemented with pelletized commercial growers' feed. Fresh and clean water was also freely available throughout the duration of the study which lasted six weeks. A significantly higher ($P < 0.01$) body condition score was observed in rabbits stocked at 10 followed by those at 14.3, 20 and 25 rabbits/m². Rabbits reared at 14.3 had better fur condition ($P < 0.01$) than those kept at 20 and 25 rabbits/m² respectively. During the open-field assessment, gait score was adversely affected ($P < 0.01$) at the highest housing density. The proportion of rabbits with fight wounds increased ($P < 0.01$) in densities of 20 and 25 rabbits/m². A higher incidence of hock and foot burns ($P < 0.01$) was recorded among rabbits at the highest stocking density. Rabbits housed at 10 and 14.3 rabbits/m² were superior to those at higher densities in packed cell volume, haemoglobin concentration, red blood cell counts and mean corpuscular volume, although differences were not statistically significant. No definite trend was observed in mean corpuscular haemoglobin or mean corpuscular haemoglobin concentration. White blood cell counts; neutrophil, lymphocyte, monocyte and eosinophil values were also not significantly different among rabbits under the varying stocking densities. However, the neutrophil/lymphocyte ratio suggests that rabbits stocked at 20 and 25 rabbits/m² were more stressed. These results indicate that increasing the density beyond 14.3 rabbits/m² elicited some negative effects on live performance of weaner rabbits in the savanna zone of a tropical environment.

EFFECT OF HANDLING IN PRE-WEANING RABBITS

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Fear is an emotion that may negatively affect rabbits' welfare, as a consequence of bad handling during the rearing period. A number of recent researches suggests that a certain level of gentle handling in the pups, scheduled very accurately, can reduce the fear stress of the animals towards new environments and humans. The fear response may be reduced by handling around nursing time during the first week after birth. The aim of this study was to investigate the effects of regular daily handling on weight gain and on the fear reaction towards a new environment and towards human beings in domestic rabbit pups. We obtained different results between handled pups and control pups in the emergence test and in the immobility test. In fact we found that a minimal human contact applied before nursing reduced the fear of humans in rabbit pups. Our handling method, requiring minimal contact, can be used to reduce fear and improve welfare in rabbits. Furthermore, the short length of the handling procedure allows stockpersons to produce tamer rabbits, which could have a better reproductive and productive capacity due to reduced stress levels.

EFFECT OF ENVIRONMENTAL ENRICHMENT AND GROUP SIZE ON BEHAVIOUR AND PRODUCTION IN FATTENING RABBITS

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During recent years the analysis of the rabbits' welfare has received increasing attention. Many scientific investigations have been carried out on the effects of alternative housing systems on productive performance, meat quality and welfare of rabbits. Intensive rabbit breeding normally involves the use of small collective cages housing 2 to 8 rabbits according to the surface area of the cage and to the local tradition. The housing system should allow adequate social contacts because rabbits are social animals living in colony; moreover, environmental enrichment may allow the animals to perform their natural behaviors, thus possibly improving their welfare. The aim of this research was to study the effects of group size and environmental enrichment on the behaviour and production of 216 hybrid fattening rabbits (*Oryctolagus cuniculus*). We compared behaviour (time budget and reactions to specific behavioural tests: "tonic immobility" and "emergence test") and weight gain of fattening rabbits housed in cages with a different number of animals (2, 3 or 4 per cage) but with the same density (714 cm² per cage). Moreover, half of the cages were enriched using a wooden stick. Wooden stick and number of animals per cage had no effect on weight gain or the reactions in the behavioural tests. No statistically significant differences between day and night on behaviour were apparent. The interaction with a wooden stick was significantly higher at the beginning of the fattening period. Principal component analysis performed on the whole period showed significant differences according to the treatments on the following behaviours: movement, sitting, lying, and allo-grooming. Increasing the number of rabbits per cage and introducing a wooden stick seemed to affect movement frequency and social interactions. Rabbits housed 3 and 4 per cage showed less lying behavior and higher movement and sitting; thus, the larger functional space could allow rabbits to perform more natural behaviors compared with rabbits housed 2 per cage. Environmental enrichment is related to higher allo-grooming, which could indicate a social behavior related to pheromonal olfactory stimulation and mutual recognition.

MEAT QUALITY AND SAFETY

ENHANCEMENT OF NUTRITIONAL QUALITY AND SAFETY IN RABBIT MEAT

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ABSTRACT

Nutritional value and safety have gained great importance among the factors that determine meat quality. The close relationship between diet and health has led to changes in consumer habits, demanding products that meet their dietary and nutritional preferences. Rabbit meat is highly valued for its nutritional and dietary properties; it is a lean meat with a low-fat content and less saturated fatty acids and cholesterol than other meats. In this review, a short description of the nutritional value of rabbit meat is provided and the role of rabbit meat as a functional food is discussed. Rabbit meat consumption could be a good way to provide bioactive compounds to human consumers, since manipulation of rabbit's diet is very effective in increasing the levels of ω 3 PUFA, CLA or Vitamin E. An overview of the possibilities to incorporate these bioactive compounds in rabbit meat is offered.

Food safety is an important and essential aspect for consumers, especially on the meat sector since numerous crises have recently affected meat production. Bovine Spongiform Encephalitis (BSE) in bovine, high dioxin levels in chicken meat, the danger of increased spread of other infectious disease (e.g. Foot and Mouth disease, Avian influenza, etc.), as well as pathogens such as *Salmonella*, *Listeria monocytogens*, *Campylobacter* and *Escherichia coli* 0157:H7 have recently contaminated the European livestock and meat chains. Microbiological quality, presence of chemical residues, animal identification and traceability issues as well as the hazard analysis and critical control points system in rabbit meat production is discussed.

INTRODUCTION

Meat quality has been traditionally determined by sensory aspects, as their appearance, texture, as well as its aroma and flavour. However, at present the nutritional value and safety have gained great importance among the factors that determine meat quality. The nutritional role of meat is controversial because consumers generally consider that high ingestion of meat contributes to excess fat, cholesterol and saturated fatty acids, which are strongly linked to obesity and cardiovascular problems. The close relationship between diet and health has led to changes in consumer habits, demanding products that meet their dietary and nutritional preferences. Rabbit meat

is highly valued for its nutritional and dietary properties; it is a lean meat with a low-fat content and less saturated fatty acids and cholesterol than other meats. Moreover, different strategies can be followed to increase beneficial meat components in order to obtain healthier rabbit meat, as will be discussed later. Food safety is an important and essential aspect for consumers, especially on the meat sector where numerous crises have recently affected meat production. These crises have led to a decline in consumer confidence and in subsequent economic losses for the meat industry. Microbiological quality, presence of chemical residues, animal identification and traceability issues, as well as the hazard analysis and critical control points system in rabbit meat production will be discussed.

NUTRITIONAL QUALITY OF RABBIT MEAT

Recently, the nutritional value of rabbit meat has been reviewed by several authors (Combes, 2004; Dalle Zotte, 2004; Combes and Dalle Zotte, 2005; Hernández and Gondret, 2006), showing that rabbit meat has a high nutritional value compared with other meats.

The main components of meat, excluding water, are proteins and lipids. Rabbit meat is a lean meat rich in proteins of a high biological value and it is characterized by high levels of essential amino-acids (Dalle Zotte, 2004). Furthermore, meat is also an important source of highly available micronutrients, such as vitamins and minerals. Also, rabbit meat does not contain uric acid and has a low content of purines (Hernández, 2007). The information available on chemical composition of rabbit meat is extremely variable, especially regarding fat content, depending on the part of the carcass studied (Pla *et al.*, 2004) and also on the different productive factors (Dalle Zotte, 2002), especially feeding factors having a strong influence on the chemical composition of rabbit meat, in particular, on its lipid composition.

Rabbit meat is characterized by its lower energetic value compared with red meats (Dalle Zotte, 2004) due to its low fat content. Fat content varies widely depending of the carcass portion from 0.6 to 14.4% (fat from edible meat with intramuscular and intermuscular fat content) with an average value of 6.8% (Hernández and Gondret, 2006) with the loin being the leanest part of the carcass (1.2% of lipids). Fatty acid composition of rabbit meat is characterized by high polyunsaturated fatty acid content. The fatty acid composition of rabbit meat and its possible modification through diet will be discussed later on. The amount of cholesterol in rabbit meat is about 59 mg/100 g of muscle (Combes, 2004), lower values than those presented in meat from other species (61 mg in pork, 70 mg in beef, 81 mg in chicken) (Dalle Zotte, 2004).

The mineral fraction of rabbit meat is characterized by its low contents in sodium (49 and 37 mg/100 g for hind leg and loin, respectively) and iron (1.3 and 1.1 mg/100 g for hind leg and loin, respectively), while the phosphorus level is high (230 and 222 mg/100 g for hind leg and loin, respectively; Combes, 2004). Rabbit meat has a low zinc concentration (0.55 mg/100 g) and the copper concentration is quite similar to the meat of other species (0.03 mg/100 g) (Lombardi-Boccia *et al.*, 2005). Selenium

levels in rabbit meat depend on diet, reported to vary between 9 µg/100 g (Díaz-Alarcon *et al.*, 1996) and 22 µg/100 g (Wiesner *et al.*, 1978).

Meat is an important source of B vitamins. Consumption of 100 g of rabbit meat contributes to 8% of daily Vitamin B₂, 12% of Vitamin B₅, 21% of Vitamin B₆, and 77% of Vitamin B₃ requirements, and provides a fulfillment of the daily Vitamin B₁₂ requirement (Combes 2004). However, heat treatments alter Vitamin B contents (Lombardi-Boccia *et al.*, 2005). In addition, rabbit meat, as is true of other meats, contains only trace amounts of Vitamin A. Nevertheless, it should be noted that a high amount of this vitamin can be found in rabbit edible liver (Ismail *et al.*, 1992). Extra supplementation of Vitamin E in the diet (200 mg/kg) to improve the oxidative stability of the meat has led to an increase of almost 50% of Vitamin E in rabbit meat (Castellini *et al.*, 2000). Also, Vitamin E content is not affected by cooking treatment (Dal Bosco *et al.*, 2001).

ENHANCEMENT OF NUTRITIONAL QUALITY OF RABBIT MEAT

Rabbit meat and its role as functional food

In recent years, much attention has been paid to the influence of diet on human health and well-being. The primary role of diet is to provide sufficient nutrients to meet the nutritional requirements of an individual. There is now increasing scientific evidence to support the hypothesis that some foods and food components have beneficial physiological and psychological effects over and above the provision of the basic nutrients (Jones and Jew, 2007). Many traditional foods contain components with potential health benefits. In addition to these foods, new foods are being developed to enhance or incorporate these beneficial components due to their health benefits or desirable physiological effects.

Consumer interest in the relationship between diet and health has increased the demand for information about functional foods. No universally accepted definition for functional foods exists. The International Life Science Institute (ILSI Europe) has proposed the following definition for functional foods: "A food can be regarded as functional if it is satisfactorily demonstrated to affect beneficially one or more target functions in the body, beyond adequate nutritional effects, in a way that is relevant to either an improved state of health and well-being and/or reduction of risk of disease. Functional foods must remain foods and they must demonstrate their effects in amounts that can normally be expected to be consumed in the diet: they are not pills or capsules, but part of a normal food pattern" (Diplock *et al.*, 1999). A functional food can be a natural food, a food to which a component has been added, or a food from which a component has been removed by technological or biotechnological means. It can also be a food where the nature of one or more components has been modified, or a food in which the bioavailability of one or more components has been modified, or any combination of these possibilities.

The nutritive value of meat has an increasing importance among the factors determining meat quality and consumer acceptability. Indeed, meat is a major source of proteins and essential amino-acids; it is a source of group B vitamins, minerals,

and other bioactive compounds. However, meat is also a major source of saturated fatty acids and cholesterol and its consumption could be related to cardiovascular diseases, hypertension, obesity and diabetes (Valsta *et al.*, 2005). However, different strategies can be effectively used to increase or reduce bioactive compounds in order to produce functional meat and meat products (see Jiménez-Colmenero *et al.*, 2006 for a review). Rabbit meat, as it has been previously discussed, is a lean meat rich in proteins of high biological values, with highly unsaturated lipids, low cholesterol content, and noticeable quantities of linolenic fatty acid (C18:3 ω 3). Also, it displays a low content of sodium and a high content of phosphorus, and can be a good source of B vitamins (Hernández and Gondret, 2006). Most research conducted in recent years on rabbit meat quality has focused on incorporating bioactive compounds in meat for the benefit of human health. Moreover, rabbit meat consumption could become a good way to provide these bioactive compounds to human consumers, since manipulation of rabbit's diet is very effective in increasing the levels of ω 3 PUFA (Hernández *et al.*, 2007; Nuchi *et al.*, 2007), CLA (Corino *et al.* 2002 and 2003), or Vitamin E (Castellini *et al.*, 1999). In addition, both selenium and iron are also responsive to dietary supplementation (Lynch and Kerry, 2000). These possibilities will be discussed in this review.

Fatty acid composition

As Hernández and Gondret (2006) state, rabbit meat fat comprises mostly saturated fatty acids (SFAs) and polyunsaturated fatty acids (PUFAs), with percentages around, 36.9%, and 34.6% of total fatty acids in the hind leg, respectively. Monounsaturated fatty acids (MUFAs) are less represented (about 28.5%) (Table 1). The most ubiquitous fatty acids are oleic (C18:1), palmitic (C16:0), and linoleic (C18:2) acids, showing percentages higher than 20% of total fatty acids. Altogether, rabbit meat has a high ratio of PUFA to SAT fatty acids (0.75 and 0.85 for the loin and the meat of hind leg, respectively; Alasnier *et al.*, 1996; Ramírez *et al.*, 2005). Among the PUFAs, linoleic (C18:2) and linolenic (C18:3) are essential fatty acids because animal organisms are unable to synthesize them. Linoleic acid is the precursor of ω 6 family of PUFA, while linolenic acid serves the same function for the ω 3 family, especially for eicosapentaenoic (EPA) and docosahexaenoic (DHA) fatty acids. A minimum intake of combined EPA and DHA of 500 mg/day is recommended for human cardiovascular health (ISSFAL, 2004). The amount of linoleic fatty acid is about ten times greater in rabbit meat than in beef and lamb and around double than the quantity reported for pork meat (Enser *et al.*, 1996). The amount of linolenic acid is also remarkably abundant in rabbit meat (3%, Hernández and Gondret, 2006) in comparison with those reported in other meats (1.37 in lamb, 0.70 in beef and 0.95 in pork; Enser *et al.*, 1996). However, rabbit meat has a very low amount of EPA and DHA (Ramírez *et al.*, 2005). The ω 6: ω 3 ratio reaches high values, 7 (Dal Bosco *et al.* 2004) or 11 (Ramírez *et al.*, 2005) for the loin and the meat of hind leg, respectively. Therefore, increasing ω 3 fatty acid and consequently decreasing the ω 6: ω 3 ratio up to 5 is an interesting goal to improve the nutritional value of rabbit meat for human benefits (ISSFAL, 2004).

Table 1: Least squares means and standard errors of fatty acids content in rabbit leg meat (mg/100 g of meat).

Fatty acids	Mean \pm s.e.
C10:0 (Capric)	3.19 \pm 1.01
C12:0 (Lauric)	6.27 \pm 0.68
C14:0 (myristic)	67.1 \pm 2.82
C16:0 (palmitic)	712 \pm 24.6
C16:1 <i>cis</i> ω 7 (palmitoleic)	78.0 \pm 5.16
C16:1 <i>cis</i> ω 9	9.36 \pm 0.36
C17:0 (Margaric)	16.9 \pm 0.63
C17:1 (Heptadecenoic)	6.74 \pm 0.58
C18:0 (stearic)	185 \pm 5.88
C18:1 ω 9 (oleic)	635 \pm 24.3
C18:1 ω 7 (vaccenic)	34.9 \pm 1.32
C18:2 ω 6 (linoleic)	777 \pm 33.2
C18:3 ω 3 (α -linolenic)	81.2 \pm 4.81
C20:1 (icosaenoic)	9.96 \pm 0.73
C20:2 ω 6 (eicosadienoic)	12.8 \pm 0.58
C20:3 ω 6 (eicosatrienoic)	6.68 \pm 0.54
C20:4 ω 6 (arachidonic)	45.4 \pm 1.24

Adapted from Ramírez *et al.* (2005).

It is well known that rabbits, and other non-ruminants, are able to incorporate dietary fatty acids into adipose and muscle tissue lipids. Therefore, fatty acid composition is strongly affected by dietary lipid composition. The effects of various dietary fat sources have been the subject of many experiments in recent years. The addition of vegetable fat compared to animal fat sources in the diet leads to differences in rabbit meat quality, especially regarding fatty acid composition of the tissues and meat flavour (Oliver *et al.*, 1997; Hernández *et al.*, 2000). For instance, sensory test panels attributed a higher “liver” taste to animals fed with an animal diet, while meats of animals fed with a vegetable diet had a higher “aniseed” or “grass” flavour. However, no differences between groups were found for the texture parameters evaluated.

Different vegetable oil sources have first been used in rabbit diets to increase the level of lipid unsaturation (see Dalle Zotte, 2002 for a review). The dietary use of linseed oil has been proposed by many authors as a way to raise the content of ω 3 PUFA (Bernardini *et al.*, 1999; Dal Bosco *et al.*, 2004; Colin *et al.*, 2005; Hernández *et al.*, 2007). Dal Bosco *et al.* (2004) studied the synergistic effect of dietary α -linolenic acid and vitamin E on the oxidative stability and nutritional and eating characteristics of fresh and stored rabbit meat. This study confirmed the ability of rabbit to synthesize long chain PUFA (EPA and DHA) from the dietary precursor, leading to an increase in ω 3 PUFA content of the meat of rabbits consuming the ω 3 diet, without any alteration of oxidative stability and sensory quality of the meat. Hernández *et al.* (2007) studied the fatty acid composition of rabbit leg meat from animals fed with three diets enriched with 3% animal fat, 3% linseed oil and 3%

sunflower oil, respectively. These authors found higher percentages of linolenic and linoleic fatty acids in the meat of the animals fed with the diet enriched with linseed oil and sunflower oil, respectively, than in the animals fed with the diet enriched with animal fat. An increase of the long chain polyunsaturated fatty acid (EPA and DHA) as well as a decrease of the $\omega 6:\omega 3$ ratio (mean value of 1.31) was also achieved with the diet enriched with linseed oil. Recently, some studies pointed out the possibility of manipulating $\omega 3$ PUFA content in rabbit carcasses during the early growth period (Castellini *et al.*, 2004; Muñiz *et al.*, 2004). These studies showed that dietary maternal $\omega 3$ PUFA are secreted in the milk, allowing $\omega 3$ enrichment in the young tissues.

Grass-based diets can also modify fatty acid composition of rabbit meat. Forrester-Anderson *et al.* (2006) suggested that grass-based diets fed to rabbits reared outdoors on pasture altered the fatty acid profile, enhancing $\omega 3$ fatty acid content. Pla *et al.* (2007) also found that meat of hind-leg of organic source rabbits was poorer in monounsaturated and richer in polyunsaturated fatty acids than meat from conventional rabbits. Other strategies for specifically increasing long chain fatty acids such as EPA and DHA are based on the dietary use of fish oils or algae (Bryhni *et al.*, 2002 in pigs; Bou *et al.*, 2004 in chicken; Nute *et al.*, 2007 in lamb). A high increase of long chain polyunsaturated fatty acids can be achieved feeding rabbits with diets enriched with fish oils. However, high levels of lipid oxidation (Nuchi *et al.*, 2007), lower growth and impair carcass and meat quality, depending of the fish oil used (Navarrete *et al.*, 2007).

Lipid oxidation is a major non-microbial factor responsible for the quality deterioration of muscle foods. It leads to discoloration, higher drip-loss, and the development of off-odours and off-flavours (Monahan, 2000). The dietary manipulation of tissue lipid composition to produce meat with a high content of PUFA could reduce the oxidative stability of meat-products and have negative effects on meat quality. The level of oxidation after refrigerated storage of rabbit leg meat has been studied by Hernández *et al.* (2008). These authors found that oxidation products evaluated by measuring peroxide value (PV) and TBARS (TBA-reactive substances) were not very high in rabbit meat, although both oxidation parameters increase with storage time. However, when rabbits were fed with a diet enriched with 3% linseed oil, rabbit meat showed higher TBARS values after 5 *post-mortem* days of refrigerated storage (Hernández *et al.*, 2007), although values were well below for apparent rancidity (Campo *et al.*, 2006). Nevertheless, diets were supplemented with 100 ppm of α -tocopherol. Dietary inclusion of 8% linseed produced higher susceptibility to lipid oxidation (TBARS) of both fresh and frozen (-20 °C for 3 or 6 months) meat batters for hamburger production (Bianchi *et al.*, 2006).

Conjugated linolenic fatty acid

Conjugated linolenic fatty acid (CLA) consists of a group of geometric and positional isomers of linoleic acid. It has potential nutritional benefits for humans, because it has anti-obesity (Lin *et al.*, 1995) and anti-carcinogenic activities (Ip *et al.*, 1996); it is also able to ameliorate diabetes (Housseknecht *et al.*, 1998), and it has a protective effect against atherosclerosis in rabbits (Lee *et al.*, 1994). Naturally occurring CLA

originates mainly from bacterial isomerisation or/and biohydrogenation of PUFA in the rumen and the mammary gland (Griinari and Bauman, 1999). Food sources originated from ruminants are known to have markedly higher CLA concentration than those from monogastric animals (Schmid *et al.*, 2006). Rabbits are able to recycle part of the end microbial fermentation products through cecotrophy, so that the CLA retained in their meat might be higher than in other non-ruminant species (Gómez-Conde *et al.*, 2006).

Conjugated linoleic acid has also received a great deal of attention as a supplement in rabbit feed. Dietary CLA supplementation is an effective tool for increasing, in a dose dependent manner, the amount of CLA in intramuscular lipids of rabbits with *cis*-9,*trans*-11 being the predominant isomer (Lo Fiego *et al.*, 2005; Petacchi *et al.*, 2005). In addition to the beneficial effect of CLA in human health, CLA can favorably modify body composition (Corino *et al.*, 2002; 2003) due to its potential to increase lean tissue deposition in various species (Dunshea *et al.*, 2005). Rabbit growth performance and carcass characteristics at standard slaughter weight (2.5 kg, 76 d) were not affected by diets supplemented with 0.25 or 0.5% CLA. However, CLA supplementation reduced perirenal fat weight at heavy slaughter weight (3.1 kg), and lowered concentration of serum triglycerides and total cholesterol (Corino *et al.*, 2002). Regarding the chemical composition of rabbit meat, a significant decrease in meat lipid content was evident only when rabbits fed diet with a high supplementation level of CLA (0.5%) were considered at heavy slaughter weight (3.1 kg) (Corino *et al.*, 2003).

Antioxidants

There has been an increasing interest in the use of antioxidants in rabbit feed formulas because the dietary manipulation of tissue lipid composition to produce meat with a high PUFA content could decrease meat oxidative stability.

Hernández and Gondret (2006) reviewed the use of vitamin E in the diet. Vitamin E is commonly used in animal feeds as an indispensable component of biological membranes with stabilizing properties and a high antioxidant activity. Vitamin E is the generic term used to describe at least eight naturally occurring compounds that exhibit the biological activity of α -tocopherol (Morrissey *et al.*, 2000). In recent years, different studies have examined the effects of dietary extra supplementation with vitamin E on the deposition of α -tocopherol in tissues, on meat quality characteristics, and on oxidative stability and the shelf life of rabbit meat. Several authors (López-Bote *et al.*, 1997; Castellini *et al.*, 1999) have shown that the deposition of α -tocopherol in rabbit muscle is very efficient and has a strong relationship with the supplementation level used in the diet. Dietary α -tocopheryl acetate supplementation has been found to stabilize color of raw meat (Corino *et al.*, 1999), even after refrigerated storage (Dalle Zotte *et al.*, 2000). Vitamin E has also been effective in reducing lipid oxidation during refrigerated and frozen storage of meat (Castellini *et al.*, 1999; Lo Fiego *et al.*, 2004). In addition, Vitamin E supplementation increases the oxidative stability of cooked rabbit meat (Castellini *et al.*, 1999), whatever the different cooking methods studied (Dal Bosco *et al.*, 2001). Also, a high α -tocopherol level improves some physical traits of meat, reducing shear values and increasing water holding capacity

(Castellini *et al.*, 1998). The effect of dietary synergetic supplementation of Vitamins C and E have been also investigated, leading to an increase in the vitamin content and reducing the oxidation of the lipids (Castellini *et al.*, 2000; Lo Fiego *et al.*, 2004). Different natural ways to improve the oxidative stability of rabbit meat have also been studied. For example, rabbit lipid oxidative stability was improved by increasing the level of oats in rabbit diet (López-Bote *et al.*, 1998). Coni *et al.* (2000) also verified the antioxidant efficiency of extra virgin olive oil and oleuropein, an olive oil biophenol, in rabbit plasma and isolated low density lipoproteins (LDL). However, it seems that oleuropein did not reduce meat susceptibility to oxidation (Paci *et al.*, 2001). Meat contains several natural antioxidants such as catalase, superoxide dismutase and glutathione peroxidase (GSH-Px). Studies on meat of several species (Pradhan *et al.*, 2000) indicate that endogenous antioxidant enzymes could potentially delay the onset of oxidative rancidity in refrigerated stored meat. Indeed, GSH-Px could have an important role controlling lipid oxidation due to its high activity in rabbit meat when compared to other species (Hernández *et al.*, 2002). There are other endogenous antioxidants such as histidine-containing dipeptides, carnosine and anserine, but contents vary according to anatomical location and species (Decker, 2000).

SAFETY OF RABBIT MEAT

The safety of meat has been at the forefront of societal concerns in recent years. Numerous crises including Bovine Spongiform Encephalitis (BSE) in bovine, high dioxin levels in chicken, the danger of increased spread of other infectious disease (e.g. Foot and Mouth disease, Avian influenza, etc.), as well as pathogens, such as *Salmonella*, *Listeria monocytogens*, *Campylobacter* and *Escherichia coli* 0157:H7, have recently contaminated the European livestock and meat chains. Major meat safety issues and related challenges include microbial pathogens, food additives and chemical residues, and animal identification and traceability (Sofos, 2008).

Microbiological quality of rabbit meat

Safety and shelf life of meat are limited by microbial growth. Dominant contaminants on carcasses and packed rabbit meat are *Pseudomonas*, lactic acid bacteria, yeasts and *Brochothrix thermosphacta*, according to Rodríguez-Calleja *et al.* (2004) with total bacteria counts between 4.01-4.96 log cfu/g.

It is established that microbial levels of 6-7 log cfu/g are critical for the spoilage of meat. Rodríguez-Calleja *et al.* (2005a) studied the self life of rabbit carcasses, overwrapped with oxygen-permeable film and stored at 3°C, over 8 days. Shelf life according to both appearance and odor was estimated at 6.8 days reaching the aerobic plate counts values of 8 log cfu/g. In fact, after 5 days of storage, most of the carcasses already showed some softening and the counts of these bacteria were about 7 log cfu/g. Other authors estimated shelf life of rabbit carcasses in 3 days at 4°C (Bobbitt, 2002). These differences could be explained by differences in initial microbial counts, since a high initial contamination of meat reduces product shelf life (Gil *et al.*, 1998). In addition, it is possible to increase the shelf life of rabbit

meat using modified atmospheres (Berruga *et al.*, 2005) or irradiation (Badr, 2004; Rodríguez-Calleja *et al.*, 2005b).

Microbial ecology of rabbit meat could also be affected by different feeding programs; some components of the feed could play a specific role on the growth rate of some microbial groups. Vannini *et al.* (2003) showed that a dietary supplementation of whole linseeds limited the growth rate of several microbial groups (except psychrotrophic bacteria) with a consequent increase in meat shelf life. In addition, dehydrated alfalfa meal at high percentages in the diet seems to also have an inhibiting effect on microbial growth in rabbit meat products (Vannini *et al.*, 2002).

The slaughtering process may cause extensive contamination of muscle tissue with a vast range of micro-organisms. Some of these micro-organisms come from the animal intestinal tract and others from the environment in contact with the animals before or during slaughter. López *et al.* (2002) have studied the evolution of the most important contaminant and pathogen biota on carcasses during the slaughter process of rabbits (Figure 1). These authors found that there was an increase of micro-organism counts during evisceration process, especially enteric micro-organisms, so an improvement of this process is required in order to reduce the final contamination of carcasses. After carcass chilling, microbial counts were reduced to a great extent. Nevertheless, final counts of total aerobic micro-organisms and yeasts and moulds were still high. *Listeria monocytogenes*, *Salmonella spp.* and *Campylobacter spp.* were not found in all steps of the slaughter process. *Staphylococcus aureus* was present during evisceration. However, after chilling this micro-organism was not present.

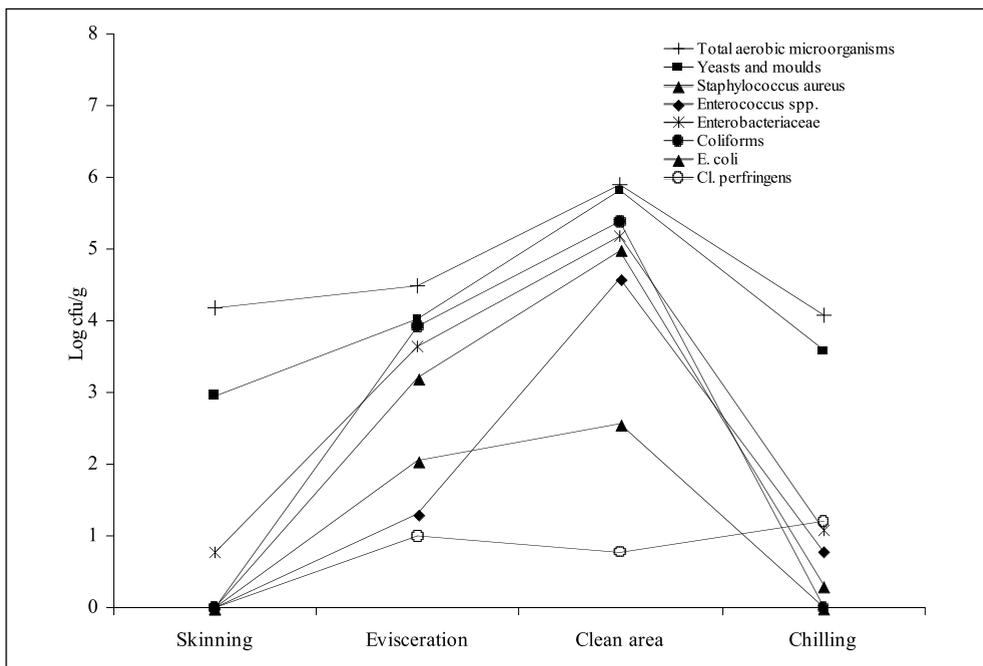


Figure 1: Evolution of microbial counts during rabbit slaughter process (from Hernández and Gondret, 2006).

Chemical contaminants

Foods of animal origin play an important role in determining the exposure of human beings to contaminants either of biological or chemical origin. Chemical contaminants are compounds that have a potential risk for consumers. They are present in relatively small amounts, usually due to human activities. In vivo, the main ways in which animals can accumulate harmful substances are poisons and the addition or voluntary administration of substances with pharmacological action (antibiotics, compounds with hormonal action and tranquilizers) or insecticides. An accidental contamination of food can be produced; water and air can be contaminated by heavy metals, halogenated hydrocarbons and other persistent pesticides, or by cleaners and disinfectants. Moreover, pollution or harmful substances can be formed during meat processing and storage.

POPs is the acronym for a group of persistent organic pollutants framed within the Stockholm Convention (Stockholm Convention on Persistent Organic Convention (POPs) 2004; available from <http://www.pops.int/>). These pollutants represent a relevant and growing concern for human beings due to their physical-chemical properties, bioaccumulative behaviour in lipid tissues, and possible toxicological effects (Brambilla *et al.*, 2008).

Recently, the presence of some of these POPs, such as dioxins, has been studied in rabbit (Ábalos *et al.*, 2007). In fact, a European Project, entitled “Feeding Fats Safety” (Food-CT-2004-07020) was developed in the 6th EC Framework Program. The aim of this project was to study different aspects of animal nutrition in order to preserve animal health and to produce safe and good quality meat products. Rabbits and chickens were the species used in this project. The ability of different fats to modify the lipid composition of meat, the effect of the level of fat oxidation and the toxicological or physiological effects of certain fat degradation compounds and undesirable contaminants (dioxins, polycyclic aromatic hydrocarbons) have been studied. Ábalos *et al.* (2007) studied the presence of PCDD/Fs (polychlorodibenzo-*p*-dioxins and dibenzofurans) and DL-PCBs (“dioxin-like” polychlorinated biphenyls) in rabbit and chicken meat samples from animals fed fish oil spiked feed at different levels of contaminants. Three different levels of contaminants under the maximum quantity allowed by the EU Directive were tested. The profile of PCDD/Fs in chicken samples from the three different treatments resembled the profile previously observed in the corresponding feeds, in general, the levels of the different compounds increased when increasing their amount in the feed. However, in rabbit meat samples, completely different bioaccumulation behavior was observed when compared to chicken samples. The profile of PCDD/Fs in rabbit meat did not correspond to that in present feeds. In fact, there were no significant differences in PCDD/F toxicity among rabbit samples from the three different treatments. For DL-PCBs, the profile was similar between feeds and meat samples, both in chickens and rabbits.

The level of polycyclic aromatic hydrocarbons (PAHs) in rabbit tissues and their rate of transfer from feed has also been studied by Devier and Budzinski (2007) in the frame of the European Project: “Feeding Fats Safety” (Food-CT-2004-07020). PAHs are a group of chemicals that are formed during the incomplete burning of coal, oil, gas, wood, garbage, or other organic substances, such as tobacco and charbroiled meat. There are

more than 100 different PAHs. Some of them are known or suspected carcinogens, and are linked to other health problems. In this study, rabbits were fed with diets containing three levels of PAHs (153, 81 and 32 ng PAHs/g ww). The most interesting results were that PAH metabolites were not transferred from feed to meat, liver or plasma, even with highly contaminated feeds, being excreted in urine and bile.

The presence of heavy metals (Cu, Pb, Cd and Hg) in rabbit meat was studied by Skřivanová *et al.* (2002b). These authors pointed out that negligible concentration of heavy metals was found in rabbit meat, concluding that rabbit meat produced in Czech Republic was safe from the point of view of the heavy metal environmental presence. Skřivanová *et al.* (2001) showed that feeding supplemental copper at 150 mg/kg (far over the amount allowed by the EU Directive) improved performance and decreased mortality of rabbits. However, the copper was accumulated in liver, although no contamination of meat was observed (Skřivanová *et al.*, 2002a).

Antibiotics are a special case within the subject of chemical contaminants. The use of antibiotics in animal production has led to a great reduction of the infectious diseases of the animals and a consequent reduction of the risk of transmission of infectious agents to consumers. Therefore, the use of antibiotics has led to an increase in the safety of the food chain. However, the presence of these residues in meat could be harmful to consumers because antibiotic residues in low concentrations encourage the development of microbial resistance, which could have serious consequences (Chander *et al.*, 2007). Antibiotics should be removed in time to ensure their elimination from the tissues. The legislation for pharmaceutical and veterinary products established a withdrawal period of antibiotics of 28 days for fattening rabbits, limiting the addition of antibiotics to the first days of fattening. The use of antimicrobials in rabbit production has been reviewed by Badiola *et al.* (2007).

Animal identification and traceability issues

Traceability is the more comprehensive concept of tracking the movement of identifiable products through the marketing chain. The main objective of traceability is to minimize any adverse health effects by a quick and complete recall. For an adequate recall, it is necessary that all food products and all of the ingredients used in producing the food are traceable at all stages of production, both processing and distribution. A review of rabbit meat traceability was provided by Cavani and Petracci (2006). Animal identification is an effective tool for meat traceability. Current livestock identification systems (ear-tags, tattoos, etc.) are not completely efficient and offer certain inconveniences. Transponders could be a way of monitoring live rabbits to improve traceability systems if its cost is reduced in the future (Crimella *et al.*, 2005). For example, the spread and then containment of BSE in various countries made the issue of animal identification and traceability a reality and was an important challenge for its adoption by various organizations and health authorities. Animal identification and meat traceability are not themselves an issue of food safety, animal disease prevention, quality assurance, or country-of-origin labelling. However, they may be important components of such programs. The need for traceability has also arisen by consumer concerns over food authenticity. It is well known that organic foods, protected designation of origin (PDO) and protected geographical indication (PGI) products and other labels are steadily

gaining in popularity. Other categories that consumers are demanding is traditional, regional, and handmade products, which are claimed to be safer because they are more natural. If one wants to keep consumers' confidence by use of such labels, the early detection of any kind of fraud must be made possible.

Hazard Analysis and Critical Control Points

The Hazard Analysis and Critical Control Points system (HACCP) is a preventive system used to safely produce food in the agricultural sector. This system is based on prevention rather than inspection. The HACCP principles are applicable to all stages of production, including the production of raw materials, preparation, handling, processing, and distribution and consumption system (Brown *et al.*, 2000).

HACCP is based on the following seven principles:

Principle 1: Conduct a hazard analysis.

Principle 2: Determine the critical control points (CCPs).

Principle 3: Establish critical limits for each critical control point.

Principle 4: Establish critical control point monitoring procedures.

Principle 5: Establish corrective actions.

Principle 6: Establish verification procedures.

Principle 7: Establish record-keeping and documentation procedures.

One of the main critical control points in the slaughter of rabbits is the evisceration since it can produce gastrointestinal tract ruptures, producing an important microbiological contamination of the carcass. There is some information about HACCP system for slaughterhouses in rabbits (Tantiñá *et al.* 2000). In table 2, the main stages in rabbit slaughter and the potential hazards associated are listed.

Table 2: Main stages and associated hazards in the slaughter of rabbits.

Stage	Hazards
<i>Ante mortem</i>	
Animal production (farms)	1. Infectious and parasitic diseases 2. Chemical residues 3. Mechanical injuries 4. Injuries when rabbits are introduced in cages
Loading, transportation, reception and unloading	5. Deficiencies in transport: trucks, cages, density, travel
<i>Ante mortem</i> inspection	6. Deficient inspection
Stunning and hanging	7. Incorrect immobilization and stunning 8. Incorrect hanging
Dirty area: slaughter, skinning, evisceration	
Bleeding	9. Incomplete severing of blood vessels 10. Incomplete bleeding
Feet and ear removal and skinning	11. Carcass contamination by deficient handling practices and deficient cleaning of utensils and automated cutting machines

Stage	Hazards
Evisceration	12. Fecal material release due to rupture of gastrointestinal tract (microbiological contamination)
Skinning of hind legs	13. Carcass contamination by deficient handling practices and deficient cleaning of utensils and automated cutting machines
Clean area	
Post-mortem inspection	14. Deficient inspection
Refrigeration	15. Insufficient refrigeration
Packaging	16. Carcass contamination by deficient handling practices

Adapted from Tantiñá *et al.* (2000).

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NIR PREDICTION FOR PROTEIN AND INTRAMUSCULAR FAT CONTENT OF RABBIT HIND LEG MEAT

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Global calibration equations were developed to predict the chemical composition of raw, homogenized rabbit meat by means of near infrared reflectance spectroscopy (NIRS). Forty-four Pannon White rabbits were housed in groups in three different pen types (16 animals/m²) and fed the same diet. Forty five animals were housed in cages (12 animals/m²) and divided by groups fed by different feeding regimes. Rabbits were slaughtered at the bodyweight of 2.4-2.5 kg. Homogenized fresh and freeze-dried left total hind leg muscles were investigated using Foss NIRSystem 6500 spectrometer with small ring cup sample holder. The ether extract and protein concentrations of all samples were determined by wet chemistry (dry matter based fat and protein concentrations were 10.7±2.39% and 84.6±2.38%, respectively). Calibration equations were developed for the two separate series of samples (n=44 or 45) and for the entire dataset (n=89). Calibration was performed for 1100-2500 nm wavelength interval, and for its two half intervals (1100-1900 nm and 1800-2500 nm). Leave-one-out cross validation was applied to test the calibrations. Best results were gained by using 89 freeze-dried samples and analysing the 1100-2500 nm wavelength range (R²=0.990 and 0.973, 1-VR=0.984 and 0.966 for fat and protein content, respectively).

EFFECT OF LIMITING ACCESS TO DRINKING WATER ON CARCASS CHARACTERISTICS, MEAT QUALITY AND KIDNEYS OF RABBITS

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Feed restriction is a common practice to reduce post weaning digestive disorders in rabbits and to get carcasses with less fat. Water restriction is commonly used to induce feed restriction in growing rabbits. Two water restriction times (2 and 4 hours per day, continuously) were used in our trial carried in good sanitary conditions.

Thirty six rabbits were divided at weaning (28 days) into three groups: R2 with restricted access to drinking water from 35 to 80 days of age of 2 hours per day (from 8 to 10 am), R4 access to drinking water 4 hours per day (from 8 to 12 am) and T (control) with unlimited access to drinking water.

Six animals from each group were slaughtered at 80 days of age. Carcass measurements and meat quality were studied. Histological study for kidneys was carried out.

Relative weight of gastrointestinal tract increased with the degree of water restriction: 16.5, 18.4 and 18.9 for groups T, R4 and R2 respectively ($P < 0.05$). Perirenal fat was lower in restricted groups, but the differences were not statistically significant. Scapular fat decreased of 2/3 or 1/3 respectively with limiting access to drinking water of 2 or 4 hours per day.

Compared to control group, meat of restricted groups contained more water: 70.3, 70.0 and 69.3% respectively for R4, R2 and T ($P < 0.01$). The same tendency was observed for protein content: 19.2, 19.3 and 17.7% for groups R2, R4 and T, respectively. Fat content decreased in meat of restricted animals: 8.4, 8.2 and 8.1%, respectively for T, R4 and R2 ($P < 0.05$). Water restriction induced decrease of carcass and meat adiposity. There were no differences between pH post mortem and pHu among the three groups. Water restriction did not affect kidney histology.

INFLUENCE OF PRESLAUGHTER FASTING ON LIVE WEIGHT LOSS, CARCASS YIELD AND MEAT QUALITY IN RABBITS

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The study was carried out on forty-eight growing rabbits (77 days-old; 2.5 kg live weight), reared and slaughtered under commercial conditions. At the farm, the rabbits were divided into three groups (n=16 per group) and feed was removed at 0, 6 or 12 h before crating. Subsequently the rabbits were transported for 1.5 h and laired at abattoir for 1.5 h prior to slaughter so that the following three groups were obtained: short fasting (SF; total fasting: 3 h), medium fasting (MF; total fasting: 9h); long fasting (LF; total fasting: 15 h).

Rabbits fasted for short time had higher live weight both at crating and at slaughter ($P<0.05$) in respect with MF and LF. This result was determined by the lower live weight loss observed between feed removal and crating (0.00 vs. 2.38 and 2.82%; $P<0.01$) which produced a lower total live weight loss (from feed removal to slaughter) (2.25 vs. 4.55 and 5.15%; $P<0.01$). The incidence of full gastrointestinal tract exhibited a decrease ($P<0.01$) going from SF (21.2%) to MF (19.8%) and LF (18.6%), determining a lower ($P<0.05$) carcass yield in SF compared to MF and LF. Moreover, in comparison with MF and SF, the long fasting produced a lower percentage (4.45 vs. 4.83 and 4.95%; $P<0.01$) and a higher pH (6.00 vs. 5.94 and 5.96; $P<0.01$) of the liver, indicating a higher depletion of energetic substrates.

Concerning the meat quality traits, the duration of fasting did not significantly modify the rate of muscle acidification and depletion of energy stores as evidenced by both pH and R-value at 45 min *post mortem*. However, the rabbits fasted for short time exhibited a lower pH at 24 h *post mortem* (5.58 vs. 5.69 and 5.70; $P<0.01$), a lighter colour (L^* , 55.0 vs. 52.2 and 52.9; $P<0.01$) and a superior cooking loss (29.6 vs. 28.1 and 27.5%; $P<0.01$) in respect to those submitted to medium and long fasting. The LF-NMR analysis of the T_2 distribution revealed that the differences in relative amounts of water populations were not significant.

Overall, the results obtained in this study confirm that preslaughter fasting allows to pursue the emptying of the gut with the aim to reduce carcass faecal contamination during slaughtering. Moreover, it was established that a long preslaughter fasting can lead to an increase in muscle ultimate pH, higher water holding capacity and darker colour of the meat. However, the differences observed in meat quality traits are not so large to determine a deterioration of product quality so that it can be concluded that the application of a correct fasting protocol by the rabbit production chain can allow to pursue the emptying of the gut, improve carcass yield and maintain satisfactory meat quality.

USE OF LINSEED OIL AND ANTIOXIDANT (VITAMIN E) IN RABBIT DIETS TO IMPROVE DIETETIC TRAITS OF RABBIT MEAT

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The aim of the study was to determine the effect of supplementing 3.4% additive linseed oil and antioxidant (vitamin E) on rabbit meat quality. Thirty New Zealand White female rabbits were investigated. Animals were assigned to three feeding groups: group I – fed a pelleted basal diet with standard components; group II – fed a pelleted diet with 3% plus linseed oil; group III – fed a pelleted diet with 3% linseed oil and 100% greater vitamin E content (increased from 50 to 100 mg/kg). The linseed oil contained alpha-linolenic acid C18:3n-3.

The experiment was terminated by the slaughter of animals at 3 months of age (6 animals per group). Hind leg muscles were analysed for fatty acids, total cholesterol, vitamin E and malondialdehyde (TBARS) after 14 and 90 days of storage. Sensory analysis included the evaluation of aroma intensity, aroma quality, tenderness, juiciness, taste intensity and taste quality on a scale of 1 to 5 points. The overall sensory quality was also calculated.

It is concluded that the addition of linseed oil to rabbit diets had a favourable effect on the composition of the lipid fraction of rabbit meat, causing a significant decrease in total saturated fatty acids (SFA) and an increase in polyunsaturated fatty acids (n-3 PUFA). Vitamin E supplementation prevented lipid oxidation processes, which shows the effectiveness of the antioxidant used. A significant ($P \leq 0.01$) decrease in meat cholesterol content was found in the experimental groups.

FEED RESTRICTION DURING SUMMER: EFFECT ON RABBIT CARCASS TRAITS AND MEAT QUALITY

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The aim of the research was to evaluate the effect of feed restriction during the whole productive cycle on the carcass and meat traits of hybrid Hyla rabbits raised during the summer season. The trial was carried out on two groups, each comprising 684 weaned hybrid Hyla rabbits fed the same commercial concentrates, supplied respectively *ad libitum* (AL group) and restricted to 80% and 90% of *ad libitum* (RES group), respectively from weaning to 60 days of age and from 61 days of age to slaughter (81 days). After slaughter, carcasses were prepared according to the norms of the World Rabbit Science Association (WRSA). Moreover, pH of *Biceps femoris*, measured 1 and 24 hours post mortem, meat quality and water holding capacity were determined. The main, significant differences in carcass traits consisted in the percentage of empty gastro-intestinal tract on the body weight (7.10 vs. 5.65%, respectively for RES and AL group, $P < 0.01$), dressing out percentage (67.64 vs. 70.21%, respectively for RES and AL group, $P < 0.05$) and carcass length (37.09 vs. 38.73 cm, respectively for RES and AL group, $P < 0.05$). The great incidence of empty gastro-intestinal tract, that affects also dressing out percentage, can be due to the higher relative intake of RES group with a consequent higher relative development of the gastro-intestinal tract.

MICROBIOLOGICAL QUALITY AND SAFETY OF RABBIT MEAT IN VENETO REGION - ITALY

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In 2006 a systematic study was carried out in the four main rabbit slaughterhouses of Veneto Region to evaluate meat health and the hygienic characteristics on 433 carcasses, 239 sectioned and jointed meats and 137 surface samples taken before the beginning of work from facilities and equipment surfaces. Hygienic indicator (total bacteria count, enterobacteria and *E.coli*) counts resulted different among slaughterhouses and correlated with different levels of application and respect for good manufacturing practices. From the results, rabbit slaughterhouses can hardly be conformed to the hygiene criteria established for other mammalian species by the European Commission Regulation (EC) N° 2073/2005: only 71.6% of the carcasses produced by slaughterhouse A, 49.5% of those from slaughterhouse B, 38.1% of those from slaughterhouse C and 55% of carcasses from slaughterhouse D had a total bacteria count lower than 10⁵ cfu/g. Only 71.1% of carcasses from slaughterhouse A, 76.3% of those from slaughterhouse B, 57.1% of those from slaughterhouse C and 53.3% of carcasses from slaughterhouse D had enterobacteria counts lower than 10³ cfu/g; instead, *E. coli* was usually present at low levels both in carcasses and meats. The 0.6% of carcasses from slaughterhouse A, 0.5% of carcasses and 7% of meats from slaughterhouse B, no sample from slaughterhouse C, 6.6% of carcasses and 11.9% of meats from slaughterhouse D resulted positive for *Salmonella spp.* The 0.5% of carcasses from slaughterhouse B, 28.3% of carcasses and 33.3% of meats from slaughterhouse D resulted positive for thermo-tolerant *Campylobacter*. The 15.7% of carcasses and 31.9% of jointed meats from slaughterhouse A, 0.5% of carcasses and 33.9% of meats y slaughterhouse B, 9.5% of carcasses and 10% of jointed meats from slaughterhouse C, 21.7% of carcasses and 33.3% of jointed meats from slaughterhouse D resulted positive for *Listeria monocytogenes*. The 0.6% of surface samples from slaughterhouse D were positive for *Salmonella spp.*, 15.8% of surface samples from slaughterhouse A and 1.2% of those from slaughterhouse D were positive for *Listeria monocytogenes*. The high prevalence of *Salmonella* and *Campylobacter* found in samples from slaughterhouse D probably comes from cross-contamination due to the fact that also poultry are slaughtered in the same building. The high prevalence of *Listeria monocytogenes* is likely linked to the general wear and tear to work surfaces and equipment and the uneffectiveness of hygienic practices.

EFFECT OF FEED RATIONING AND PARITY ORDER OF RABBIT DOES ON GROWTH PERFORMANCE AND MEAT QUALITY OF THEIR OFFSPRING

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Thirty hybrid female rabbits of 15 weeks of age were randomly divided into three groups and fed with one of the three following diets: "C diet" for young females (DE=11.71 MJ/kg DM) fed *ad libitum*; "R diet" was the C diet fed at 80% of *ad libitum*, "F diet" rich in fibre (24.6% vs. 18.7% of C diet; DE=9.77 MJ/kg DM) fed *ad libitum*. The rabbits were first time inseminated at 19th weeks of age. The three diets were administered until the first parturition, afterwards all the does received the C diet *ad libitum*. The offspring received a commercial pelleted diet balanced for growing rabbits. The trial examined the offspring's performance and meat quality of two parity orders. Two newborn rabbits per litter were chosen at the first and second parturition of does and their growth performance, carcass yield and meat chemical composition were determined. For each doe, one rabbit was slaughtered at 36 days of age (weaning), the other one at 81 days of age. After weaning the remaining rabbits were caged by pairs in standard commercial cages. Maternal feed rationing did not affect offspring's performance and carcass and meat quality, with exception of a reduction in daily growth during the first 36 days of life on rabbits belonging to does rationed with diet F if compared to diet C (18.4 vs. 21.5 g/d; P<0.10) that elicited lower live weight at weaning (708 vs. 813 g; P<0.10). The feed intake of weanling rabbits of the second parity was significantly lower than that of first parity (134 vs. 150 g/d; P<0.001) but the live weight at 81 days was not significantly different between the two groups. Rabbits of the second parity showed higher slaughter yield at 36 and 81 days of age (P<0.05) and furnished leaner carcasses on the slaughter at 81 days (P<0.05). Also hindleg meat tended to be leaner, but only for rabbits slaughtered at 36 days of age (P<0.10). Parity order x maternal diet interaction was found on the protein content of hindleg meat of offspring at 81 days of age (P<0.001): while the protein content of rabbits that derived from mothers fed C and F diets decreased from the first to the second parity, that of rabbits of R-fed mothers showed an opposite trend.

EFFECT OF MOTHER'S FEEDING, PHYSIOLOGICAL STATE, PARITY ORDER AND OFFSPRING'S AGE ON THEIR *POST-MORTEM* PH EVOLUTION OF *LONGISSIMUS DORSI* MUSCLE

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The muscle pH evolution and its final value (pHu) have important influence on the keeping qualities of meat, as affecting proteins structures and water holding capacity. Thirty hybrid female rabbits of 15 weeks of age were randomly divided into three groups and fed with one of the three following diets: "C diet" for young females (DE=11.71 MJ/kg DM) was fed *ad libitum*; "R diet" was fed at 80% of *ad libitum* (quantitative rationing), "F diet" was rich in fibre (24.6% vs. 18.7% of C diet; DE=9.77 MJ/kg DM) and fed *ad libitum* (qualitative rationing). The does were inseminated at the 19th week of age. The three diets were administered until the first parturition, afterwards all the does received the C diet *ad libitum*. The offspring received a commercial pelleted diet balanced for growing rabbits. Two newborn rabbits per litter were chosen at the first and second parturition of does. They were then slaughtered at 36 d of age (weaning) and at 81 d of age, respectively. The trial considered the offspring's *post mortem* pH evolution of muscle *Longissimus dorsi* (LD). To analyze the muscle pH evolution according to the time, pH was measured at 1, 2, 3, 4, 5, 6, 24 hours *post mortem*. Each measure was performed in duplicate to obtain reliable data. These analyses evaluated the evolution of m. LD *post mortem* pH according to the offspring's age (36 and 81 days of age), maternal diet (C, R, F), physiological state (pregnant, non-pregnant), and parity order (first, second). Significant differences (P<0.01) were found in muscle pH according to the age, having younger rabbits higher m. LD pHu reached with lower decline. Quantitative maternal feed rationing originates higher m. LD pHu of offspring of 81 days of age (P<0.01). The physiological state of does had no any effect on the *post mortem* pH of offspring's m. LD. Offspring of the first kindling exhibited significantly higher muscle pH from 6 h *post mortem* onward (P<0.01).

UNUSUAL SPOILAGE IN RABBIT CARCASSES CAUSED BY *JANTHINOBACTERIUM LIVIDUM*

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A rare case of meat spoilage was identified in rabbit slaughterhouses in the regions of Veneto and Emilia Romagna (Italy). The first cases were detected in May and June 2005, but the problem was only brought to our attention in July 2005. As noted by the meat inspector, 2-3 days after slaughtering, the surfaces of some of the carcasses began to show pin-point violet colonies. Microbiological analyses established the origin of this spoilage, which was not due to fungi or *Pseudomonas* spp. A psychrophilic strain of *Janthinobacterium lividum* was isolated from all the carcasses as absolutely predominant microflora. This is probably the first report of this rare spoilage identified in meat in Italy.

THE EFFECT OF DECREASING THE OMEGA 6/OMEGA 3 RATIO IN FEED ON FATTY ACID CONTENT OF RABBIT MEAT TO MEET HUMAN DIETARY RECOMMENDATIONS

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Consumers are concerned about their health and the nutritional value of their diet. In response, the meat industry is concentrating its efforts on improving the nutritional value of meat. Moreover, a publication on recommended daily intakes underlines the importance of a high level of omega 3 fatty acids in our diet and advises a ratio of omega 6/omega 3 fatty acids of 5. The human diet contains varying levels of omega 3 and 6 fatty acids and one way to enhance the beneficial ratio is to include these fatty acids in animal feeds.

The aim of this study was to evaluate the impact of decreasing the omega 6/omega 3 fatty acid ratio in feed on the final fatty-acid content of rabbit meat and to carry out an initial investigation as to when the omega fatty acid feeds should be fed in the rearing period. Four different diets were formulated with decreasing omega 6/omega 3 fatty acid ratios from 12.4 to 1.6. Four batches of 60 rabbits were fed from 35 to 71 days of age with either omega 3⁻, standard, omega 3⁺, or omega 3⁺⁺ diets. A fifth batch of 60 animals were fed with the standard diet from 35 to 50 days of age, and then with the omega 3⁺⁺ from day 51 to 71. Lipids, fatty acid content and dry weight were analysed in a homogenous ground sample of all the meat from each rabbit.

A close relationship between omega 3 content in meat and feed was identified. Moreover, omega⁻ and standard diets did not result in the recommended ratio in meat. On the other hand, the other three batches showed a ratio below 5, with 4.8, 2.5 and 1.9 for omega⁺, the finishing omega⁺⁺ and continuous omega⁺⁺ diets respectively. Thus, the meat from these animals can carry the official label: “source of omega 3”. With the omega⁺⁺ (finishing or continuous) diet, rabbit meat can be labelled as being “rich in omega 3” since 30% of the dietary requirement is met. Satisfactory results were obtained when the omega rich diet was fed only in the finishing period and this opens the way to further work to identify precisely the optimum conditions (feed quantity and/or feeding period) to obtain the required dietary results at the lowest cost.

EFFECT OF THE DIETARY N-3 AND N-6 FATTY ACIDS ON TEXTURE PROPERTIES AND SENSORY CHARACTERISTICS OF RABBIT MEAT

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The effect of enriching rabbit meat with long-chain n-3 and n-6 PUFA through dietary fat on texture properties and sensory characteristics was studied. Rabbits from the same genetic type were fed *ad libitum* with three different diets. The experimental diets included a 3% of added fat: animal fat (A), sunflower oil (SF) or linseed oil (L). The three diets were supplemented with 100 ppm of α -tocopherol. 144 rabbits were used in this experiment, 48 rabbits of each group from both sexes. Animals were slaughtered at 63 days of age. *Longissimus* muscles from the right and left side of the carcasses were used for sensory and texture analysis, respectively. Textural properties were measured by Warner-Bratzler (WB) shear device and by the texture profile analyses (TPA) test. A sensory analysis was performed by six trained tasters of rabbit meat. The parameters evaluated were: juiciness (J), hardness (H), intensity of rabbit odor (IRO), aniseed flavor (AF), liver flavor (LF) and metallic/acid flavor. Changes in instrumental texture and sensory characteristics during 3 and 7 days of aging have been evaluated. A Bayesian analysis was performed.

There was a storage time effect for shear force and area, having the meat after 7 days of aging lower values than after 3 days of aging. In the texture profile analysis, rabbit muscle after 3 days of aging had 2% higher values for cohesiveness, 4% higher values for springiness and 8% higher values for chewiness than muscle after 7 days of aging. In the sensory analysis, rabbit loins after 3 days of aging were 12% harder than after 7 days. Nevertheless, there was a decrease in the intensity of rabbit odor with aging time. No storage time effect was found in juiciness and aniseed, liver and metallic flavor. Regarding the diet effect, the largest differences appeared between diet A and the others. Diet SF and diet L showed a 9% and 7% higher shear force than diet A. The total work performed to cut the sample (area) was an 11% higher in SF group than in A group. L group showed an 8% higher area than A group. No diet effect was found between SF and L diets. The results from the TPA showed no diet effect for most of the traits. Some of the texture variables measured indicates that the meat from group A could be more tender; however, no diet effect was found in hardness evaluated by sensory analysis. No evidence of diet effect was found for all the sensory characteristics measured.

Our results show that enriching rabbit meat with long-chain n-3 and n-6 PUFA through dietary fat has a small effect on instrumental texture properties but seems to have no effect on sensory characteristics. Moreover, instrumental texture and sensory analysis confirms that ageing improved the tenderness of rabbit meat.

COMPARISON OF THE SLAUGHTER CHARACTERISTICS OF GROWING RABBITS REARED ON WIRE NET OR COMBINED (WIRE NET/STRAW) FLOOR

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One hundred twenty-four rabbits were placed into 12 pens (50×170 cm) differing in the type of floor: wire net (6 pens) or a combined (half of the floor wire net and half of the floor straw litter) floor (6 pens). Within floor types, three stocking densities (8, 12 and 16 rabbits/m²) were applied. Thirty-six rabbits were placed into conventional cages (2 rabbits/cage) as control. The experiment took place between the ages of 5 and 11 weeks. At 11 weeks of age animals were slaughtered and carcasses dissected. Rabbits on the wire net floor had significantly higher liveweight at slaughter than rabbits on the combined floor (2575 vs. 2498 g). The dressing percentage (ratio of warm carcass to the live weight) was higher in animals reared on the combined floor (60.5% vs. 61.1%). The type of floor had a significant effect also on the ratio of the hind part to the chilled carcass (30.6% vs. 31.3%; $P < 0.01$). The ratio of *m. Longissimus dorsi* to the chilled carcass differed also significantly between the two pen-raised groups (9.17% vs. 9.48%; $P < 0.05$). The heart and the lungs ratio was very similar, while the ratio of the liver and kidneys was higher in rabbits on the wire net floor (6.25% vs. 5.94% and 1.38% vs. 1.26% to the chilled carcass, respectively). The perirenal fat was significantly higher on the wire net than on the combined floor (1.64% vs. 1.34%; $P < 0.01$). Rabbits reared at the highest stocking density (16 rabbits/m²) had the highest ratio of the fore part (25.3%), and these animals accumulated the highest amount of fat (1.68%) in their abdominal region. Comparing the experimental groups to the control it was found that rabbits in the conventional cages reached the highest live weight at 11 weeks of age (2690 g). Rabbits in pens on wire net reached 93-124 g, rabbits in pens on combined floor 174-239 g less slaughter weight than the controls. Similar tendency was observed also in the ratio of perirenal fat to the chilled carcass. Presumably due to the higher moving activity, rabbits in pens on the combined floor had higher ratio of hind legs to the chilled carcass (29.0-29.5%) than rabbits in pens on wire net floor (28.3-28.8%) or rabbits in the conventional cages (28.2%). In conclusion the advantage of the combined floor (wire net/straw) could be that rabbits on this floor have higher hind part in the carcass and lower amount of fat in the perirenal region, which could be favourable for the slaughterhouses and consumers.

EFFECT OF DIETARY SUPPLEMENTATION WITH RAPESEED AND FISH OIL MIXTURE AND ANTIOXIDANT ON RABBIT MEAT QUALITY

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The aim of the study was to determine the effect of a rapeseed oil (2%) and fish oil (1%) mixture and antioxidant on rabbit meat quality. The natural antioxidant vitamin E was added to the diet to protect fat from oxidation.

Thirty New Zealand White female rabbits were investigated. Animals were assigned to three feeding groups: group I – fed a pelleted basal diet with standard components; group II – fed a pelleted diet with 2% rapeseed oil and 1% fish oil; group III – fed a pelleted diet with 2% rapeseed oil, 1% fish oil, and 100% greater vitamin E content (increased from 50 to 100 mg/kg). Adding a mixture of rapeseed oil (2%) and fish oil (1%) to rabbit feed had a favourable effect on the composition of the lipid fraction of meat. There was a decrease in total saturated fatty acids and a significant increase in n-3 polyunsaturated fatty acids (especially EPA and DHA). The use of the natural antioxidant vitamin E in the diet of group III caused a significant increase in EPA and total n-3 PUFA as well as having a favorable effect on reducing the susceptibility of muscle lipids to oxidation during the frozen storage of meat. The addition of a rapeseed and fish oil mixture to rabbit diets had no effect on the sensory evaluation of the analyzed meat.

COMMERCIAL AND MICROBIOLOGICAL QUALITIES OF FROZEN RABBIT CARCASSES IN BENIN

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A survey of the commercial and microbiological qualities of rabbit frozen carcasses was undertaken in Benin in order to contribute to the improvement of the conditions of slaughtering and distribution of the rabbits. The survey showed that the transformation and the marketing of the rabbit meat do not generally respect the norms. From the survey of the commercial quality, it came out that the carcasses are presented traditionally and escorted to the stockists in jute bags.

The microbiological analyses done on 30 samples of carcasses revealed that: 100% are contaminated by the Total Mesophile Aerobic Flora (TMAF); 93.33% are contaminated by the Thermotolerant Coliformes; 26% are contaminated by the Presumed Pathogenic Staphylococci (PPS); 3.33% are contaminated by the Anaerobic Sulfito-Reducing (ASR); no sample revealed the presence of *Salmonella*.

In relation to the norms, 83.3% of the samples analyzed were conform whereas 16.7% were not conform. The quasi-totality of the samples analyzed were contaminated by the TMAF and the Thermotolerant Coliformes. Thus the conditions of hygiene were deficient during the technology of slaughtering. The construction of modern slaughtering areas, the refrigerators' purchase for the transportation of the carcasses, and the maintenance of a cold weather chain in the stations of sale would permit to reduce the bacteriological contamination and to improve the commercial quality of rabbit carcasses in Benin.

FATTY ACID COMPOSITION OF RABBIT MEAT WHEN FED A LINSEED BASED DIET DURING DIFFERENT PERIODS AFTER WEANING

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The aim of the study was to investigate the dietary use of extruded linseed on the fatty acid (FA) profile of rabbit meat when fed during different weeks of the fattening period. Two diets with comparable energy and protein content but largely different in ω -6/ ω -3 ratio were fed to weaned rabbits (29 d old). The ω -3 enriched diet (ω -6/ ω -3=1.03) was obtained by incorporation (12.8%) of a concentrate, based on extruded linseed (L) at the expense of raw materials rich in ω -6 PUFA, in a control (C) fattening diet (ω -6/ ω -3=4.22). Both diets were fed continuously during 6 weeks (L and C groups) or rabbits shuttled after 4 weeks from one diet to the other (LC and CL groups). At the age of 71 days, out of each treatment group, 12 rabbits with a weight near to the average group weight were slaughtered and the FA profile in the hind leg was determined. Differences in slaughter yield, pH₂₄ or colour characteristics were not significant. However, significant differences in FA profile according to the diet as well as the duration of feeding were observed. The α -linolenic acid content amounted to 6.33, 20.91, 12.39, and 15.51%, for C, L, CL and LC treatments, respectively. Thus, even a short distribution (2 weeks) of an ω -3 rich diet allows to produce rabbit meat with a twice as high content of ω -3 FAs. The ω -6/ ω -3 ratio dropped from 5.39 to 1.26, 2.61 and 1.84% in C, L, CL and LC hind legs, respectively. The same pattern was found for DPA and DHA, while EPA content was not significantly different between treatments. These results indicate that the decrease of ω -3 FAs is slower compared to the incorporation and that rabbits are able to elongate FAs.

EFFECT OF DIETARY TYPE AND LEVEL OF FIBRE ON CARCASS YIELD AND ITS MICROBIOLOGICAL CHARACTERISTICS

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The aim of this trial was to study the effect of level and type of fibre during the last week of the fattening period on carcass characteristics. A total of 6602 New Zealand × Californian rabbits, weaned at 35 days of age, were fed with a commercial diet (C0: 35% of NDF, 13.4% of starch and soluble fibre, beet pulp as fibre source) from weaning to 56 days. The last week of fattening period (56-63d) animals were fed with three different diets (C0, C1 and C2). Diet C1, was formulated to have a similar NDF content as diet C0, but the fibre source was replaced by a more insoluble fibre (straw) and diet C2 was formulated to reduce the NDF to 32.4%, but keeping the same fibre sources as diet C1, and increasing the starch content (17.3%). Seven multi-floor cage rolling stand (MFRS) with 290 and 280 animals, were assigned to C0 and C1 diets, respectively, and other six MFRS (288 animals) to diet C2. In four animals per MFRS were determined the carcass yield. In two of them (14 animals/treatment) a sample of caecal content was taken to determine the same day the *C. perfringens*, *Enterobacteriaceae*, *E. coli* and *Coliform* counts. Temperature and pH in *Biceps femoris* were measured after the cold time (2 hours after slaughter at -1°C) and 24 h *post mortem* and samples from the *Longissimus dorsi* and *Biceps femoris* were also taken to study the carcass bacteriological quality at days 1, 7 and 11 after slaughter (*Total Aerobic*, *Enterobacteriae*, *Coliform* and *Coagulase-positive Staphylococcus*). Animals fed with the lower dietary fibre content (C2) increased (P=0.05) its carcass yield. Carcass temperature was higher in the muscle of the animals fed diet C1 (P=0.001). The log cfu/g of *Enterobacteriae*, *Coliform* and *Coagulase-positive Staphylococcus* analysed in caecum content were higher for the animals fed treatment C1. The highest values of microorganisms content of the carcass were reached 11 days after slaughter (P<0.001). From these results, it might be concluded that a decrease of dietary fibre (from 35 to 32% NDF) when insoluble fibrous sources are included enhances the carcass yield and the carcass microbiological quality. For the same level of fibre, the inclusion of moderate amount of beet pulp (10%) improved the microbiological characteristics without impairing the carcass yield.

RESULTS OF AVILAMYCIN RESIDUES MONITORING PLANS FOR THE EXPERIMENTAL USE IN ITALY

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The digestive disease is the main cause of mortality in industrial fattening rabbit farms. Recently, avilamycin has been experimentally used by rabbit producers in Italy as a new option to control digestive syndrome. This experimental use was exceptionally authorized by the Italian Health Ministry in order to reduce the losses due to Epizootic Rabbit Enteropathy (ERE) in the rabbit breeding. Although the ERE pathogenesis is not yet completely known in all its aspects, the presence of *Clostridium perfringens* has been reported as associated agent in most of cases. The objective avilamycin experimental use was to evaluate the efficacy of the drug in feed for the control of digestive signs associated with *Clostridium* spp. in rabbits at a dose of 5 mg avilamycin/kg body weight/day, equivalent to 60÷120 g/1000 kg of feed on the basis of age, body weight and feed consumption for all animals in the weaning phase.

During the experimental use some different residues monitoring plans were performed by Italian health authorities and Elanco Animal Health with the aim to ensure public health on treating food-producing animals with an experimental therapy. According to the guidelines of the Italian Health Ministry an official avilamycin residues monitoring plan was conducted by Elanco during the last two years and other experimental plans were performed by regional authorities and foodstuff producers. An HPLC-MS/MS method was developed by Elanco, performed and validated by the “Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna” according to European legislation (EEC/657/2002) and applied to monitor avilamycin residues in the different plans actuated in Italy. The Avilamycin was analyzed by hydrolysis to dichloroisoverminic acid (DIA), the residual marker analyzed. The avilamycin linear range was from 50 to 250 µg/kg (approximately concentration of DIA from 10 to 50 µg/kg). Within laboratory data (reproducibility intra-laboratory) were from 11% to 17% for muscle and liver. Repeatability was included between 10% and 19% for both tissues. The mean recovery was 85% for muscle and 81% for liver. According to different monitoring plans, more than 250 samples of rabbit treated with avilamycin were collected and their tissues (muscles, livers or both tissues) were analyzed. The results obtained from these analyses demonstrate the very low risk due to residues and the high level of safety for avilamycin used in rabbit as food-producing animal.

EFFECT OF NUTRITIONAL STATUS OF KITS ON CARCASS TRAITS AND MEAT QUALITY (PRELIMINARY RESULTS)

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The aim of the experiment was to study the effect of nutritional supply during foetal, suckling and growing periods on carcass traits and meat quality of rabbits. New-born rabbits (n=224) were divided into three groups according to their birth weight (low/L/: 35-50 g, medium/M/: 58-62 g, high/H/: 70-88 g). Half of the litters were nursed by one doe (O), while the other half by two does (T). After weaning at 3 weeks, from the age of 4 weeks half of the rabbits were fed *ad libitum*, while the other half was reared on a restricted feeding regime corresponding to 85-90% of the *ad libitum* feed intake level. Rabbits were slaughtered at 11 weeks of age. Body weight at slaughter of L rabbits was 124 g lower than M and 255 g lower than H rabbits (P<0.05). L group had the highest (58.0%) while M rabbits the lowest dressing out percentage (57.3%) (P<0.05). Ratio of hind part was significantly (P<0.05) higher in H group (39.0%) than in L group (38.1%). Ratio of perirenal fat was the highest in L rabbits (2.10%) and the lowest in H group (1.64%) (P<0.05). Value of WHC was 1.8% lower (P<0.05) in H rabbits compared to L and M animals. Slaughter weight of T rabbits was 198 g higher (P<0.001) than that of group O. Dressing out percentage was 0.7% (P=0.002) lower in T rabbits. In group T the cooking loss of *m. Longissimus dorsi* was 1.6% lower (P=0.003) than in group O. In consequence of feed restriction the slaughter weight and dressing out percentage decreased by 141 g (P<0.001) and 0.8% (P=0.001), respectively. Feeding regime influenced all the meat quality parameters except the b* value of meat colour. Rabbits in restricted group had 0.08 (P<0.001) lower pH, while 1.1 (P<0.001) higher L* and 0.7 (P=0.008) lower a* values of *m. Longissimus dorsi*. The cooking loss of *m. Longissimus dorsi* of restricted rabbits was 1.2% higher (P=0.03). Birth weight x number of nursing does x feeding interaction had significant (P<0.05) influence on the lipid content of *m. Longissimus dorsi*: the highest lipid content was found in MOA rabbits (2.30%) while the lowest in HOA (1.18%) and in LOR (1.21%) groups. Birth weight x number of nursing does interaction was significant on the proportion of *m. Longissimus dorsi* to reference carcass weight (P=0.03) and on meat to bone ratio (P=0.02). Highest ratio of *m. Longissimus dorsi* was found in LO and MO groups (11.9%), while LT rabbits showed the lowest value (11.4%). Meat to bone ratio was the highest in HT rabbits; it differed significantly (P<0.05) from other groups except MT. These results show that the nutrient supply during foetal and suckling ages has a long term effect which could be important in terms of carcass traits and meat quality as well.

PASTURE AVAILABILITY AND GENOTYPE EFFECT IN RABBIT: 3. PERFORMANCE, CARCASS AND MEAT CHARACTERISTICS

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To verify the effect of pasture availability and genotype on performance and meat quality, 80 weaned rabbits (40 Leprino of Viterbo and 40 New Zealand White) were assigned to two homogeneous groups (sex, weight and genotype): Control (C), reared in standard bicellular cages, and Pasture (P), kept in a wired pen provided of an external grassed pasture area. To evaluate fatty acid composition and tocopherol content of feed and pasture two sampling were carried out and grass was taken from different areas of external paddock. Live weights, feed intake and feed:gain ratio were recorded. At 90 days of age 20 rabbits per group were sacrificed. Respect to feed, pasture showed higher percentage of saturated fatty acids (28.41 vs. 19.67%) and lower of monounsaturated fatty acids (8.00 vs. 19.55%). Regarding polyunsaturated fatty acids, great differences were observed in relation to linoleic and linolenic acids (13.89% vs. 47.25% and 47.72% vs. 11.93%, respectively). Productive performance, carcass traits and fatty acid composition of meat were strongly affected by genotype and housing system. In particular, the possibility of performing movement and ingesting grass caused lower daily gain (Leprino 29.6 vs. 31.9 g; NZW 40.0 vs. 44.2 g) and slaughter weight (Leprino 2418 vs. 2563 g; NZW 2650 vs. 2902 g). Chilled carcasses of pasture reared rabbits followed trend of live weight; bone (Leprino 14.0 vs. 11.6 kg/cm²; NZW 14.0 vs. 10.0 kg/cm²) and meat (Leprino 3.4 vs. 2.9 kg/cm²; NZW 3.4 vs. 2.5 kg/cm²) shear force were higher and meat fatty acid profile was richer in n-3 and total polyunsaturated fatty acids. Regarding genotype effect, Leprino showed lower daily gains, live and carcass weights. Pasture availability improved the meat nutritional quality and in particular the n-6/n-3 ratio was optimal and the total tocopherols content was suitable to assure a good oxidative stability.

A CRITICAL APPRAISAL OF RABBIT PRESLAUGHTER CONDITIONS IN A COMMERCIAL PRODUCTION CHAIN

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A study was conducted in order to assess the preslaughter conditions of rabbits in a commercial chain and to determine the effect of the season, journey (short: <220 min; medium: 220-320 min; long: >320 min) and lairage (short: <134 min; medium: 134-235 min; long: >235 min) on mortality, live weight loss, slaughter yield and carcass quality. A total of 831 flocks (average of 2,207 rabbits/flock) from 79 farms of 9-12 weeks-old growing rabbits shipped for slaughtering during the year 2006 towards a major Italian abattoir was considered. Transport and lairage at abattoir of the rabbits were mainly conducted during the night and early morning.

The overall average of mortality rate and live weight loss were found to be 0.082 and 3.39%, respectively. As for carcass evaluation, incidence of downgraded and condemned carcasses were 0.40 and 0.46%, respectively, while bruised carcass level was 2.22%. During the winter, it was observed lower ($P<0.01$) live weight loss (3.12%), while carcass yield (57.9%) was higher ($P<0.01$) during the summer. The shortest transport time (<220 min) exhibited lower mortality rate (0.053 vs. 0.080 vs. 0.113%; $P<0.01$) and live weight loss (2.43 vs. 3.47 vs. 4.26%; $P<0.01$) as well as higher slaughter yield (58.0 vs. 57.3 vs. 57.0%; $P<0.01$) in respect with medium and long transport times. Flocks laired for less than 150 min also exhibited a significantly lower mortality rate (0.065 vs. 0.075 vs. 0.105%; $P<0.01$) and higher carcass yield (57.8 vs. 57.4 vs. 57.1%; $P<0.01$) if compared to medium and long lairage times. Carcass bruising had the higher ($P<0.01$) incidence during the summer in respect with other seasons, while downgraded carcasses prevalence was higher ($P<0.05$) during the autumn. Longer journeys determined a higher ($P<0.01$) incidence of bruised carcasses, but did not influence downgraded and condemned carcass rates.

This study has shown that the preslaughter conditions of the rabbits in a commercial integrated chain production are rather satisfying as a consequence of the effective coordination of transport and lairage based on slaughtering daily programme. Main critical points are represented by long transport and lairage which can impair mortality rate, slaughtering yields and carcass quality. On the other hand, a minor role is played by the season probably because transport was mainly conducted during the night and early morning moderating the effect of high temperatures during the summer.

PREVALENCE OF PATHOLOGICAL LESIONS IN MEAT RABBITS AT SLAUGHTERING

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The slaughterhouse is considered an important control point for the monitoring of rabbit diseases. In our study, 59,440 rabbit carcasses were examined, but only 1% of pathological lesions were recorded at *post-mortem* inspection. Mainly tegumentary, digestive and urinary systems were affected. The most consistent lesion was the subcutaneous abscess; nephritis, probably caused by *Encephalitozoon cuniculi*, was also frequent. A pathological alteration of the liver, classified as “necrotizing hepatitis”, localized at the caudate lobe, was observed and here firstly described.

GROWTH AND CARCASS CHARACTERISTICS OF RABBITS HOUSED IN OPEN-AIR OR STANDARD SYSTEMS

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A study was carried out to investigate the effects of two housing systems on the performance, carcass traits and meat quality of growing rabbits. Ninety-six rabbits were used from 59 to 87 days of age. Forty eight rabbits were housed in 12 wired cages (0.5×0.6 m; 4 rabbits/cage) and 48 rabbits were housed in one open-air pasture pen (20×9 m). The growth performances were measured during the growing period. At 87 d, 12 rabbits per treatment were slaughtered and carcass characteristics determined according to the WRSA norms. Rabbits reared in cages showed higher ($P<0.05$) weight gain (45.9 vs. 34.2 g/d), pellet intake (163.4 vs. 110.2 g/d) and live weight (3062 vs. 2705 g) than those reared in open-air pens. At slaughter the open-air rabbits had higher ($P<0.05$) proportion of hind part (34.9 vs. 33.1%) but the dressing out percentage was similar (about 60%). The open-air rabbits had lower ($P<0.05$) percentage of dissectible fat in reference carcass (1.66 vs. 5.29%) and proportion of muscle in hind leg (79.3 vs 81.9%) and their femur had higher diameter. The housing system had no effect on dressing out percentage, viscera proportion and meat pHu.

EFFECT OF THE DIETARY N-3 AND N-6 FATTY ACIDS ON RABBIT CARCASS AND MEAT QUALITY

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Three groups of rabbits from the same genetic type were fed respectively with three experimental diets supplemented with 100 ppm of α -tocopherol and including a 3% of added fat: animal fat (A), sunflower oil (SF) or linseed oil (L). Fifty-two rabbits per group were used. Animals were slaughtered at 63 days of age. Live weight, dressing out percentage and carcass and meat quality traits were measured. A Bayesian analysis was performed. Animals fed with diet A showed higher live weight and higher carcass weight than animals fed with diets SF and L. SF and L animals had 96% and 95% of the LW that A group showed. The reference carcass in SF and L animals was 96% as heavy as in A animals. No differences were found between the SF and L animals for these traits; however, some differences appeared for dressing out percentage (DoP), having L animals higher DoP than SF (1% higher values). L animals also showed higher DoP than A animals, but the evidence of the differences was lower ($P>1=0.93$). Nevertheless, the effect of dietary fat on these traits was small even if its presence can be detected. Animals fed with a diet enriched with animal fat showed higher lumbar circumference (around +3%), liver weight and carcass and retail cuts weights than animals fed with diets SF and L. No differences between SF and L animals were found for the main carcass traits except for meat to bone ratio (M/B). SF and A had higher M/B than L animals (5 and 3.4%, respectively). No diet effect was found for carcass fatness among the three groups of animals. Meat color was little affected by the diet. The evidence of a diet effect in redness was low. Carcass L^* was higher in A than in SF and L animals but differences were small (around 3%). The highest distance between diets appeared for yellowness (b^*). L animals showed lower carcass b^* values than SF and A animals. Carcasses from SF animals had 78% higher b^* values than carcasses from L animals, and carcasses from A animals showed double b^* values than carcasses from L animals. Regarding the fat color, fat from SF and L animals had 92% and 93% of the L^* values that A group showed, respectively. Fat yellowness was lower in A animals. SF and L had a 27% and 17% higher b^* fat values than A ($P>1=1$). The evidence for the differences in fat b^* between SF and L was low. SF had a 16% higher meat b^* values than S. Effects on pH and moisture were not relevant. Diets enriched with n-3 and n-6 fatty acids have small effects on rabbit carcass characteristics. However, meat quality traits were not modified by the type of dietary fat.

EFFECT OF OUTDOOR REARING SYSTEM, IN FLOOR CAGE, ON MEAT QUALITY OF SLOW GROWING RABBITS

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The present work investigated the effect of two housing systems (Indoor vs. Outdoor) on the qualitative characteristics of meat deriving from “Grigia Rustica” rabbits, a slow growing local population. Forty eight growing females were divided into two groups: Indoor group, housed in indoor colony cages (cm 65x40x32 h, 4 animals per cage), and Outdoor group, in wire net floor colony cages (cm 100x150x76 h, 8 animals per cage), in outdoor pen in a wooded area. The animals received *ad libitum* a commercial pelleted diet plus alfa-alfa hay and were slaughtered at 103±2 days (19 animals/group). About one hour after slaughter, carcasses were put in a ventilated cold room (+4°C) and chilled for 24 hours and the loin region and the right hind leg were excised from each carcass. The following variables were measured: pH_u, L* a* b* colour, water holding capacity (filter paper press method, M/T ratio) of *Longissimus lumborum* and *Biceps femoris* muscles; drip loss and cooking loss of *L. lumborum* muscle; chemical composition and fatty acids (FA) profile of hind leg meat. The muscles pH_u values were similar within groups. The *L. lumborum* and *B. femoris* muscles of Outdoor rabbits had lower lightness (55.59 vs. 59.15 and 53.02 vs. 55.50 respectively, P<0.01) and slightly higher a* value than those derived from rabbits reared in Indoor cages, probably due to increased movement. The M/T ratio of *L. lumborum* and *B. femoris* and drip loss of *L. lumborum* were similar among groups; cooking loss was significantly lower in Outdoor group (15.9% vs. 18.1%). The housing system significantly affected the dry matter (25.5% vs. 24.9%), protein (22.9% vs. 22.6%) and fat contents (1.4% vs. 1.2%) that were higher in Outdoor group. The hind leg meat of the Outdoor rabbits was significantly lower in saturated fatty acids (SFA, 36.4% vs. 37.9%) and higher in monounsaturated fatty acids (MUFA, 23.2% vs. 21.9%), while no difference was observed for polyunsaturated fatty acids (PUFA) contents. PUFA/SFA and MUFA+PUFA/SFA were similar in both groups. The meat showed similar n-6/n-3 ratio between experimental groups (10.5) and slightly higher than the recommended value in human. In conclusion, meat from outdoor rabbit appeared less pale, showed a significant higher fat content that was probably positively related to lower cooking loss, and a positive lower content of SFA and a higher content of MUFA. Outdoor rearing seems to be a possible alternative housing system that satisfies the ethical concern of modern consumer, even furnishing good meat quality.

RESPONSE OF GROWING RABBITS TO DIETARY ANTIOXIDANT VITAMINS E AND C. 2. EFFECT ON MEAT QUALITY

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A seven-treatments experiment was carried out to study the response of seventy, 6 weeks old New Zealand White rabbits to dietary supplementation with the antioxidant vitamins, α -tocopheryl acetate (vitamin E) and ascorbic acid (vitamin C), provided individually or in a combination, on some meat quality traits at the end of 7-weeks experimental trial. Rabbits were equally allocated to one of the following supra-nutritional levels of the two vitamins/kg diet up to the 13th week of age: (1) control with no additional vitamin supplementation (40 mg vitamin E provided through the vitamin and mineral premix); (2) 40 mg vitamin E (E40); (3) 80 mg vitamin E (E80); (4) 200 mg vitamin C (C200); (5) 400 mg vitamin C (C400); (6) 40 mg vitamin E+200 mg vitamin C (E40C200); (7) 80 mg vitamin E+400 mg vitamin C (E80C400). Meat α -tocopherol and ascorbate contents of the meat at days 10 and 20 of frozen storage were higher ($P<0.01$) in the vitamin-supplemented groups, especially the E80C200 treatment for α -tocopherol, also C400 group at day 10 and E40C200 group at day 20 for ascorbate content. TBARS levels of stored meat was significantly ($P<0.01$) lower in the vitamin-supplemented groups, substantially in the C200 fed group. Again, C400 and E40C200 groups significantly ($P<0.01$) maintained the higher desirable polyunsaturated fatty acids (PUFAs) content of the stored meat. It is concluded that vitamin E and/or vitamin C successfully ameliorated the quality traits of meat produced in terms of improved oxidative stability and the introduction of a highly nutritional food (rabbit meat) source rich in vitamins E and C for human.

ESTIMATION OF RABBIT HIND LEG MUSCLE WEIGHT AND MUSCULARITY BY REAL TIME ULTRASONOGRAPHY

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Forty-four New Zealand White x Californian rabbits were used to evaluate the real time ultrasonography (RTU) in assessing hind leg muscle weight and hind leg muscularity. For *in vivo* muscularity determination animals were scanned over the hind leg using a RTU machine equipped with a 5 MHz probe. The RTU images were taken on hind leg at three positions perpendicular to femur bone. At each position a section of hind leg muscle was analysed and the area of muscles was determined. The muscle volume of the hind leg was calculated by combining the muscle area measurements with the femur length. The weight of hind leg muscle was estimated from this volume of the hind leg and knowing the rabbit muscle density. Information related to hind leg muscle weight and femur length was used for hind leg muscularity. After scanning, animals were weighed, slaughtered and the reference carcass was cut. The right hind leg was dissected and the muscle was weighed and femur length was measured. The hind leg muscularity in carcass was calculated as previously described for RTU measurements. Correlations between the RTU measurements and their corresponding on carcass were determined. The LW varied from 1200 to 3410 g (average 2202 g) and reference carcass weight ranged between 561 and 1940 g (average 1199 g). The correlations between carcass and RTU hind leg muscle weight were high (r from 0.56 to 0.83, $P < 0.001$) and the total hind leg muscle weight was the trait that presented the highest correlation between carcass and RTU measurements ($r = 0.83$; $P < 0.001$). Lower correlation coefficients were found for muscularity (r from 0.15, $P > 0.05$ to 0.46, $P < 0.01$). Our results show that RTU is able to estimate hind leg muscularity and hind leg muscle weight of rabbits.

IN VIVO RABBIT CARCASS COMPOSITION AND *LONGISSIMUS DORSI* MUSCLE VOLUME PREDICTION BY REAL TIME ULTRASONOGRAPHY

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The real-time ultrasonography (RTU) was used to measure *in vivo* the *Longissimus dorsi* muscle (LM) volume and to predict carcass composition of 63 New Zealand White X Californian rabbits. Animals were scanned between 6th and 7th lumbar vertebrae with RTU equipment with a 7.5 MHz probe, then weighed and slaughtered. Chilled carcass and reference carcass weight (RCW) were recorded and carcass dissected into meat, bone and total dissectible fat. The LM volume measured by *in vivo* RTU (LMVU) was calculated by multiplying the LM area obtained *in vivo* by RTU between the 6th and 7th lumbar vertebrae by the loin length measured on live animals by palpation on bone anatomical basis of the end points. Equivalent measurements to those taken *in vivo* were obtained on the carcass and the LM volume was measured using a digital image analysis (LMVC) and directly in loin LM muscle by Archimedes principle (LMVAr). Single regression equations were used for the estimation of carcass composition and LM volume (LMVC and LMVAr) using the LMVU measurement as independent variable. The carcass and *in vivo* measurements were also compared by ANOVA. The live weight varied from 1200 to 3410 g (average 2093 g) and the RCW from 472 g to 1773 g (average 997 g). Bone and total dissectible fat represented 78.0, 16.4 and 5.5% RCW, respectively. The LM measurements in carcass and *in vivo* by RTU were not significantly different for LM area and LM volume (LMVC). The LMVAr (57.4 cm³) was lower (P<0.05) than LMVC (64.5 cm³) and LMVU (65.5 cm³). Carcass and *in vivo* loin lengths differed significantly, being the carcass lower (11.2 cm; P<0.05) than the *in vivo* measurement (12.4 cm). Regression equations showed a strong relationship (P<0.001) between LMVU and the correspondent volume in carcass (r²=0.811 and 0.796 for LMVC and LMVAr, respectively). LMVU was also useful in predicting the amounts of carcass tissues (r²=0.801, 0.718 and 0.414 for meat, bone and total dissectible fat weight). Lower determination coefficients were obtained between LMVU and carcass tissues expressed in percentage of RCW (r² from 0.003, P>0.05 to 0.329, P<0.001). In conclusion, carcass LM volume may be predicted from the loin length and LM area measurement obtained *in vivo* from a RTU scan between the 6th and the 7th lumbar vertebrae, and the amount of carcass tissues can be predicted from LM volume measured *in vivo* by RTU.

MICROBIAL CONTAMINATION LEVELS IN RABBIT CARCASSES OBTAINED FROM POPULAR MARKETS IN TOLUCA VALLEY, MEXICO

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Microbial load in 125 rabbit carcasses from popular local markets (supermarkets, butcheries and markets) in Toluca Valley, Mexico was evaluated using plate microdilution to estimate mesophilic and coliform total count (colony forming units/surface, CFU), and *E. coli*, *S. aureus*, *Salmonella spp.* and *Listeria monocytogenes* isolations. The lowest mesophilic and coliform CFUs was observed in samples from supermarkets (1.50 ± 0.92 and 0.8 ± 0.61) compared to higher microbial load in markets (2750 ± 1310 and 2463 ± 918) ($P < 0.001$). Isolation frequency of pathogens considered as public health risk were: *E. coli* (62.4%), *S. aureus* (36.0%) and *Salmonella spp.* (1.6%). *E. coli* hemolytic (HLYI) and CR+ strains were found and *S. typhimurium* and *S. anatum* serotypes ($P < 0.001$). No *Listeria monocytogenes* isolations were obtained with the bacteriological procedures used. It is concluded that the microbial contamination levels of rabbit carcasses evaluated in the different popular markets in Toluca Valley is related with its marketing, in which markets are considered as critical risk for food safety and public health.

EFFECT OF A HIGH FIBROUS DIET IN THE FINISHING PERIOD ON CARCASS YIELD AND MEAT QUALITY OF RABBITS

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Effect of dietary insoluble fibre level during the last week of fattening period on carcass and meat quality traits was investigated in rabbits slaughtered at 9 weeks of age. Eighty eight rabbits, identified at weaning (28 d) with a glass-encapsulated transponder, were randomly selected at 56 d of age from two groups of rabbits fed a moderate (33% NDF) or a high (41% NDF) insoluble fibre diet. Within treatment, half rabbits were fed an experimental diet containing a very high concentration of NDF (49%) from defatted grape seed meal and the remaining animals continued receiving the same diet than before up to 63 d of age. Without fasting, rabbits were carried to a slaughterhouse and processed following standard techniques. Rabbits fed a high fibrous diet in the last week of the fattening period (56-63 d) maintained their growth rate when NDF dietary level increased from high commercial levels (41%) up to near 50%. However, when this diet was fed to rabbits following a moderate NDF (33%) diet in the growing period, feed intake increased greatly, but not enough to maintain growth rate. Dietary treatments did not significantly influence live weight before and after transport. Full gastrointestinal weight decreased by feeding the highest fibrous diet (49% NDF) in the last week of the fattening period if a high fibre diet (41% NDF) was also fed in the growing period, but the opposite effect was observed when the growing diet had a moderate fibre content (33% NDF). Expressed as live weight percentage, full gastrointestinal weight of rabbits fed moderate fibre levels (33% NDF) for the whole fattening period was significantly lower than in rabbits fed higher fibre levels (41% NDF), and dressing percentage of the former was higher. These slaughtering traits were not influenced by increasing fibre level during the finishing period from 41 to 49% NDF. The meat ultimate pH was not significantly affected by treatments. Carcass colour of rabbits with the lowest growth rate in the fattening period considered was darker, and that of animals fed the 33% NDF diet over the whole fattening period were more red coloured, compared with rabbits fed the 41% NDF diet. In conclusion, the gastrointestinal content weight, cold carcass weight and dressing percentage of rabbits may be modified by feeding a high fibrous diet in the last week of the fattening period (56-63 d). Effects depend on the fibre and energy dietary levels in the growing diet (28-56 d). No significant relationship is observed between dietary factors and carcass quality traits of rabbits slaughtered at the same age.

COLOUR AND PH OF RABBIT MEAT AND FAT DEPOSITS AS AFFECTED BY THE SOURCE AND DOSE OF DIETARY VITAMIN E SUPPLEMENTATION

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A trial was carried out to determine the effect of level and source of vitamin E supplementation on the pH and colour of rabbit thigh and loin meat as well as on perirenal and scapular fat (N=15 per treatment). The animals received a compound feed containing 2% sunflower oil and 2% linseed oil. Natural vitamin E (a by-product of the oil industry, i.e. fatty acid distillate) or synthetic dl- α -tocopheryl-acetate was used as vitamin E source. The feed was supplemented with 150 mg/kg and 300 mg/kg synthetic dl- α -tocopherylacetate, or 60+90 mg/kg and 60+240 mg/kg synthetic plus natural (d- α -tocopherol) vitamin E. Higher level of vitamin E compared to the lower level significantly increased loin pH (5.96 and 5.90; P<0.05). Vitamin E level had not further significant effect on the examined parameters. L* (53.12 vs. 50.74; P<0.005) of loin; a* of loin (0.07 vs. -0.39; P<0.05) and fat deposits (4.44 vs. 3.38; P<0.01) furthermore thigh b* (7.10 vs. 6.37; P<0.01) were significantly higher at natural vitamin E supplementation. Fat deposit L* (76.44 vs. 78.27; P<0.03) was lower in this group. Overall colour difference was well visible for thigh meat and perceptible for fat deposit what could influence consumer's choice and decision for shopping.

**EFFECT OF DIETARY VEGETABLE OIL (SUNFLOWER, LINSEED)
AND VITAMIN E SUPPLEMENTATION ON THE FATTY
ACID COMPOSITION, OXIDATIVE STABILITY
AND QUALITY OF RABBIT MEAT**

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This experiment was conducted to investigate the effect of dietary vegetable oil (sunflower and linseed) and different vitamin E supplementation on the fatty acid (FA) profile, vitamin E content and oxidative stability of rabbit meat. In addition, the FA composition of cooked and roasted meat samples was analyzed. A total of 200 New Zealand White rabbits were randomly assigned to one of four dietary treatments (50 rabbits per treatment) and fed the experimental diets between 35 and 80 days of age. All diets were supplemented with 60 mg/kg dl- α -tocopherol-acetate. A negative control (NC) diet with no added oil (energy content 10.61 MJ/kg) and three diets supplemented with 2% sunflower and 2% linseed oil (energy content 11.36 MJ/kg) were used. In addition to the 60 mg/kg dl- α -tocopherol-acetate content, no more vitamin E (PC), 240 mg/kg synthetic vitamin E (dl- α -tocopherol-acetate) (SE) or 240 mg/kg natural vitamin E (fatty acid distillate, d- α -tocopherol, a by-product of the oil industry) (NE) were added to the oil supplemented diets. The oil addition to the diets (PC, SE and NE groups) significantly increased the UFA, PUFA and decreased the SFA and MUFA content in limb muscle compared to the NC rabbits. The meat samples of the PC, SE and NE rabbits had a lower n-6/n-3 PUFA ratio (2.39, 2.14, 2.76 vs. 4.26) and a significantly ($P < 0.05$) higher PUFA/SFA ratio (1.92, 1.86, 2.01 vs. 0.66) in comparison to the NC group, respectively. It was found that supplementing the diets with vitamin E (SE and NE groups) significantly increased the α -tocopherol content of limb meat in rabbits and improved its oxidative stability compared to the NC and PC groups. The utilization of the synthetic form of vitamin E (SE group) was higher compared to the natural form (NE group), but the natural source of vitamin E was more efficient in improving the oxidative stability of the rabbit meat samples as indicated by a decreased MDA value. Our results proved that the fatty acid composition of rabbit meat cannot be altered by cooking or roasting.

MANAGEMENT AND ECONOMY

A CHALLENGING ROLE FOR ORGANIC RABBIT PRODUCTION TOWARDS POVERTY ALLEVIATION IN SOUTH EAST ASIA

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ABSTRACT

This paper addresses the challenging role of organic rabbit production applied towards poverty alleviation. The objective of the paper is to provide information on organic rabbit production with applications to food security and income generation. Rabbits are easily incorporated into integrated farming systems as they convert plant materials, which often are of low nutritive value, to high quality meat, as well as providing faeces whereby nutrients are returned to the soil. Fresh faeces from rabbits can be the raw material for biodigesters used to produce gas and effluent for applying to crops, and for use by earthworms for production of casings are a source of organic fertilizer. In general, the linkage between livestock and crops improves the sustainability of the farming system. The potential of locally feed resources has been used successfully for growing rabbits as an alternate feed supply in terms of cost effectiveness and sustainability. These feed resources can be cultivated in the farmers' plots to provide diets with high contents of digestible energy and protein without the need for cash resources to buy off-farm supplemental feeds. Feed sources from forages, vegetables and aquatic plants (e.g., Cassava, Mulberry, Leucaena, Gliricidia, Sweet Potato vines, Water Spinach, and Stylo) can be used in diets to replace or in combination with a protein source from conventional feed ingredients (soybean and fish meals). Therefore, poor families with limited resources could benefit through increased income and increased consumption of rabbit meat to meet the families' nutritional needs. Farmers can also re-invest their capital to expand their rabbit operation as a means to abandon poverty, but an emphasis on market accessibility and creating a high consumer demand for rabbit meat must also be developed.

INTRODUCTION

Countries in South East Asia, such as Cambodia, Laos, Myanmar, and Vietnam, are the poorest in the region and most of these populations depend mainly on agriculture for their food, income, and mere subsistence. In all four countries, growing inequalities exist between rural and urban populations (Ngo Van Man and Luu Trong Hieu, 2005). CelAgrid and ILRI (2007, unpublished data) reported in Cambodia that the average

monthly income of urban households was US\$ 723 while in rural households was only US\$ 210. This income gap might reflect consumption patterns of local people between both areas, especially in the demand for meat from fish and livestock. Taucher (2000) indicated that the gross national product (GNP) in Cambodia is about US\$ 300 per person per year, similar to that of Vietnam and Laos, which is considerably lower when compared to Thailand, Indonesia, and Malaysia, which are better off by 3.5 to over 9 times.

Livestock play a significant role in improving living conditions of the poor. The purpose of livestock keeping of small-scale farmers in the Southern region is for the draught power, food, cash income, and organic fertilizer from faeces. Perry *et al.* (2003) reported on the animal species kept by smallholders and their contributions to the families' assets (Table 1). Poor households rarely kept only one species, due to the risk of the diseases, and so raise several livestock species based on market value.

Table 1: Livestock species kept by the poor and their contribution to household assets (Perry *et al.*, 2003).

Species	Financial	Social	Physical	Natural	Human
Cattle/ buffaloes	Sales for milk, meat, hides, animals, draught power services, transport and savings	Networking mechanisms and social status indicators	Draught power for crop cultivation and transport	Faeces for maintenance of soil fertility	Household consumption for milk and meat
Goats/Sheep	Sales for milk, meat, hides, animals and savings	Networking mechanisms and social status indicators	-	Faeces for maintenance of soil fertility	Household consumption for milk and meat
Pigs	Sales of meat, animals and savings	Networking mechanisms and social status indicators	-	Faeces for maintenance of soil fertility	Household consumption for meat
Poultry	Sales of eggs, meat and fowl	Networking mechanisms	-	Faeces for maintenance of soil fertility	Household consumption for meat and eggs

According to CelAgrid (2007, unpublished data), the reported numbers of livestock kept per household in Takeo, Kandal and Pursat provinces of Cambodia, in terms of the average number of cattle, pigs, and chickens, were 3.15, 2.91 and 16.0 heads, respectively. In Thailand, 120 million village chickens are distributed over a small number on rural farms (Chantalakhana and Skunmun, 2002). Recent outbreaks of Avian Influenza in birds have occurred in several countries in the world, such as in Italy, Spain, and Hong Kong. In 1997, outbreaks also reached several Asian countries including Cambodia. Human deaths from the disease have been reported in Indonesia, Vietnam, Thailand, and Cambodia (CelAgrid and AED 2007; unpublished data).

Rabbit production is a new development in the region, which plays an important role in view of the economic risks by the spread of Asian bird flu (Otte *et al.*, 2007). According to the FAO (2001), backyard rabbit keeping provides additional income and supplies additional protein for poor rural and urban households with low investment and labor inputs. Rabbits have small body size, short generation interval, high reproductive potential, rapid growth rate, genetic diversity, and the ability to utilize forages and by-products as major diet components that make the animal appropriate for small livestock keeping in developing countries (Cheeke, 1986). As quoted from Rastogi (2000) the advantages of small-scale rabbit production are shown below:

- Small size and quiet nature of rabbits makes it easy to raise them in cities, suburbs and village communities;
- Large litter size and short generation interval allows for economic returns in the short term (12-15 fryers/doe/year);
- Rabbits are 2.5 and 4 times more efficient in extracting protein from forages than sheep and beef cattle;
- Rabbits can easily subsist on waste materials from the vegetable garden, family kitchen and institutional canteens/cafeterias;
- Low investment is required for establishing a small rabbitry with 3-5 breeding does;
- Meat of rabbits is an excellent, alternative source of healthy food being low in fat, salt and cholesterol.

The aim of the paper is to describe the potential of organic rabbit production in improving income and food security of smallholder families by utilizing feed resources in countries of South East Asia.

RABBIT-BASED FARMING SYSTEM

Integrated farming systems play an important role for small-scale farm families in rural areas in terms of sustainability (“environmentally-friendly”), while minimizing economic risks (Lukefahr and Preston, 1999). According to Pok Samkol *et al.* (2007), livestock convert plant materials that are low in nutritive value into high quality products, such as meat and milk, and return nutrients to the soil in the form of faeces. This synergistic interaction between livestock and crops can improve the sustainability of the farming system and improve soil fertility. According to Lukefahr (2007), using solar energy and ensuring that there is an efficient flow of nutrients in components of the farm ecosystem, there is reduced dependence on off-farm inputs (e.g., commercial feed, fertilizer, and wire) with lower capital investment and hired labor requirements.

Livestock faeces, including rabbits’ wastes and human excreta, have been used as materials for biodigesters to improve soil fertility. The soil organic matter content may be an important source of energy for organisms that oxidize methane (Keller *et al.*, 1990). In many situations, the effluent from biodigesters has become the most important product for improving soil fertility (UTA, 2001). The advantage of the biodigesters is that it can become a major source of fuel for cooking, providing

organic fertilizer for crop/tree/water plants and fish ponds, and organic fertilizer (Preston and Rodríguez, 2002) and as fuel for engines (Ho Thi Lan Huong, 2002). The appropriate use of biodigesters can also give rise to a number of related socio-economic benefits through improvements to the quality of life for rural women and children (e.g., reduction of labor in fuel wood collection and in cleaning the kitchen, cooking pots and utensils). The fertilizer value of faeces aids to improve the environment by reducing methane emissions and preventing deforestation. When applying graded levels of effluent from biodigesters loaded with pig or cattle faeces at 0 to 140 kg N/ha, the response of biomass yields of Water Spinach, an excellent forage for livestock, was linear from 6.66 to 23.6 tons/ha ($R^2=0.97$) (Kean Sophea and Preston, 2002). Also, the growth rate of the fish applied with effluent fertilizer was higher than with U-DAP or fresh faeces from cattle and pigs (Table 2) (Pich Sophin and Preston, 2002).

Table 2: Daily weight gains of fish species according to the fertilizer treatments (Pich Sophin and Preston, 2002).

	Effluent	Faeces	U-DAP	SE
Tilapia	0.499	0.348	0.358	0.045
Silver carp	1.326	0.716	1.049	0.114
Bighead carp	0.572	0.207	0.276	0.078
Silver barb	0.682	0.551	0.651	0.133
Mrigal	0.946	0.831	0.996	0.004

Using the gas produced from biodigesters as fuel for engines can be appreciated as the price of the oil is at peak levels (Leng and Preston, 2005). According to Ho Thi Lan Huong (2002), 1 m³ of gas can replace 0.85 liters of gasoline by adding a gas-air mixer to the original carburetors, and then the gas can be used to replace petrol to power the engine.

The role of the earthworms in improving soil fertility has long been appreciated by farmers, but until the last two decades there has been no major attempt to cultivate them as a component in an organic recycling system. The potential value of earthworms as a source of high quality protein to supplement poultry diets is now being recognized, but their major role is in the recycling of animal excreta for production of high quality organic fertilizer in the form of worm casings (worm compost). The biomass yield response to fertilization with worm compost was higher than urea (Figure 1) (Tran Hoang Chat *et al.*, 2005a).

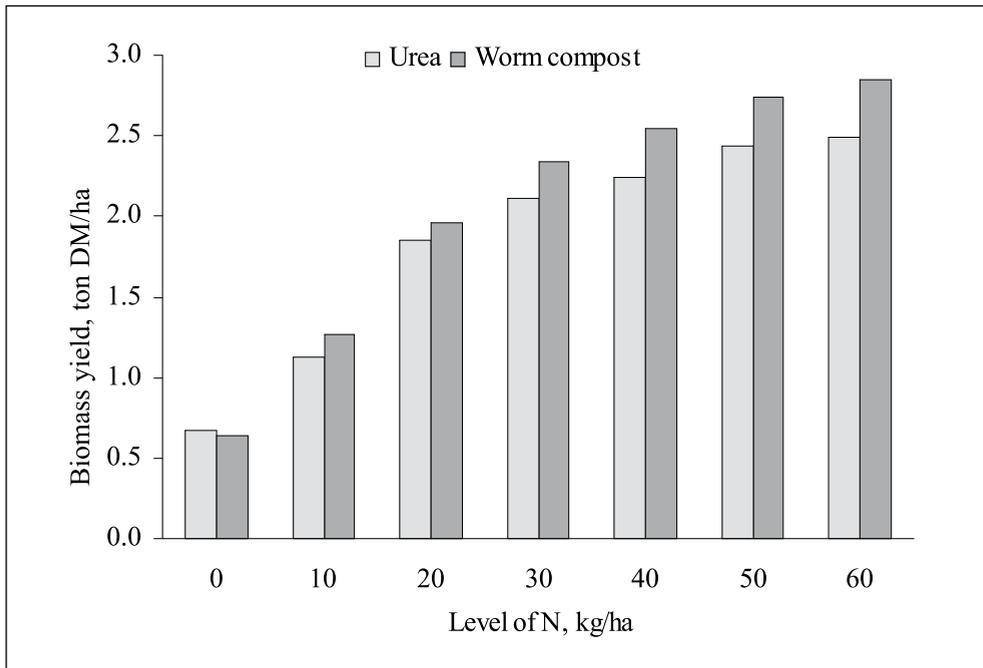


Figure 1: Biomass yield of Water Spinach fertilized either urea or worm compost (Tran Hoang Chat *et al.*, 2005a).

Earthworms can be cultivated on the faeces from virtually all species of livestock. However, they have the comparative advantage over other forms of recycling when the faeces are from pigs, goats, cattle or rabbits (UTA, 2001). The faeces from rabbits make excellent compost, which is rich in organic matter, and nutrients that can produce remarkable results for the home garden and flowers. On the other hand, California earthworms grown in rabbit faeces produce a superb and fairly odorless organic material that resembles peat moss. As demonstrated by Nguyen Quang Suc *et al.* (2000), rabbit faeces is rich in nutrients that provide growth rates of California earthworms higher than faeces from buffaloes or cattle but lower than faeces from goats (Table 3). The value of the worm casts as a source of nutrients for growth of maize was twice that of the raw faeces and, within animal species, the faeces and worm casts derived from goats and rabbits were superior to that from buffaloes and cattle (Nguyen Quang Suc *et al.*, 2000).

Table 3: Consumption of faeces and production of earthworms and conversion of faeces to worms (Nguyen Quang Suc *et al.*, 2000).

	Goats	Rabbits	Buffaloes	Cattle	SE
Faeces, kg DM	85.0	108	77.6	72.3	3.00
Earthworms, kg fresh	5.61	5.38	3.65	2.93	0.25
Conversion rates, kg DM/kg fresh	14.7	18.2	21.6	28.3	0.60

In general, growth rates of rabbits range from 10 to 20 g/day in the tropical regions compared to temperate countries where growth performance typically is between 35 to 40 g/day. The differences may be largely due to heat stress and quality of the diets. High ambient temperatures can cause infertility in breeding rabbits, bucks being more sensitive than does (Lukefahr and Cheeke, 1991). The establishment of the house for rabbits can be made from local materials, such as small poles or sticks and bamboo. However, the house should be well designed to prevent losses from predators. Lukefahr and Cheeke (1991) stated that proper hygiene and management of cages could prevent the spread of certain epidemic diseases. Studies in Vietnam by Nguyen Quang Suc *et al.* (1996) compared traditional cages to an underground system consisting of a shelter using stones, bricks, clay or concrete, which can be covered by earth. The measurements were made at 7:00 am, 12:00 pm and 7:00 pm, and the temperatures did not change for houses constructed underground, which ranged from 25 to 27°C, while the temperature for above-ground cages ranged from 28-30.5°C. The results indicated that there was a significant difference in growth rate of rabbits accommodated underground than rabbits confined in traditional cages which were 21.3 g/day compared with 17.7 g/day of feeding the same diets.

In addition, it is recommended to also use local feed resources which farmers can produce in plots to provide diets that have high contents of digestible energy and protein. However, Lukefahr and Cheeke (1991) reported that many tropical feeds contain toxins, such as mimosine in *Leucaena* and HCN in Cassava. There are practical ways to minimize toxicity problems (mentioned in a later section). Due to such anti-nutritional factors, in general, the feeds that contain toxins should only be partially used by mixing with other ingredients.

Khieu Borin (2005) reported that in South East Asia, farmers keep animals as part of their traditional culture. Limited resources, such as small land holdings and shortage of capital, prevent farmers from fully developing integrated farming systems. Devendra and Chantalakhana (2002) confirmed that the average farm size in Indonesia is only 0.4 ha. Baseline surveys conducted in the Phnom Kravagn district of Pursat, Cambodia, revealed that families had a mean farm size of 0.26 ha, a paddy field of 1.15 ha, and chamka of 0.47 ha (CelAgrid, 2006). Lukefahr (2007) recommended that all farming activities or components should be complementary, using animal faeces for compost to add to forage and garden plots, fish ponds, and earthworm bins, while forages are mostly fed to rabbits and other livestock.

Chantalakhana and Skunmun (2002) pointed out that crop/livestock interactions evolved through various process stages: (i) the pre-intensification phase of crop/livestock development, (ii) intensification phase of the integration crop/livestock system, (iii) income diversification phase, and (iv) specialized/commercial production phase. These phases depict the nature of this evolution involving crop/livestock systems in Asia and could reflect some directed changes of Asian livestock production systems in the future (Table 4). At present, the majority of livestock production systems in Asia fall under phase (ii), while some systems are moving to phase (iii). Cambodia, Laos, Myanmar and Bangladesh are still in phase (i), while Japan has moved to phase (iv).

Table 4: The evolution of crop/livestock systems in Asia (from Chantalakhana and Skunmun, 2002).

Region	Phase			
	(i) Pre-intensification	(ii) Intensification	(iii) Income diversification	(iv) Specialization
East Asia:				
Japan				x
Korea			x	x
Taiwan				x
China		x	x	
Southeast Asia:				
Indonesia		x		
Philippines		x	x	
Thailand		x	x	
Malaysia				x
Vietnam		x	x	
Cambodia	x	x		
Laos	x	x		
Myanmar	x	x		
South Asia:				
India		x	x	
Pakistan			x	
Sri Lanka		x	x	
Nepal	x			
Bangladesh	x			

FEED RESOURCES FOR RABBITS

Feed preferences and feeding systems of rabbits

Rabbits are very selective in their feeding behaviour and in the wild will select specific plant parts. They generally select leaves rather than stems, young plant materials rather than old and green rather than dry materials, resulting in a diet that is higher in protein and digestible energy and lower in fiber than the total plant material available. They are much more sensitive to slight changes in the feed than other livestock. Sometimes they will refuse to accept a new diet and will starve rather than accept the new feed for several days (McNitt *et al.*, 2000).

According to Chiv Phiny (2007), many different forage crops, including water plants and agricultural products and by-products, can be used for animal feeding in tropical regions. For example, Water Spinach, Mulberry and Cassava leaves, and Sweet Potato vines, are rich in protein and can be grown by farmers, and these match with sources of energy available in villages, such as sugar cane juice, sugar palm juice, and cassava root. The leaves of most water plants are more digestible than the leaves of trees and shrubs, but the high water content limits high levels of inclusion in diets, while forage crops often have low palatability and high fiber contents that can negatively influence feed intake and therefore reduce the availability of nutrients to monogastric

animals (Cheeke *et al.*, 1980). In backyard rabbit rearing systems, as is now widely practiced in many parts of Africa, the feeding of green herbage is advantageous, since greens are available year-round in the coastal regions and low plains of the continent. In some cases, palatable greens are fed *ad-libitum* which can reduce the amount of concentrate pelleted feeds by 50% with no adverse effects on performance of rabbits (Cheeke *et al.*, 1987).

Lukefahr (1992) presented information on suitable feed sources and basic primary dietary requirements and concluded that feeds for rabbits could be obtained from a variety of sources. These include: wild, indigenous plant stands, cultivated forage plots, farm crop residues, farm surplus foods, agricultural by-products, kitchen wastes, and market sources. However, wild plants may be poor in palatability and some forages may only be seasonally available. Pound *et al.* (1984) reported that the more appropriate approach for smallholder farmers is to grow trees, shrubs and water plants that produce much higher unit area yields of protein in the form of leaf biomass rather than cultivating traditional protein crops, such as soybeans, groundnuts or sunflowers, as components of their farming systems. Strategies to efficiently utilize these unconventional feeds are more likely to succeed when the production system is matched with the available resources (Preston and Leng, 1987).

According to Honthong Phimmasan (2005), the palatability of forages is important in rabbit production, particularly in situations when the forages are expected to provide a major part of the daily nutrient intake. Raharjo and Cheeke (1985) reported that tropical legumes were preferred over grasses and agricultural by-products, with the exception of *Gliricidia* (*Gliricidia sepium*), a legume which proved to be unpalatable. *Leucaena* (*Leucaena leucocephala*) is a very palatable to rabbits, even though it contains the toxin, mimosine. *Erythrina* (*Erythrina lithosperma*), another legume, was well accepted. Sweet Potato vines were palatable to rabbits, while banana and papaya leaves were poorly accepted. Most of the grasses (e.g., *Setaria*, *Brachiaria*, and Elephant grass) were less palatable than legumes (Raharjo, 1987).

Tree leaves with potential for feeding include the Mulberry (*Morus spp.*), which has been used in India, Brazil and Costa Rica as a forage and black Locust (*Robinia pseudoacacia*), grown extensively in China for rabbit feed. Ramie is utilized in Brazil, where it is considered a highly palatable and nutritious green feed for rabbits (Raharjo, 1987).

Water Spinach (*Ipomea aquatic*)

Water Spinach can be planted either in the soil or water and has been used traditionally in South East Asia as a vegetable for consumption by people and animals. Preston (2006) reported that it appears to be devoid of non-nutritional elements. It has a short growth period and is resistant to many common insect pests. Among aquatic weeds, Water Spinach has great potential for use as a forage for livestock and it is also effective in waste water treatment systems. Average annual fresh weight production of 90, 70 and 100 tons/ha have been reported in Hong Kong, Fiji and the Netherlands, respectively, and the dry weight production during an eight-month period exceeded 20 tons/ha when cultivated in a culture solution (Jain *et al.*, 1987).

Water Spinach contains approx. 29% crude protein (CP) in DM and it may be suitable as a protein source more so than alfalfa (19.6% CP) (Shurson *et al.*, 2002). Moreover,

Water Spinach has a lower fiber content than alfalfa leaves (Bruemmer and Roe, 1979).

Pok Samkol *et al.* (2006) fed Water Spinach as the sole diet to crossbred (Local x New Zealand) rabbits, which can support satisfactory growth rates from 14 to 20 g/day. When fed different levels of the Water Spinach plant, rabbits consumed more leaves than stems, resulting in increased protein intake (Figure 2 and 3).

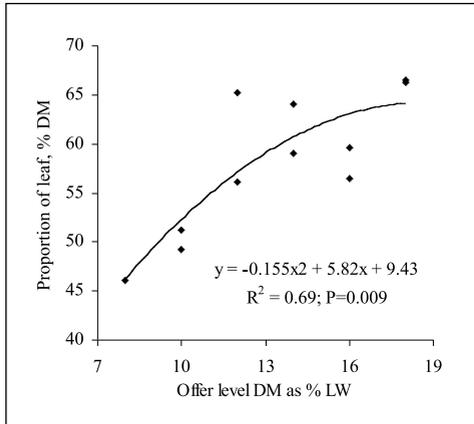


Figure 2: Proportion of Water Spinach leaves (%) in DM consumed, according to offer level (Pok Samkol *et al.*, 2006).

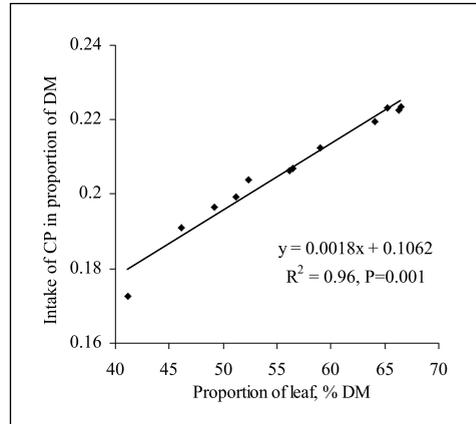


Figure 3: Relationship between proportion of leaves of Water Spinach consumed and proportion of CP in diet DM (Pok Samkol *et al.*, 2006).

When weight gains were plotted against the proportion of CP, the relation was negative (Figure 4). However, the relationship between the proportions of the DM consumed as crude fiber (CF) and weight gains was positive (Figure 5) (Pok Samkol *et al.*, 2006).

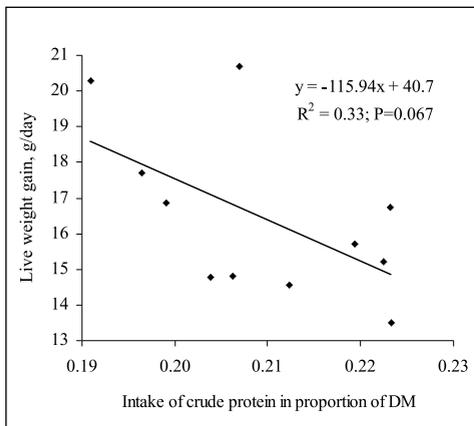


Figure 4: Relationship between proportions of DM of Water Spinach consumed as crude protein and daily weight gains (Pok Samkol *et al.*, 2006).

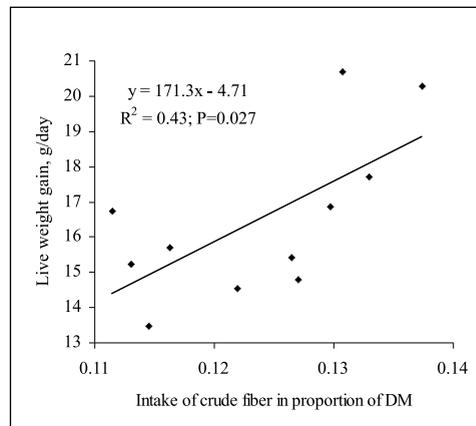


Figure 5: Relationship between proportions of DM of Water Spinach consumed as crude fiber and daily weight gains (Pok Samkol *et al.*, 2006).

Supplementation of fiber in the feed from Water Spinach added to diets based on Stylo (*Stylosanthes guiensis*), Para grass (*Panicum maximum*), or rice bran, fed to crossbred (Local x New Zealand) rabbits, supported growth rates in the range from 18 to 23 g/day (Khuc Thi Hue and Preston, 2006). According to Tran Hoang Chat *et al.* (2005b), an experiment conducted on the replacement of Guinea grass with Water Spinach to complement use of concentrates showed a general increase CP intake. When Water Spinach was used to replace Guinea grass, there was an improvement of body weight gains of growing rabbits and milk yield and litter size of does (Tables 5 and 6). They concluded that fresh Water Spinach foliage was 25% superior to Guinea grass in supporting growth and lactation performance of rabbits when offered *ad-libitum* (contributing about 50% of the diet DM) as a supplement to fixed amounts of concentrates (fed 3% of LW) and a molasses block (2% of LW).

Table 5: Effect of replacement of Guinea grass with Water Spinach on intake, live weight gains and feed conversion of growing rabbits (Tran Hoang Chat *et al.*, 2005b).

	Water Spinach replacing guinea grass (% DM basis)					
	0	20	40	60	80	100
Feed intake, g/day DM:						
Guinea grass	54.0	37.8	26.9	19.9	12.8	0.00
Water Spinach	0.00	17.2	29.9	38.4	44.5	50.5
Concentrate	39.4	39.4	39.1	39.8	40.6	40.5
Mineral block	29.9	28.6	31.1	26.4	25.8	29.8
Total DM	123.5	123.2	127.1	124.5	123.7	120.8
Total crude protein	16.0	17.6	19.1	20.8	20.8	21.0
Growth performance:						
Initial live weight, g	1390	1443	1413	1403	1473	1420
Final live weight, g	2462	2571	2557	2587	2705	2740
Weight gain, g/day	25.5	26.9	27.2	29.2	29.3	31.4
DM feed conversion	4.79	4.66	4.64	4.28	4.26	3.87

Table 6: Effect of replacement of Guinea grass with Water Spinach on feed intake, weight change, milk yield of the doe mother and growth of the litter (Tran Hoang Chat *et al.*, 2005b).

	Water Spinach replacing guinea grass (% DM basis)					
	0	20	40	60	80	100
Feed intake, g/day DM:						
Guinea grass	65.1	49.1	33.0	21.0	18.1	0.00
Water Spinach	0.00	17.9	36.0	49.0	66.4	81.2
Concentrate	73.0	72.6	71.0	70.0	71.7	70.8
Mineral block	56.2	56.7	57.0	56.0	51.7	49.6
Total DM	194	196	197	196	208	201
Total crude protein	25.7	28.0	30.0	32.0	34.5	34.6
Performance of does:						
Initial live weight, g	3400	3570	3400	3430	3500	3470
Final live weight, g	3660	3900	3730	3900	3967	3760
Weight change, g	267	333	333	566	466	300
Milk yield, g	83.2	86.0	90.2	94.4	97.8	101
Survival rate, %	95.2	95.8	96.3	95.2	96.3	96.3
Weight of litter:						
At birth, g	403	399	420	402	436	435
At 21 days, g	1883	1930	2125	1903	2177	2230
At 30 days, g	3267	3900	3933	4567	5133	5300
Weight gains (0-20days), g/day	15.9	18.5	18.7	19.8	19.5	21.1

Sweet Potato vines (*Ipomea batatas* L)

Sweet Potatoes can be cultivated for tuber or forage production depending on the purpose and season. It is considered as a small farmer's crop that grows well under many farming conditions. It can also be grown in poor soils with little fertilizer. Sweet Potatoes are relatively easy to plant for the purpose of harvesting vine cuttings rather than seeds. In addition, the crop is highly tolerant of weeds, allowing farmers to devote more time to other crops (CGIAR, 2004-2005). It is an important crop in many areas of the world, and is cultivated in over 100 countries. It has been ranked among the five most important food crops in tropical regions where a high population of the world's poorest people live (Woolfe, 1992). About 80% of the Sweet Potato crop in the world is grown in Asia, under 15 % in Africa, and about 6% in the rest of the world (Horton, 1988). With the advantages of Sweet Potato cultivation and its high nutritive value, the Sweet Potato has been developed as an alternative crop to supply food for human and feed for livestock.

Sweet Potatoes can be planted once and cut for vines as animal feed for a whole year with daily harvesting (Le Van An *et al.*, 2003). Le Van An (2004) concluded that the best options for the proportion of the stems and leaves would be the cutting interval of 20 days and a defoliation of 50% of the total branches. Defoliation reduces tuber production. There appears to be considerable differences, depending on variety, in the content of CP and CF in DM of Sweet Potato vines. CP contains approx. 26.2%

DM, Neutral Detergent Fiber (NDF) 31.0% DM, and Acid Detergent Fiber (ADF) 22.7% DM (Doan Thi Gang *et al.*, 2006).

Doan Thi Gang *et al.* (2006) fed Sweet Potato vines as the sole diet to growing rabbits; the daily weight gains were similar to that from feeding Water Spinach (Table 7). Allowing rabbits access to Guinea grass with Water Spinach, or Sweet Potato vines, or the combination of the two, depressed nutrient digestibility (Figure 6). They concluded that when rabbits were fed a basal diet of concentrates (15% soybean meal, 25% cassava root meal, 20% rice bran, 5% minerals and 35% molasses) and highly digestible foliages (Water Spinach and/or Sweet Potato vines and access to Guinea grass) there were beneficial effects on growth rate, even though there was a decrease in digestibility of the overall diet.

Table 7: Feed intake and growth rate of rabbits fed a molasses block and either Sweet Potato vines, Water Spinach or a mixture of the two foliages, and access or no access to Guinea grass (Doan Thi Gang *et al.*, 2006).

	WS	WSGG	SP	SPGG	WSSP	WSSPGG	SE
<i>Feed intake, g DM/day:</i>							
Water Spinach	48.0	36.1	0.00	0.00	33.0	24.3	0.38
Sweet Potato	0.00	0.00	44.3	26.8	23.6	15.7	0.4
Guinea grass	0.00	40.2	0.00	43.9	0.00	33.6	0.68
Molasses block	74.3	73.5	67.2	76.3	68.1	73.8	1.18
Total DM	122	149	112	148	125	147	1.68
Crude Protein	20.5	21.8	23.9	24.8	25.4	25.3	0.26
<i>Growth performance:</i>							
Initial live weight, g	980	940	925	970	930	950	0.04
Final live weight, g	2700	2890	2530	2900	2760	3060	0.06
Weight gain, g/day	21.9	26.4	21.1	26.7	23.1	27.2	1.11
DM feed conversion	10.7	8.23	7.68	7.26	6.21	7.03	0.52

WS: Water Spinach, **WSGG:** Water Spinach and guinea grass, **SP:** Sweet Potato vines, **SPGG:** Sweet Potato vines and Guinea grass, **WSSP:** Water Spinach and Sweet Potato vines, **WSSPGG:** Water Spinach, Sweet Potatoes vines and Guinea grass.

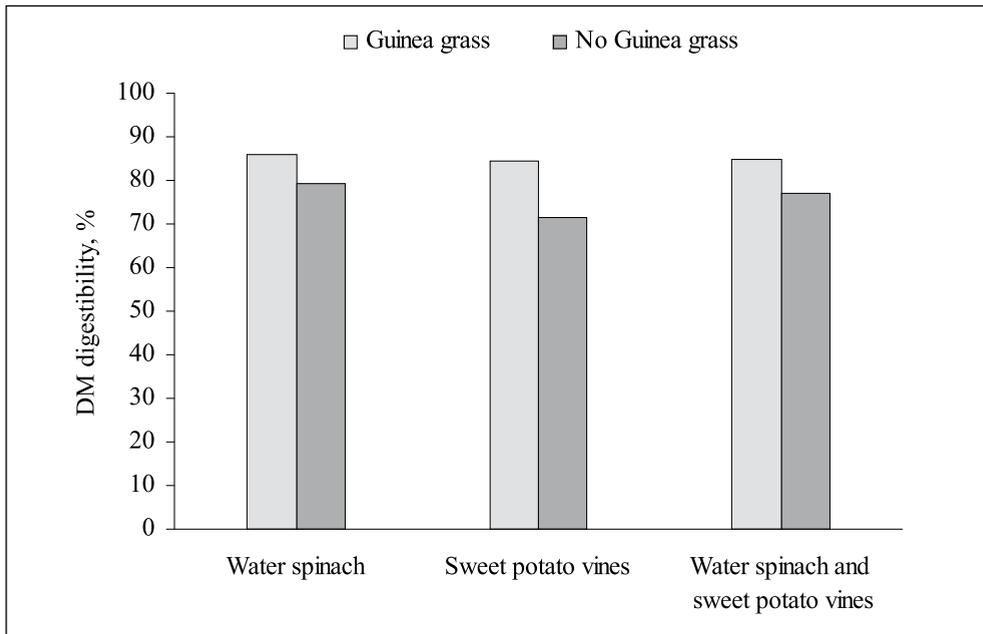


Figure 6: Effect of Guinea grass on digestibility in rabbits fed a molasses block and either Sweet Potato vines, Water Spinach or a mixture of the two foliagees (Doan Thi Gang *et al.*, 2006).

Mulberry (*Morus alba*)

Mulberry has been planted as substrate for growth of the larvae of the silkworm. It is a perennial tree, capable of being periodically cut in a plantation style, resulting in high biomass yields, which in turn can be improved when the plant is irrigated with effluent from biodigesters. It is also grown as a shade tree on wastelands and along roadsides, and as a border of fields and around farmers' houses (Sánchez, 2000).

Mulberry grows very well in most soils and produces a high biomass yield with proper management. Proper planting density and fertilization and irrigation rates are important strategies to increase yield. In East China, it is recommended to cultivate 10,500 to 15,000 seedlings/ha to harvest 26.25 tons/ha/year of leaves. However, in South China, the average annual leaf production can even reach 37.5 to 52.5 tons/ha/year by increasing planting density to 90,000 to 120,000/ha (Yongkang, 2000). In the tropics, Mulberry grows best with a sunlight range of 9 to 13 hours a day (Datta, 2000). Biomass yields were increased when applying fertilizer from livestock wastes in the form of compost or from biodigesters (Rodriguez and Preston, 1996). The yield of Mulberry can reach nearly 35 to 45 tons of fresh leaf/ha/year with CP of 20 to 23% DM and minerals of 12 to 18% in DM. The cell wall constituents have NDF content of 45.6%, cell contents 54.4%, ADF 35.0%, hemicellulose 10 to 40%, cellulose 21.8%, lignin 10%, and silica 2.7% (Lohan, 1980).

Lara y Lara *et al.* (1998) fed rabbits *ad-libitum* mulberry leaves, replacing 85% of a conventional concentrate diet. Although live weight gains were reduced slightly from 22 to 18 g/day, the feed cost was decreased by 50%. The potential of Mulberry

leaves for rabbits was confirmed in a preliminary report from Colombia (Preston, 2006; unpublished data) in which rabbits fed only Mulberry leaves had average live weight gains of 20 g/day. Singh *et al.* (1984) studied the effect of replacing Mulberry leaves *ad-libitum* to concentrate diets when fed to Angora rabbits on production of wool. The results indicated that average intake of Mulberry leaves was 10.4 g/day/kg LW^{0.75}, while the total DM intake was 29.5 g/day/kg LW^{0.75}. The digestibility coefficients for DM, CP, CF and NFE were 69, 66, 72 and 78%, respectively. They concluded that Mulberry leaves can be used to supplement the diets of Angora rabbits for wool production. Mulberry leaves may be supplemented up to a level of 40% in DM in rabbit diets. Mulberry leaves fed to rabbits as a replacement of concentrates was studied by Bamikole *et al.* (2005) in Nigeria (Table 8). Live weight gains were depressed when the level of Mulberry leaves was higher than 50%. However, there was an improvement in the DM feed conversion ratio when increasing the level of Mulberry leaf diet. Nutrient digestibility of DM, CP and CF were high in all diets. They concluded that Mulberry leaves when fed up to 50% of the total diet had a high nutritive value for feeding rabbits with comparable DM intake, digestibility and weight gains to an all-concentrate ration. In overall, satisfactory growth rates were achieved at lower costs.

Table 8: Effect of Mulberry leaves replacing concentrate fed to rabbits on intake, growth performance, and nutrient digestibility (Bamikole *et al.*, 2005).

	Level of mulberry leaves replaced concentrate (%)					SE
	0	25	50	75	100	
Feed intake, g/day:						
Dry matter	38.0	38.5	38.4	37.4	36.5	0.34
Crude protein	7.22	7.73	8.10	8.27	8.47	0.07
Crude fiber	4.56	7.16	9.64	11.8	14.0	0.10
Growth performance:						
Weight gain, g/day	5.72	5.14	4.72	3.43	2.27	0.40
DM feed conversion	0.15	0.13	0.12	0.09	0.06	0.01
Digestibility, %						
Dry matter	82.3	75.7	75.7	77.0	79.7	2.39
Crude protein	84.0	77.7	76.3	80.3	83.7	2.17
Crude fiber	81.7	86.7	79.7	83.7	88.7	2.19

Cassava foliage (*Manihot esculenta* Crantz)

Cassava is one of the world highest calorie producers for human food and is generally grown without fertilization on soils with poor fertility and where other crops would fail (Howeler and Cadavid, 1990). It is possible to obtain from cassava leaves more than 6 tons CP/ha/year with proper agronomic practices directed towards foliage harvesting (AFRIS, 2004).

Cassava leaves have been used to replace soybean in conventional diets for pigs in Vietnam (Bui Ngu Phuc, 2000) and for goat and cattle in Cambodia (Seng Sokerya and Rodríguez, 2001; Seng Mom *et al.*, 2001). Omole (1977) observed that Cassava leaves are a good source of protein, fiber, minerals and vitamins. It contains approx.

25.8 to 27.3% CP, 7.6 to 10.5% fat, 5.7 to 8.8% ash, 4.8 to 7.9% CF, and 50.1 to 51.9% NFE, on the DM basis. The lysine content is considerably high (6.33 to 7.20% of CP), but methionine, and probably tryptophan, are deficient (Rogers and Milner, 1963). Cassava leaves may be useful in rabbit nutrition as it compares favorably with alfalfa meal, which of course is a very popular feed for rabbits and is the largest single component of commercial rabbit feeds in the United States (Cheeke, 1987). It also compares favorably with *Aspilia africana*, which is often used as a major forage feed for rabbits in Africa. However, there are limitations of the utilization of cassava leaves and roots as animal feeding due to the content of anti-nutritional factors, such as HCN and tannins (Awoyinka *et al.*, 1995).

Techniques for reducing the level of anti-nutritional factors in Cassava have been developed through ensiling, sun-drying, boiling, and fermentation. However, the ensiling of Cassava, either with sugar palm or rice bran, has been used as the best methods because drying requires sunlight and boiling requires fuel wood (CelAgrid 2007; unpublished data). The HCN content of fresh Cassava leaves was 508 mg/kg DM (Chhay Ty and Preston, 2005) and this was reduced to 70.7 mg/kg DM when ensiling within 21 days with 5% of sugar palm syrup (1:1 of sugar and water) (Du Thanh Hang, 1998).

Khieu Borin (2005) reported that for several decades now Cassava has been given considerable attention by a number of research institutes in developing countries, in particular at the International Centre for Tropical Agriculture (CIAT). However, research has mainly been concentrated on tuber production, and less attention on evaluating Cassava for forage production. In the last few years, with financial support from SIDA/SAREC to the MEKARN (Mekong Basin Agriculture Research Network) programme, researchers in South East Asia have focused their efforts on Cassava with respect to forage production and its utilization as an animal feed.

Pok Samkol *et al.* (2007; unpublished data) reported that rabbits fed fresh Cassava foliage in combination with Water Spinach had daily weight gains of 11 g/day, while Water Spinach fed as a sole diet resulted in weight gains of 14 g/day. However, the intake of Cassava was only 23% of the total DM intake. Akinfala *et al.* (2003) reported that growth rates ranged from only 11.2 to 12.4 g/day when whole Cassava plant meal was included up to 45% of the diet. They concluded that whole Cassava plant meal can be used to replace maize in diets for weaned rabbits without any adverse effects on performance or on apparent nutrient digestibility.

Leucaena (*Leucaena leucocephala*)

Leucaena is a legume tree that originated in Mexico where its fodder value was first recognized by the Spanish conquistadors who carried Leucaena seeds on their galleons to the Philippines to feed their stock, where it is called Ipil Ipil in Pilipino. In the Philippines, Leucaena has been cultivated in alley cropping schemes to reduce soil erosion. It produces a high biomass yield of relatively high nutritive value for animal production (FIRA, 1980). The yield of Leucaena can range from 3 to 30 tons DM/ha/year and the CP in the leaf is about 22%. The level of protein depends, in part, on the quality of contents of soil, potassium and phosphorus.

Cultivation of Leucaena improves the soil fertility and aids in the control of soil erosion. It has been used as vegetables for humans and as foliage for animals. Therefore, the major

land-use system is subsistence farming. Mixed cropping is very popular with main crops such as coffee, tea, bananas, maize, wheat, potatoes, and beans. However, *Leucaena* has also been cropped in backyards as “living fences” around houses (NAS, 1977).

During seed propagation, the bed should be of sandy soil mixing with animal manures and rotten leaves or rice husks if possible. During transplanting, animal manures or rotten leaves should be used as a basal fertilizer. After planting, farmers can frequently fertilize with animal manure or effluent from the biodigesters.

Leucaena contains the anti-nutritional factor, mimosine, which causes loss of hair and poor growth and reproductive performance. It is recommended that *Leucaena* should not be used as the sole diet for rabbits. However, there is a method to reduce mimosine, which is to soak it in the water and drying.

Leucaena and *Arachis* in the form of foliage meal were used in an experiment at the level of 30 to 40% as a complement to concentrates of 70 and 60% of the basal diet (consisting of soybean meal 20, maize meal 30, wheat bran 40, sugar cane molasses 8, CaCO₃ 0.4, CaPO₄H₂O 0.8, vitamins and minerals 0.5, and NaCl 0.3%) (Nieves *et al.*, 2004). Feed intake and feeding time were increased when feeding *Leucaena* compared to *Arachis* (Table 9). It was concluded that diets containing 30 to 40% *Leucaena* meal were also more palatable than diets containing the same levels of *Arachis* meal.

Table 9: Feed behavior and intake of growing rabbits fed foliage meals in the diet (Nieves *et al.*, 2004).

	Leucaena (% inclusion)		Arachis (% inclusion)		MSE
	30	40	30	40	
Times eating, mins	5.79	7.35	2.6	3.12	1.82
Feed intake, g/day	73.9	73.2	58.1	63.2	2.80

Ruiz-Feria *et al.* (1998) conducted a rabbit experiment involving the partial replacement of concentrate with *Leucaena* leaves (10, 20, and 30%). When using *Leucaena* at the 10% level, rabbits grew faster than at the levels of 20 and 30%. They discussed that the reduction of growth rate may have resulted from the effects of mimosine contained in *Leucaena*. The recommendation was made of the feeding level of *Leucaena* in the range of the 0 to 30%, and should depend on economics in terms of realized feed cost savings in relation to growth response. Onwudike (1995) reported that *Leucaena* fed to rabbits produced reddish-brown urine and loss of hair. Kidneys of *Leucaena*-fed rabbits showed serious degenerative changes in the tubules. The livers of the *Leucaena*-fed rabbits showed a dense chronic inflammatory reaction in the portal tracts. It was recommended that *Leucaena* should not supply more than 50% of the green feed given to rabbits. Martínez *et al.* (2005) compared the hay from *Leucaena* and Mulberry that substituted diets consisting of barley grain, soybean meal, animal fat, and minerals. The results indicated that intake from the *Leucaena*-based feed was significantly higher than Mulberry at 144 g/day compared to 102 g/day, while daily weight gains were 46 g/day for *Leucaena* and 34 g/day for Mulberry. However, digestibility of CF was higher in Mulberry than in the *Leucaena* diet, and there was no significant difference between the two diets in terms of DM and CP digestibility.

Gliricidia (*Gliricidia sepium*)

Gliricidia is a legume tree that has been used for many years in Colombia and in other countries as living fences around houses. It is a tropical species that grows at altitudes from 0 to 1,500 m above sea level. It can be grown in acidic soils of low to medium fertility. It is also planted in plots involving intercropping with other crops such as Cassava and Maize.

High biomass yields of Gliricidia occur when fertilizer is applied. Farmers can apply animal manures as the basal fertilizer during plot preparation. Animal manures or effluent from biodigesters are very important to apply to plots once Gliricidia is first harvested. The chemical composition of Gliricidia is 24.3% CP, 2.12% EE, 16.0% CF, 9.50% ash, and 37.9% NFE, on the DM basis (Onwudike, 1995).

However, there are many anti-nutritional compounds in Gliricidia, and the most significant one is tannins. According to Phimpachanhvongsod (2001), Gliricidia was found to contain 40.7 g/kg DM of condensed tannins that many animals cannot tolerate when consuming large quantities. The tannins bind to proteins and decrease the nutritive value of the plant.

Onwudike (1995) studied two different foliage supplemental diets from fresh Gliricidia and Leucaena added *ad-libitum* to a pelleted feed made from yellow maize, groundnut cake, fish meal, brewer's dried grain, dicalcium phosphate, oyster shell, palm oil, salt, and a premix. Results indicated that there was a significant difference in growth rates of the rabbits when fed Gliricidia compared to Leucaena versus the feeding of pellets alone (Table 10). However, there was less feed consumed for Gliricidia than for Leucaena, which might have been due to Gliricidia being less palatable than Leucaena. Feed conversion of the rabbits was improved 3.07 when fed Gliricidia compared to 3.91 with a Leucaena diet. The study concluded that Gliricidia is suitable as a green feed for rabbits and its use helps to ensure an increase in growth rate and improved efficiency compared to concentrated pelleted feeds. This will help to reduce the cost of rabbit production in developing countries of the world where the costs of animal proteins and concentrate feeds are high.

Table 10: Growth performance of rabbits fed Gliricidia and Leucaena as a substitute to pelleted feed (Onwudike, 1995).

	Control	Gliricidia	Leucaena	MSE
Feed intake, g/day:				
Pelleted intake	58.7	65.1	52.7	2.66
Foliage intake	-	5.86	7.17	0.143
Total intake	58.7	70.96	59.87	0.705
% of foliage intake	-	8.26	11.9	-
Growth performance:				
Initial live weight, g	552	554	559	-
Final live weight, g	1582	1741	1314	19.2
Weight gains, g/day	18.4	21.2	13.8	0.342

Stylo (*Stylosanthes guianensis*)

Stylo is a short-lived (2 to 3 years), perennial legume that grows into a short shrub with some woody stems. It is adapted to a wide range of soils and climates; however, it is one of the few herbaceous legumes that grow well on infertile, acid soils. It is usually grown as a cover crop, which is cut every 2 to 3 months. It does not tolerate close cutting to the ground since there are few buds on the lower stem for re-growth. Cuts must be made higher than 25 cm to ensure good re-growth (Horne and Stür, 1999).

Stylo has the chemical composition of 19.7% DM, 19.9% CP, 13.3% CF, 9.38% ash, 1.34% EE, and 56.0% NFE (Omole *et al.*, 2007). The percentage of DM digestibility of young plant material ranged from 60 to 70%; however, when the age of the plant increased the digestibility was reduced by 40% due to lignification (Mannetje and Lones, 1992). Kryothong (2003) studied the effects of cutting height and time on DM yield of Stylo. The results indicated that when increasing the cutting height from 20 to 30 cm that there was a reduction of DM yield from 4.7 to 4.3 tons/ha.

A recent study by Hongthong Phimmasan *et al.* (2004) investigated the *ad-libitum* feeding of Stylo with graded levels of broken rice as the energy supply. Results indicated that when rabbits were offered high levels of broken rice that they consumed less quantities of Stylo. However, total intakes of DM and CP of rabbits fed graded levels of the broken rice were not different. Also, there was no significant difference in growth rate between experimental and control groups (the latter being rabbits fed only Stylo), which ranged from only 5.61 to 6.67 g/day. Rabbits fed different forages from Stylo, Lablab and Sunflower leaves as sole diets were studied by Omole *et al.* (2007). Rabbits fed Stylo had higher feed intake than either Lablab or Sunflowers, and had more rapid daily weight gains (Table 11). Also, there were no reported deaths observed from the feeding of these forages.

Table 11: Growth performance and health of rabbits fed different source of foliage (Omole *et al.*, 2007).

	Stylo	Lablab purpureus	Sunflowers	MSE
Initial weight, g	516	515	519	-
Final weight, g	1232	1119	1124	15.3
Live weight gain, g/day	7.95	6.71	6.72	0.16
Feed intake, g/day	39.2	35.6	35.8	0.35
Feed conversion ratio	4.93	5.31	5.32	0.21
Survival rate, %	100	100	100	-

RABBITS FOR FOOD SECURITY AND INCOME

Many of the developing regions of the world are now facing a double burden of a growing population and malnutrition (Weingartner, 2005). According to the World Health Organization (WHO), the estimated number of cases of diabetes in developing countries is likely to increase more than two-fold in the next 30 years from 115 million in 2000 to 284 million in 2030 (WHO, 2003). In most developing countries in Asia, and even in food surplus countries such as Thailand, malnutrition still exists

(Valyasevi and Winichagoon, 1992), especially in rural areas. It was indicated that most animals and animal products produced on rural farms are usually sold for cash by farmers and therefore flowed out of the communities. It is suggested that malnutrition has remained a basic cause of poor health for rural people. Children, pregnant and post-partum mothers, and sick children and adults can get infected more easily due to their low resistance to infected diseases, which often causes their absence from school or work. Malnutrition results in ignorance and poverty among rural people (Chantalakana and Skunmun, 2002).

Animal products have contributed from 3 to 45% of total food calories for humans in the Asia-Pacific region in 1999 with the lowest (3.0%) in Bangladesh and highest in Mongolia (45%), while Australia, Japan and New Zealand also consume high quantities of animal products, accounting for 20 to 33% of food calories from animal products. Livestock products, such as meat, milk and eggs, are not only foods used to overcome hunger but are critical to develop healthy minds and bodies. Without the availability of animals that largely use agricultural by-products, and forages and vegetative plants, there would certainly be less total food production (Anonymous, 2001). One clear advantage of rabbits is their ability to directly consume forage and convert proteins into animal protein (Lukefahr, 1992), which is appropriate in traditional agriculture systems in Asia that largely maintain mixed crop and livestock farming systems where the economic viability of animal products is realized by the small-holder farmers (Devendra and Chantalakhana, 2002).

Rosegrant *et al.* (1995) projected the demand per capita of animal products in Asia as shown in Table 12. In East Asia (including Japan), between 1990 and 2020, the rates of increase for demand were more than double for beef, pork, and poultry meat and were approximately double for eggs. The projection based on the population growth in Asia that would reach to 4,689 million people in 2020, which will account for almost 58.2% of the total population in the world (Nygaard, 1994). Despite trends that the population is becoming more and more urbanized with time, the actual number of people in rural areas of LDC's is still increasing, and they depend directly or indirectly on agriculture for their survival. According to Chantalakhana and Skunmun (2002), much of the world's children born today can expect to live longer and be better educated than their parents, and there is no doubt that much of the improvement will come from the increase of consuming more animal food products (e.g., meat, milk and eggs).

Table 12: Projected per capita demand for livestock products in Asia (kg/year) (Rosegrant *et al.*, 1995).

Livestock products	South Asia			Southeast Asia			East Asia (including Japan)		
	1990	2010	2020	1990	2010	2020	1990	2010	2020
Beef	1.2	1.4	1.5	2.5	4.5	6.0	1.3	2.3	3.1
Pork	0.3	0.4	0.4	5.5	8.6	10.5	18.8	30.6	38.2
Sheep meat	1.0	1.1	1.2	0.3	0.5	0.7	1.0	1.0	1.2
Poultry meat	0.5	0.6	0.7	4.2	6.9	8.5	3.0	5.2	6.5
Eggs	1.3	1.6	1.8	3.3	5.5	7.0	6.8	10.9	13.6
Milk	63.4	84.9	95.3	3.2	3.8	3.5	7.7	9.3	10.2

The forecast for ruminant and non-ruminant meat production and the demand per person in 2010 for selected Asian countries were presented by Vercoe *et al.* (1997) (Table 13). It is projected that there will be a deficit of ruminant meat production in every country, and a deficit of non-ruminant meat production in most countries. According to Devendra (2001) the demands for animal products will be more than double in the next two decades which is the meat and eggs from non-ruminant animals in industrial systems will continue to be the main source of animal proteins, and it is unlikely that these systems will meet all of the projected demands. However, the consumption of ruminant meat and milk is increasing in most of Asia, and 95% of ruminants are found in the mixed farming systems. Mixed farming systems will continue to be the main avenue for intensification of food production, with some specialization in crop or animal activities. This is clear evidence that countries in Asia need more animal products to be produced either through the expansion of production or an increase in productivity.

Table 13: Forecast of ruminant and non-ruminant production and demand (kg capita) in the year 2010 in Asia (Vercoe *et al.*, 1997).

Countries	Ruminant meat demand	Ruminant meat production	Non-ruminant meat demand	Non-ruminant meat production
Bangladesh	3.0-3.4	1.7	1.0-1.2	0.8
Cambodia	5.2-5.9	3.3	14.2-16.3	9.1
China	5.8-7.4	8.5	54.5-71.3	49.8
India	5.1-6.2	3.8	1.5-1.9	26.2
Indonesia	4.6-6.0	2.5	12.3-16.7	26.2
Laos	4.8-5.4	3.0	12.4-14.2	7.8
Malaysia	6.3-7.9	0.6	73.7-93.9	139.6
Pakistan	16.9-20.3	14.6	19.8-24.5	9.8
Philippines	4.4-4.9	2.7	27.8-31.7	29.8
Sri Lanka	2.7-3.3	1.2	3.4-4.3	3.8
Thailand	10.1-14.8	6.3	35.5-53.5	42.3
Vietnam	10.1-14.8	3.0	21.5-25.2	18.9

Lebas and Colin (1992) calculated that the world production of rabbit meat is of the order of 1.5 million tons. This would mean a per capita annual consumption of roughly 280 g of rabbit meat; however, most inhabitants in many countries do not consume rabbit meat as compared to the consumption of 2.5-3 kg/year in France and 4-4.5 kg/year per capita in Italy. Europe is indeed the centre of world rabbit production. The major world producers far surpass all other countries, which include Italy, the Commonwealth of Independent States countries (particularly Russia and the Ukraine), France, China and Spain. Europe, collectively accounts for 75% of total world production. China ranks second, which specifically involves the central Chinese provinces, such as Sichuan and Szechuan. Less major production areas are found in some regions of Africa, Central America, and Southeast Asia, particularly Indonesia. Colin and Lebas (1996) indicated that countries such as Indonesia, the Philippines, Thailand, and Vietnam account for 87% of the region's total doe population, and

Brunei has the largest number of breeding does per 1,000 inhabitants. Vietnam led other countries in the total value of rabbit meat produced per 1,000 USD of the country's total gross national product. Rabbits are not reared in significant numbers in most countries of the Near East.

According to Lukefahr and Cheeke (1991), whose report involved a survey of literature reports from lesser developed countries and provided a summary of production parameters, stated that a breeding doe could produce 20 marketable offspring from 4 litters/year and ten does could yield 200 fryers annually. This first set of figures assumes a forage-based diet and using supplements produced from the farm. The inclusion of purchased concentrated feeds could increase doe production to 6 litters per year. Depending on the family size and its age and body weight composition, 2 to 5 fryers could be consumed weekly, and the rest sold for income. As a guide, a live fryer weighing an average of 2.5 kg with a 60% carcass yield should produce about 1.0 kg of edible meat of which there should be approx. 200 g of protein. As estimated by Lukefahr (2007) a small farm family that raises 10 breeding does and consumes only 2 fryers a week, would yield 86 fryers at a final weight of 2.5 kg, which could generate additional income of US\$ 262 with the market price of US\$ 1.22/kg. This production level could contribute a 19.8% increase in the average income of farmers in Indonesia, while in Cambodia, Laos, and Vietnam this same figure could represent an increase of approx. 87.3% of income for the family (CelAgrid and ILRI, 2007; unpublished data; Taucher, 2000). In general backyard rabbit farming with the size of 4 to 5 breeding does can produce meat with low investment and operating costs. It takes little time or money to either down-scale or expand the size of the operation. Moreover, the labor input can be shared among the family members. These features associate the rabbit enterprise with minimum economic risk (Lukefahr, 1992). Lukefahr (2007) emphasized that opportunities for expansion should carefully consider the market demand. Also, farmers should certainly avoid flooding the market with rabbits. In addition, rabbit meat should be competitive with other meats by setting the price lower than that of broiler chickens.

CONCLUSIONS

- This paper has presented the topic of the role of organic rabbit production for improving the income and food security of poor families in the case of risk aversion from crop failures and natural disasters, and also for farmers who can re-invest funds to expand their enterprises to eventually alleviate poverty.
- Development of locally grown feeds has a high priority and it is important that the alternative feed resources can be cultivated on local farms to directly benefit poorer farmers who do not have cash resources to purchase supplements from outside the farm.
- Forages such as Cassava, Mulberry, Leucaena, Gliricidia, and vegetables such as Sweet Potato vines, together with water plants such as Water Spinach, and Stylo can be used successfully in diets for rabbits to replace or in combination with a protein source from conventional feed ingredients, including soybean and fish meals.

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STATUS AND PROMOTIONAL STRATEGIES FOR RABBIT PRODUCTION IN NIGERIA

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Status and promotional strategies of rabbit production in Nigeria were examined in this study. This was because such data were lacking and rabbit production offers a great potential for the attainment of food security in terms of provision of high quality animal protein intake. Data were obtained from field survey, questionnaires administration, personal observations and experiences of authors, structured personal interviews of identified rabbit farmers, agents, personal interviews and visitations to markets and retail outlets for rabbit, proxy information from distant collaborators, recorded data from government establishments and published data. All the information collected was synthesized to provide an informative blend on major aspects of rabbit production patterns in Nigeria. From the data collected, rabbit production in Nigeria is largely traditional, non-commercially oriented, family consumption targeted, and smallholder type operation comprising 2-7 does and 3 bucks. About 3.4-5.2% of the Nigeria population may be keeping rabbits with women and children being mostly involved. Rabbit keeping is both intensive and semi-intensive, though some scattered free range backyard rearing was recorded. Unlike the rural rabbit keepers, space is limiting for most urban rabbit keepers. Diets of rabbits in Nigeria are primarily forages, grasses and legumes, kitchen wastes, while feeding pelleted commercial feeds is rare and, when fed, the commercial feeds are mostly broiler poultry mash. Although feed millers do advertise their capabilities in production and sale of rabbit pellets, production is largely for specialized research based institutions. Bucks and does are generally under-utilized, with does producing up to 20 weaned rabbits per year, and are usually offered for sale after four parities. Exchanges of males for breeding was observed among some traditional rabbit keepers. In fact, advertisements are made for availability of rented breeding bucks for a paltry sum. Nigerian rabbits do not suffer any peculiar disease; however, mange and coccidia infections are very common. There are high peri- and post-natal mortalities, and overall mortality between birth and marketing was estimated at 30-40%, being highest in the young rabbits. The productive and reproductive performances of rabbits in Nigeria relate with the level of management. However, there is a latent and growing market for rabbit meat in Nigeria especially as a compliment and/or an alternative to broiler meat. Some promotional strategies and policy suggestions for sustainable expansion of rabbit production were highlighted in this paper.

CHARACTERIZATION OF TRADITIONAL RABBIT BREEDING SYSTEM USED IN THE SOUTH OF TUNISIA

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This study consisted of an inventory of rabbit genetic resources at the governorates of the western South Tunisian. An inquiry was led in the regions of Tozeur and Gafsa concerning the various types of animals and their management systems.

A total of 199 rabbit farmers were touched by the inquiry. The survey conducted at each farm was performed by a questionnaire on animals and their behavior and an illustration with taking photos of the various animal phenotypes and the raising sites.

The extensive rabbit raising system with weak input and productivity is the most common system encountered in the region, while the semi-intensive system is practiced by only 5 farmers in the delegation of Dguech and 8 rabbit raisers in the region of Gafsa.

ECONOMIC SUSTAINABILITY OF RABBIT FARMING INNOVATIONS

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Although multiple definitions have been proposed for the concept of animal welfare, the interpretation offered by Webster is of particular interest for the purposes of this study. The author, despite basing himself on the definition proposed by the UK Farm Animal Welfare Council (FAWC), lists five determining factors of animal welfare, including the prevention of the animal's physical and mental exhaustion induced by intensive productive and reproductive activities. With specific reference to rabbit farming, it is necessary to underline that research predominantly focuses on evaluating the impact which technical innovations - in terms of animal population density, cage sizing and the improvement of environmental conditions - have on animal welfare, and is underpinned by a predominantly anthropocentric viewpoint. A limited number of studies focus on evaluating the impact of organisational innovations which are aimed at protecting and respecting the physiology of the animals. The purpose of this study is to evaluate the economic sustainability of adopting a less intensive insemination rhythm with a view to safeguarding the welfare of breeding does. The findings of this study reveal that the innovation in question is able to guarantee positive financial returns for the business, as well as a substantial reduction in the risks associated with the production activity.

POSSIBILITY OF USING THE NATIVE BREED OF POPIELNO WHITE RABBITS FOR MEAT PRODUCTION

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Popielno White rabbits are a native breed created from the no longer existing Polish Albino breed. Research on the creation of the breed began in 1950. In 1989, Popielno White rabbits were included in the farm animal genetic resources conservation programme, financed by the Ministry of Agriculture and Rural Development. The aim of the study was to determine the possibility of using the native breed of rabbits for the production of carcasses of high culinary value. Twenty female and 4 male Popielno White and New Zealand White rabbits were studied. Females of the foundation stock were kept on litter in pens in a closed facility. Reproductive and growth performances and carcass parameters of Popielno White and New Zealand White rabbits were evaluated. The slaughter was carried out when rabbits were 90 days of age old. The study showed the high prolificacy of Popielno White rabbits. Growth parameters were lower in Popielno White than in New Zealand White rabbits, but they were compensated by better reproductive performance. The most valuable cuts (loin, hind part) of Popielno White rabbits were comparable in size with those of New Zealand White, which is a typically meat breed. Overall sensory quality also confirmed the culinary value of meat from Popielno White rabbits.

CONSUMERS' OPINION ABOUT RABBIT MEAT CONSUMPTION IN HUNGARY

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The aim of the study was to get information about the rabbit meat consumption habits of Hungarian consumers. Data were collected by questionnaires (n=1274). The official rabbit meat consumption data (0,1 kg/person/year) is calculated by the difference of the carcass weight of the purchased and slaughtered rabbits and the weight of the exported rabbit meat. However, most of the decreasing number of small and medium scale rabbit farmers, rabbit breeders has a regular self-consumption, and they sell living animals and carcasses to friends, neighbours and also to local markets. Differences were found between the answers of the asked sample population by living in Budapest (n=543) or in the countryside (n=731). In this study countryside means all regions of Hungary except Budapest. A lot of people can not make difference between rabbit and hare, so our first task was to make clear the difference between the two species. 75% of the asked population in Budapest has already tasted rabbit meat, but 70% of them eat it only one or twice a year. Those people who have a negative attitude towards rabbit meat are vegetarian or refuse consumption due to emotional reasons. One third of the people buys living animals or the whole carcasses (8% and 26%) and 46% is looking for different parts of the rabbit. 66% of the consumers (almost all from the centre of the city) would like to buy semi-finished or ready-made products. The culture of rabbit meat cooking is very poor, only 14% of the people could list more than 2 rabbit recipes. The housewives of the suburban area prefer the rabbit carcass from the farmer or from small butcher shops instead of the supermarkets. Most of them do not trust in the quality and the freshness of the meat in the supermarkets, and they wouldn't like to pay much more for the carcass than the price of the poultry meat. More people have a negative attitude towards rabbit meat in the countryside than in Budapest. Most of them have never tasted it. However the most frequent problem is the lack of rabbit meat and rabbit products in the supermarkets in the country, so the urban citizens who like rabbit meat can not buy it in their preferred shops. The culture of processing and cooking domestic rabbit meat is very poor in the Hungarian cuisine. Only 7% of the people could list more than 2 rabbit recipes. Several possibilities were found to improve the rabbit meat consumption. The marketing can be ineffective only in the vegetarian group.

EFFECT OF REPRODUCTIVE RHYTHM AND FRESH CHICORY (*CICHORIUM INTYIBUM L.*) INCLUSION IN THE PRODUCTIVE PERFORMANCE OF RABBIT DOES

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The effect of two reproductive rhythms (11 or 18 d *post-partum*) and dietary inclusion (LCh) or not (L) of fresh chicory (*Cichorium intyibum*) on productive performance of rabbit does was assessed. Four groups of 20 multiparous selected New Zealand x California does were submitted to the following diets and reproductive rhythms: L11: commercial lactating diet, 11 days *post partum*; L18: commercial lactating diet, 18 days *post partum*; LCh11: commercial lactating diet+chicory, 11 days *post partum*; LCh18: commercial lactating diet+chicory, 18 days *post partum*. Females were inseminated for four consecutive cycles. Within each experimental group, the number of suckling kits was adjusted to 7-8 per litter. Kits were nursed once a day and were weaned at 32 days. Fertility rate, number of kits born alive, pre-weaning mortality, litter size at weaning, weight of kits at 21 days and weight of rabbits at weaning were recorded during the four cycles. Data were analysed according to a randomised complete design using PROC GLM; the model evaluated the fixed effects of reproductive rhythm, diet and their interaction. Means were compared by Tukey test ($P<0.05$). The presence of statistical interaction between reproductive rhythm and diet was high. Chicory inclusion significantly increased the fertility rate, the number of kits born alive and kit weight at 21 days in the 11 d *post partum* groups but did not influence these data in the 18 d *post partum* groups. At weaning, the total weight of rabbits of four reproductive cycles was positively influenced by chicory inclusion (37.5 kg vs. 30.1 kg for LCh and L respectively; $P<0.01$) and also by the reproductive rhythm, even if less significantly, (36.2 vs. 31.5 kg for 18 d and 11 d respectively; $P<0.05$). In conclusion, chicory inclusion improved the productive performance of does submitted to the standard 11-d reproductive rhythm; for the semi-intensive 18-d group, chicory improved only the weight of rabbits at weaning. L11 does showed the poorest results making evident the progressive reduction of body reserve due to the partial overlapping of pregnancy and lactation.

EFFECTS OF SEASON, GENERATION, NUMBER OF MATING, PARITY AND DOE NUMBER OF TEAT ON DOE AND LITTER BIRTH CHARACTERISTICS IN DOMESTIC RABBIT

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Records of 866 kits born in 203 litters over a period of 132 weeks were used to evaluate the effects of season of birth (raining, dry) on litter birth characteristics. Other factors investigated included the effects of generation of birth (1, 2), number of mating (1, 2), parity (1, 2, 3, 4) and doe number of teats (8, 9, 10). Other traits investigated included gestation gain (GG), kindling loss (KL), litter size at birth (LSB), neonatal mortality (NEOM), live birth percentage (LB), mortality per litter (ML), litter weight (LWT) and mean kit weight (MKW). Gestation gain was taken as the difference between doe weight at mating and the weight taken one day prepartum. Kindling loss was taken as the difference in doe weight one day prepartum and its weight immediately after kindling. Litter weights divided by number of individuals for each litter gave the mean kit birth weight, while the gestation length was obtained as the difference between the dates of successful mating and kindling. Out of the 866 kits produced in two generations, 371 were born in the rainy season (April to September) and 495 in the dry season (October to March). Season of birth did not significantly ($P>0.05$) influence litter size at birth, live birth percentage and mean kit birth weight, although the percent survival was slightly higher during the dry season. The percentage survival at birth for the first four parities were 4.07 ± 1.63 , 4.04 ± 1.29 , 3.96 ± 1.10 and 3.56 ± 1.46 , respectively; corresponding values for gestation length were 31.6 ± 2.7 , 31.6 ± 1.8 , 31.9 ± 1.6 and 32.2 ± 1.7 , respectively. Litter size at birth and mean kit birth weight for single mating were 3.95 ± 1.36 and 38.95 ± 7.87 , respectively. Corresponding values for double mating were 3.82 ± 1.45 and 39.14 ± 10.22 , respectively. Litter size at birth and mean kit birth weight for does with 8, 9 and 10 teats were 4.12 and 41.73 g, 4.11 and 42.31 g and 4.48 and 41.01 g, respectively. Second generation kits were born in lower litter size and litter weight, they had lower mean kit weight and live birth per litter. Parity, number of mating and doe number of teats had no significant effect ($P>0.05$) on the investigated bio-economic traits. The present results showed that selection of replacement does may not be done with accuracy using external characteristics like doe number of teats. It may be possible to undertake an all year round breeding in commercial rabbitry under tropical condition. The rainy season however remained the best season that favours farmer productivity, especially under forage based backyard rabbitry.

EFFECTS OF WEANING LITTER SIZE AND SEX ON POSTWEANING BODYWEIGHT, MORTALITY AND CARCASS CHARACTERISTICS OF DOMESTIC RABBIT IN THE HUMID TROPICS

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Records of 101, mixed-breed, four weeks-old rabbit kits were used to assess the effects of weaning litter size and sex on post-weaning bodyweight, mortality and carcass characteristics under a tropical humid climate with rainy and dry season temperature and relative humidity of 30.4°C and 81.8% and 34.8°C and 61.1%, respectively. Kits for postweaning bodyweight assessment were fed according to their age (59 g to 129 g per day) on diets containing 46.59% rice bran, 25.00% palm kernel cake, 15.50% rice offal, 1.50% oyster shell, 0.55% bone meal and 0.50% salt. About 140 ml of water were supplied daily to each kit. A total of 22 rabbits (11 males and 11 females) were used to evaluate carcass characteristics. The investigated carcass characteristics were shrunk-weight (SW), bled-weight (BW), dress carcass weight (DCW), dressing percentage (%), carcass length (CL), gastrointestinal tract (%GIT), pelt (%P), external offal (%EXO), edible offal (%EDO), adipose fat (%AF), meat in cut (%MIC) and primal cuts (shoulder-rib%, rack%, loin% and leg%). Mean kit bodyweights at weaning (4 weeks) and at 16 weeks of age were 227 g and 862 g, respectively. Mean bodyweights and age of male and female rabbits used for the carcass evaluation were 1373±247 g and 1182±191 g, and 192±49 days and 166±25 days, respectively. Mortality was higher than 26% in all the litter size groups. The main cuts (as percentages of shrunkweight for female rabbit) were 12.6%, 5.2%, 10.2% and 19.1%, for the shoulder-rib, rack, loin and leg cuts, respectively. Corresponding values for the males were 14.6%, 6.0%, 10.0% and 20.5%, respectively. Post-weaning bodyweight of rabbit kits was significantly ($P < 0.05$) influenced by the weaning litter size. In general term, post-weaning bodyweight decreased with increasing weaning litter size. Sex effect was not significant ($P > 0.05$) for post-weaning bodyweight and carcass characteristics. The present work suggests that the consideration of sex of kits to be used as fryers may only be necessary in rabbitries experiencing very high postweaning loss. Kits weaned in lower litters may need to high quality diets to ameliorate the adverse effect of weaning litter size on post-weaning bodyweight of fryer rabbit. High mortality calls for improved nutrition, and other management practices like the use of prophylactic medicants such as vaccines, longer nursing period, gradual change from milk to mash feeds and the use of therapeutics like antibiotics and coccidiostat, in addition to the exploitation of crossbred livability.

MEAT PRODUCTION OF RABBIT: AN ALTERNATIVE FOR THE SEMIDESERT QUERETANO AND PEOPLE IN EXTREME POVERTY

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The municipalities of Tolimán and Ezequiel Montes (state of Querétaro, Mexico) are located in the so-called semi-desert queretano, a region characterized by limited natural resources, high marginalization, discrimination and child malnutrition. The rabbit breeding in 10 villages in the municipalities of the semi-desert queretano under a system semi-technical and infrastructure paperback has been an option for 51 Indigenous women, the elderly (vulnerable population) and their families to have access to better food, besides obtaining revenue from the sale of the rabbit meat and crafts produced with rabbit skin. The initial aim of this work was to produce meat for personal consumption and to realize that with a good handling within RPU (Rural Production Units). Production exceeded what expected, the mentality changed and was directed towards increasing the production of meat for sale to the public. This was accomplished through the interaction with a technical training, advising, coordinating and managing resources support (grants) from government institutions through programs aimed at improving the quality of life of rural people and the most vulnerable poor people, women and the elderly. There are five groups and each group has been integrated into a cooperative that, in turn, has become a rabbit breeders Association. Each producer works individually on his RPU, teamwork is for training, processing, marketing, procurement of inputs, resource management and promotion of their products. Fifty one producers and their families now eat 308 g per person per week of meat in addition to an average weekly income of € 13.37 per family coming from the sale of rabbit meat and crafts made with rabbit skin.

ANALYSIS OF THE EFFICIENCY AND THE REPRODUCTIVE SEASONALITY OF AN ALTERNATIVE RABBIT KEEPING SYSTEM

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With the aim of analysing the reproductive seasonality of an alternative rabbit keeping system, the reproductive and productive parameters were checked for a whole year (from the spring of 2006 to the winter of 2007 included) in a farm located in the Viterbo province (Italy). Fifty-four does and 9 bucks belonging to the Leprino di Viterbo breed were kept in an alternative underground cells outdoors system. Animals were managed by natural mating 10 days after parturition according to a two-weeks cycle. Prophylaxis was done by stamping out any suspected animal. Does gave an average of 7.3 births per year, producing 56.7 total kits born, 52.1 kits born alive, 42.0 weaned kits, and 40.7 fattened kits per year. Fecundity was 80.3% and fertility was 67.6%. Observed differences according to season were not significant. Average fertility was slightly lower than in the Italian industrial rabbit farming. Total kits born (7.7), kits born alive (7.1), kits born dead (0.6) per litter, and perinatal mortality (9.0%) did not show differences between seasons. Mortality during lactation (19.8%) and the number of weaned kits per litter (5.7) showed variability throughout the seasons, being the mortality during lactation higher ($P < 0.001$) and the number of weaned kits lower ($P < 0.05$) in summer. Mortality during the fattening period was lower than in industrial farming. The analysis of the management of this alternative farm that raises the Leprino di Viterbo breed under an open air system without pharmacological treatments highlights that this alternative system can produce about 100 kg of high quality meat per doe and year. Rabbits are sold at the price of € 3.13/kg live weight offering a good income to small farmers that raise rabbits to integrate their income with a part-time work. Furthermore, this alternative system makes it possible to reduce the productive seasonality due to the fact that underground cells fit well with bioclimatic and ethological needs of the rabbit.

EFFECT OF REMATING INTERVAL, WEANING AGE AND PARITY ON RABBIT DOE PERFORMANCE UNDER HEAT STRESS

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Forty three cross-breed New Zealand, California, Butterfly, Dutch and Satin rabbit does were first mated with an average weight of 3.58 ± 0.44 kg. Once confirmed pregnancy, rabbit does were randomly assigned to two treatments: in the treatment A the does were mated 14 d after parturition and litters were weaned at 35 d of age; in the treatment B the does were mated 21 d after parturition and litters were weaned at 42 d of age. A commercial pelleted diet was offered *ad libitum*. The following measurements were recorded for six months (the first three parities): eliminated does, doe weight at birth and at weaning, doe feed intake, number of mating per doe to get pregnant, number of kits and kit mortality, litter weight and litter feed intake, feed efficiency. Inside the farm the temperature-humidity index during the sun light period ranged from 28 to 32, which can be considered as moderate/severe heat stress. In the rabbit does of treatment B the number of kits at weaning decreased by 36% ($P=0.026$) in comparison to does of treatment A and the parturition interval increased (+2.6 d with respect the expected interval). Feed efficiency tended to be lower by 25% ($P=0.093$) in the B group. No effect of treatments was detected on total eliminated does (7.55% on average), weight of rabbit does at birth (3.66 kg) and at weaning (3.79 kg), feed intake ($72.0 \text{ g/kg BW}^{0.75}/\text{d}$), fertility (91.7%) and the number of kits born alive (5.80). Parturition interval tended to increase between the third and the fourth parity ($P=0.073$). Kit mortality at birth was high (15.9%), and tended to be lower at the first parturition compared the following two ones ($P=0.12$). Kit mortality during lactation increased with parity number ($P=0.029$). Most of mortality of suckling rabbits (95%) occurred during the first 21 days of lactation. Accordingly, feed efficiency impaired with parity number ($P=0.014$). Parity number did not affect total eliminated does, weight of rabbit doe at birth or at weaning, feed intake, fertility and number of kits born alive. These results show that rabbit doe productivity impairs when lactation is extended and along successive reproductive cycles.

ENTERPRISE ORGANIZATION AND CAPITAL REQUIREMENTS IN HUNGARY

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This study focuses on enterprise organization and capital requirement of two basically different animal enterprises, namely dairy cow and rabbit enterprises. Dairy cow enterprise plays an important role in the national agriculture in Hungary, while rabbit enterprise has a tiny significance, but at the same time it has dominantly good export possibilities. It was supposed that investigation of enterprise organization of traditional dairy cow sector ensures to draw some relevant conclusion for rabbit enterprise. Agricultural resources altogether affect on farming and their roles in income-generating process is also equally important. According to our analysis, the capital structure of the examined enterprises has some differences. Fodder production area of dairy cow enterprise has greater importance than that of rabbit enterprise. Rabbit enterprise may be based on even exclusively purchased fodder. However, four of the investigated dairy farms do not have any fodder production area. The situation of these farms is the most critical from this aspect. Livestock and fodder require more than half of the capital in the dairy sector, while it is lower than ten percent in the rabbit sector. Marketing of livestock can be one of the solutions for solvency problems in both enterprises in order to have possibilities for paying short term loans from the sales. At the same time rabbits can be sold more easily than cows. The structure of rabbit enterprise has significantly changed recently caused by the reducing number of small-scale farms. The consequences of liquidity of enterprises differ from an important economic aspect. Non-marketable and unused assets have some fixed costs even if they are not utilized, so profitability declines further. In order to increase the capital effectiveness, one of the possibilities is to create producers groups. Common technical investments, which are sponsored by the national government and European Union, may reduce depreciation costs. On the basis of our calculations, same capital supply results more than twice effectiveness in the rabbit enterprise than in the dairy one.

COMMERCIALIZATION OF RABBIT'S MEAT IN TIZI-OUZOU AREA, ALGERIA

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This paper provides an overview of the rabbit meat marketing system in the Tizi-Ouzou area. To meet this objective the descriptive survey method was employed, using a structured questionnaire to provide data on commercialization of rabbit meat. Butcheries (n=192 representing 67.3% of the total butchereries), poulterers (n=49 representing 30.6% of the total poulterers), restaurants (n=184 representing 43.4% of the total ones) and hotels (n=11 representing 25.5% of the total ones) were concerned. Rabbit meat is sold in 8.48% (n=37) of stores visited and do not sold in 91.51% (n=399) ones. It's marketed more cooked (restaurants and hotels) than fresh (butchereries and poulterers). 75.44% of respondents who do not sell rabbit meat justifies it by the lack of demand and 10.78% (n=43) because of its unavailability. Rabbits are bought from producers generally as whole carcasses (86.49%) of about 1.4 kg for an average price of 312.16 DA per kg (1U\$=85 DA). In the butchereries and poulterers, rabbit meat is sold to consumers at an average price of 470 DA. 44.56 kg of rabbit meat are sold per week. Although the local demand continues to increase, the marketing for rabbit meat in Tizi-Ouzou stays neglected. The marketing is fragmented, inefficient so the distribution chain of this meat is disorganized. This inefficient marketing system has resulted in higher costs and low availability of rabbit meat with a possibility for expansion through appropriate promotion. Despite those problems, there are also opportunities for expansion of this market. Given the current low levels of consumption of rabbit meat, there is a potential for total consumption of this meat to increase substantially as production and availability increases. The ultimate objective is to educate the public about the high nutritional quality of rabbit meat. More attention must be given to the market outlets and promotions of rabbit meat in Tizi-Ouzou area.

**SURVEY REPORT FOR PRESENT SITUATION OF TECHNOLOGY
SYSTEM OF CHINA RABBIT INDUSTRY NATIONAL SURVEY GROUP
FOR TECHNOLOGY SYSTEM OF RABBIT INDUSTRY**

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Through the survey of China rabbit industry in main rabbit production provinces/cities, we know the basic situation of rabbit production, development status of rabbit products, organization level of rabbit industry, technologies research, and existing main questions in China, which will provide the scientific basis for building a modern rabbit industrial system for decision-maker.

RABBIT PRODUCERS ORGANIZATION IN THE STATE OF MEXICO

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Rabbit producers in Mexico have been characterized by developing their activity in an individual and disintegrated attitude which have maintained them anonymous and marginated from promotion state policies. In spite of this, they have accomplished a great demand for rabbit meat and to make the most of business opportunity it has been necessary to overcome inefficiencies. Thus, it is essential to promote the integration of rabbit producer organizations, in accordance to the law and taking into consideration that a renewed association would be useful for social integration, to motivate new projects adapted to each region's, sector's, organization's and people's reality. This study aimed to motivate rabbit producer organization and integration in the productive chain, to promote inclusive, creative and proactive participation, to contribute in enterprise development for the organized people well being, timely attending demands and expectations of final consumers and to consolidate state rabbit production as a social and economically important activity. An ideal, appropriate or proven method was not selected, we have turned to experience and knowledge of the social, cultural and economical surroundings of Mexico rabbit producer characteristics, demanding a gradual fulfillment of: association, social invention, social learning, social compromise, strategic participation planning and participation research, having as a result regional, state and national work meetings where producers, professionals, students, public staff, goods and services people have met. These meetings have cleared out that rabbit production in the State of Mexico and in the country contributes with its richness to society that demands an everyday employment, food and health as inalienable human rights. Rabbit producers from Toluca Valley volunteered and declared their interest in associating, promoting thirteen local associations and two regional unions. With these two unions, in August 31st, 2007 the Rabbit Producers Federation was legally integrated. The development plan considers: a) promotion of municipal, regional and state organizations with values, mission and vision declaration, b) generation of basic statistics, c) integration of system-rabbit product state committee, d) training and professionalization for human capital development with enterprise point of view and e) the incorporation as official promotion program to invest in infrastructure and social capital development. It is concluded that at the end of the first integration period of rabbit producers more leaders should integrate from other important municipalities of the State of Mexico, so they can build a free, autonomous, inclusive, participant, cooperative and competitive organization to take advantage of the real market opportunities and biggest potential in the country.

SOCIAL AND ECONOMIC CONTRIBUTION OF RABBIT PRODUCTION IN THE STATE OF MEXICO

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The objective of this study was to find out the contribution of rabbit production in the State of Mexico, located in central Mexico at 500 to 5000 meters over sea level and average temperature of 15°C. Direct interviews were made to obtain data in 517 rabbit production units located in 36 municipalities in which a non probabilistic intentional sampling was used. Data were analyzed using the Statistical Package for the Social Sciences (SPSS), and referred to as frequencies and percentages. In the social variables the following indicators were obtained: 75.4% were men producers and only 24.6% were women, 23.8% were young producers (18 to 30 years of age), 57.6% were adults (31 to 60 years of age) and 18.6% were mature adults (over 61 years of age). Average age of producers was 47.1 years; 77.5% were married and 22.5% were single, 78.8% studied up to secondary school and 21.2% studied up to preparatory school or career. In the economic variables, 94.6% of the people used rabbit production as complement to their primary occupation and in only 5.4% of the cases rabbit production represents in between 51 to 100% of their income. 90.1% of the production units were financed using own resources. 64.2% of the farms had walls and ceiling, 2.5% had rabbits on the floor, 88.2% used cages and only in 26.1% of cases productive registers were used. 28% of the farms were whole cycle and 98.3% had 21 reproductive females. Predominant breeds were: New Zealand, California and Flandes Giant. Regarding health, 64.2% referred mange, respiratory and digestive infection as common problems; 86.3% of the producers had no biosecurity programs and 85.5% slaughtered the rabbits in the same place with no knowledge of sanitary regulations. 6.5% of the producers used their production for family purposes, 48.4% sold bunnies and 45% rabbits in carcass; commercialization was local with no marketing strategies. There are 10,933 reproductive female rabbits, with potential to generate 70 direct employments and producing 12,026 kg in carcass/week with a retail price of 5.5 dollars. 89.6% of the producers were not organized and 84.5% had no technical assistance. It is concluded that rabbit production in the State of Mexico is a low scale activity that produces innocuous and nutritive meat for family and local market, promoting direct working opportunities and economic income even though there are no official programs to promote and support experience and vocation of participants as valuable strengths and opportunities that the physical, demographic and commercial environment offers for this activity in the State of Mexico.

INFLUENCE OF TEMPERATURE-HUMIDITY INTERACTION ON HEAT AND MOISTURE PRODUCTION IN RABBIT

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The heat and moisture production rates of mature rabbits were studied under different building environmental conditions in a tropical area of Ile-Ife, Southwestern Nigeria. The conditions considered were those of buildings having inlet openings of 30 and 50% of the sides and orientations of 45 and 90 degrees to the prevailing wind. Sixteen rabbits fed *ad libitum* a commercial pelleted feed were used for the experiment which was conducted for thirteen weeks. The indoor temperature and relative humidity of the pens were recorded twice daily throughout the period of experiment. The heat and moisture production of the rabbits in the pens were then calculated. The results obtained showed that the heat and moisture production of the rabbits are affected by temperature and relative humidity. The heat and moisture production of the rabbits increased with increase of the temperature-humidity index (THI). Rabbits in buildings with higher thermal comfort level (lower THI of 32.49°C obtained for 50% opening/90° orientation pen) produced less amount of heat (21.16 W/hr/animal) and moisture (16.5 gm/hr/animal) than those in the more thermally stressful enclosures THI of 33.82°C (50%/45°) with 25.37 W/hr/animal, 20.40 gm/hr/animal; THI of 34.21°C (30%/90°) with 26.18 W/hr/animal, 29.68 gm/hr/animal; THI of 35.40°C (30%/45°) with 30.26 W/hr/animal, 35.62 gm/hr/animal, respectively. The relationship existing between total heat production, moisture production and THI was determined and found to be expressed by regression models with high R² ranging from 0.98 to 0.99 and 0.77 to 0.92, respectively.

A PROPOSAL FOR THE GENETIC IMPROVEMENT OF RABBITS FOR SMALLHOLDER UNITS IN NIGERIA

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Sustainable long-term genetic improvement programmes designed to assist smallholder rabbit producers in sub-Saharan Africa have recorded low levels of success. Reasons for this trend could be attributed to uncoordinated and poorly defined research agenda, research priorities that are not tailored towards the needs of end-users (backyard rabbit units) and heavy dependence on foreign aid, exotic breeds and lack of facilities needed to implement such programmes. The current presentation gives a conceptual framework for the implementation of a genetic improvement scheme specially designed for smallholder rabbit farms, so as to serve as a model to stabilize and promote such operations in sub-Saharan Africa. The dual objectives are: (a) to develop a line of domestic rabbits with productive adaptability to the conditions in smallholder units in southern Nigeria, and (b) to establish a management support package based on renewable local resources for the developed line. To achieve these objectives, and based on detailed consultations with village rabbit project leaders, a 120-doe closed nucleus breeding unit, comprising of a heterogeneous population of rabbits, will be established. Selection criteria will be based on a combined index for annual numerical doe productivity, a composite trait that reflects productive adaptability under smallholder conditions. Concurrently, a management support package based on renewable local resources (e.g. *ad libitum* forage and palm kernel cake-based concentrate, due to its relative low-cost and year-round abundance) will be included. A dissemination process for the genetic stocks with the accompanying management package, as well as a feedback system for backyard rabbit keepers will be established. Details on project funding and sustainability are discussed. The ultimate goal is for the nucleus centre to serve as a major source of quality genetic stocks and an accompanying management support package specifically designed for smallholder units.

**RESEARCH OUTPUT IN RABBIT SCIENCE AND PRODUCTION
EMANATING FROM SUB-SAHARAN AFRICA – IMPLICATIONS FOR
FUTURE RESEARCH AND DEVELOPMENT EFFORTS**

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This study examined the trend and quantum of research output in all aspects of rabbit science and production emanating from sub-Saharan Africa, with emphasis on their relative contributions to the development of viable and sustainable smallholder rabbit units. The hypothesis was that a high proportion of research works in rabbit science and production is not client-based and, therefore, has poor uptake or adoption rate by the preponderant smallholder rabbit units in the region. The objectives of the study were: to examine the trends in scientific research outputs in all aspects of rabbit science and production and to assess the contributions of Research & Development (R&D) in rabbit science and production that are oriented towards smallholder units. Publications were sourced from journals, conference proceedings, bulletins, technical reports and grey literature from 1990 to 2007. Data recorded from each publication included names of authors, year of publication, country of origin, area of publication. Each publication was categorized based on the following criteria: basic or applied research, on-station versus on-farm research, uni- or multi-disciplinary, or conducted with external collaboration or funding. An index of possible applicability of research findings was used to classify the papers. In all, a total of 115 publications were sourced from the literature for the period covered by the study. Results showed that over 80% of the publications were basic research output, uni-disciplinary in nature, and were conducted under on-station conditions, while about 10% of the research studies had some form of foreign collaboration through technical support and/or funding. Publications in journal papers accounted for 88% of the publications, while the rest were from Conference Proceedings (mainly World Rabbit Congresses) and other reports. The index of potential applicability of research output showed that over two-thirds (or 68%) of all the research results needed further trials to adapt the technologies to existing conditions of backyard rabbits units. Strategies for a new research agenda to boost R&D efforts to promote smallholder rabbit production are discussed.

SMALLHOLDER RABBIT PRODUCTION IN SOUTHWESTERN NIGERIA: CURRENT STATUS, EMERGING ISSUES AND WAYS FORWARD

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The preponderant nature of smallholder rabbit production units and their contributions to food security are cardinal reasons to design a clientele-based approach to Research and Development (R&D) for such units. An assessment of critical factors affecting such units will help to design appropriate intervention measures to boost and enhance their contributions to food security. This study examined the current trends in small- and ultra-small-holder rabbit units in southwestern Nigeria with emphasis on current constraints, prospects, benefits and challenges. The methodology combined questionnaire surveys with on-farm monitoring and recording of data in relation to socio-economic characteristics of rabbit keepers, features and attributes of such units, reproductive management, annual doe productivity and economics and marketing outlets. Results showed that a combination of ultra-small- (units with at most two breeding does) and smallholder units (>two breeding does and less than 10) constitute 80% of all the rabbit farmers, while medium (>10 and less than or equal to 50 does) and large scale (>50 does) had frequencies of 15 and 5%, respectively. The rabbit farmers cut across all ages and professions, including retirees. The primary reason for keeping rabbits was for home consumption, with occasional sale of exceeding stocks, while the sale avenue involved direct sale of live animals to consumers or other farmers. Majority of the rabbit keepers (57%) indicated that all members of the family were involved in animal care and management. Breeds used were invariably crosses among imported commercial meat type rabbits (mainly New Zealand White, Californian and Chinchilla). There was no reliable and steady supply of breeding stocks anywhere. Instead, foundation and replacement stocks were mostly acquired from friends and other smallholder farms. With respect to animal housing, there were no prototypes for the backyard units since 50% of the rabbit farmers used assorted materials (wood, wire mesh, tires, etc) for the construction of rabbit cages and the designs varied widely. Cages were mostly (67%) single-tiered and placed outside the house. Mating of does follows a seasonal pattern, due to seasonal heat stress. About 70% of the farmers noted that doe receptivity and conception rates were markedly low during the dry season. Principal constraints facing the units include getting reliable and stable sources for foundation/replacement stocks, feeds, theft, access to information on rabbit management under smallholder units, etc. Detailed proposals were made on ways to streamline R&D activities in favour of smallholder rabbit units.

OUTDOOR REARING SYSTEM FOR FATTENING RABBITS: EFFECT OF GROUP SIZE

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This study aimed to evaluate in outdoor rearing system the effect of group size on the performance of a slow growing local rabbit population "Grigia Rustica". The rabbits were weaned at 35 d and housed indoor in colony cages until 56 ± 3 d of age. During this period all the animals received a coccidiostatic treatment for 5 d. At 56 ± 3 d of age, 88 rabbits were transferred to outdoor pens, located in a wooded area, housed in wire net floor colony cages and divided into three groups *at random*: thesis 4 animals/cage (T4), thesis 8 animals/cage (T8), thesis 16 animals/cage (T16), maintaining a density of 5 animals/m². The animals were fed a complete feed and alfalfa hay *ad libitum*. Growth, feed intake and health status were checked from weaning to slaughter. At 103 ± 3 d, 12 animals for each group were slaughtered and carcasses chilled for 24 h in a ventilated cold room (+4°C). Hot carcass, skin, full gastrointestinal tract, chilled commercial carcass and reference carcass weights were recorded; the left hind leg was dissected to establish the meat to bone ratio. The productive performance were analyzed by least squares means considering as categorical variable the group size. The carcass parameters were analyzed by least squares means considering rearing system as main categorical factor nested within cage and using slaughtering weight as covariate. No mortality was observed, even if during the last fifteen days of the trial one rabbit of the T8 group and three rabbits of the T16 group showed aggressiveness and, for this reason, they were excluded by the experiment. The slaughtering weight, studied at parity of initial live weight, showed differences between groups ($P<0.01$): generally lower productive performances were observed with increasing group size. Rabbits in group T4 showed the highest live weight at slaughtering age than T8 that showed the lowest values. The rabbits of the group T4 showed a significantly higher weight gain than groups T8 and T16. T4 group showed a significantly higher slaughtering weight and the lowest skin percentage. The highest values of meat to bone ratio were observed in T4 and T16 groups: in the first case it is probably due to the more rapid growth than the other groups, while in the second case it may be explained by the more intensive physical exercise. In conclusion the best performance of the rabbits reared with the lowest group size showed that it is possible to reduce the rearing period of about one week, improving the productive efficiency of this kind of rearing system. The onset of the aggressiveness was observed during the last days of rearing in the higher group size that need a longer time to growth. These results may suggest the opportunity to raise even the slow growing rabbits with low group size.

**STRATEGY ON THE DEVELOPMENT OF SMALL- AND MEDIUM-
SCALE RABBIT FARMING BASED ON FARMERS COOPERATION.
A CASE OF RABBIT PRODUCTION IN INDONESIA**

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The situation in Indonesia and unique potential of the rabbits provides the opportunity for rabbit development. Rabbits form an integral part of the complex farming activities. Its farming activity is characterised by small in scale, limited capital investment, limited knowledge of technical farming operation, and in some areas lack of ability to market products and also lack of cooperation among raisers. In this last 4-5 years, rabbit farming has been gaining its popularity in Indonesia. A significant increase, not only in rabbit population, but also in numbers of areas and farmers interested in raising rabbits is recorded. A shift from improving family health and nutrition, through rabbit meat consumption, to an increased income-oriented farming occurs almost everywhere. It is a profitable farming practise, with the Benefit/Cost ratio that varies from 1.1 to 2.7 depending on the system and scale of farming. Scale of farming varies from 5 to 500 does, with different farming system operations. At micro- (<20 does) to small-scale operation (20-50 does) a self forage-providing is very common, while at larger scale operation (>50 does) a combination of forages and commercial feed is practised. For micro and small scale operation, especially in a new growing area, where market is initially less available, a strong cooperation of farmers is suggested. A strategic development, as partly applied to an area in Central Java, involving a group of farmers, is considered succesful. This strategy, introduced as 'Rabbit Village Concept', includes training of member-farmers, village breeding, strengthening the program and management of the organization, creating market and promotion of rabbits.

PRODUCTIVE PERFORMANCE OF SIX GENOTYPES OF FATTENING RABBITS FED WITH SEVEN COMMERCIAL FEEDS

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Thirty-five-day old weanling rabbits from three breeds, New Zealand White (NZ), Californian (CA), and Chinchilla (CH), and their F1 crosses were used to evaluate body weight (BW) and average daily gain (ADG) from 35 to 70 days of age when they were fed with seven commercial feeds. Four hundred and thirty eight rabbits of both sexes were used in a 6x7 factorial (genotypesxfeeds) repeated measurement (week) experiment. The effects of genotypes and feeds were significant on BW and ADG, with a superior performance of genotypes involving the NZ breed and feed 3. Means for BW at 70 days of age for genotypes were 2199±13^a, 2163±12^b, 2107±13^c, 2088±14^c, 1895±16^d, and 1882±15^d g, for NZ, NZxCA (NCA), NZxCH (NCH), CAxCH (CCH), CH and CA, and were, 2159±13^a, 2080±15^b, 2068±19^{bc}, 2046±15^{cd}, 2024±14^d, 2023±15^d, 1991±14^e g for feeds 3, 1, 5, 6, 4, 7, and 2. Performance through the fattening period varied according to genotype, feed, and week age.

HUNGARIAN RABBIT MEAT EXPORT IN THE ASPECT OF THE WORLD MARKET

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Hungary is at the 10th place in the rabbit meat production (8,000 tons in 2005). Since 97% of slaughtered rabbits are exported and Hungary is at the 2nd-3rd place in the rabbit meat export, that is why the continuous estimation of available and potential markets is essential. According to the database of FAOSTAT our main competitors are China, Argentina, France, Spain and Italy. On the basis of the data of 2005, Hungary exports most of the rabbit meat to Italy (42.2%), Switzerland (26.0%), Germany (19.6%) and Belgium (6.3%). On the other hand, these countries are the main rabbit meat importers (in 2005 Germany: 6.141, Belgium: 3.694, France: 3.502, Italy: 2.887 and Switzerland: 2.636 tons). In terms of the Hungarian rabbit meat export, the continuous decrease of the Italian import is disadvantageous, furthermore there is no prospect of increasing the 75.7% (2005) of Hungarian sharing. The Swiss import also decreases and the Hungarian sharing is also high there (52.2% in 2005). The German rabbit meat import has been improving during the last years. Although Hungary is one of the main exporters (16.7% in 2005) the highest numbers of exporters compete for this market. The Hungarian rabbit meat export could be increased only in new markets. In terms of stability of the Hungarian rabbit production the stimulation of home consumption has a great importance.

CIRCADIAN CHANGES OF RECTAL TEMPERATURE AND FEED AND WATER INTAKE IN ADULT RABBITS UNDER HEAT STRESS

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Forty six cross-breed New Zealand, California, Butterfly, Dutch and Satin rabbit does with an average weight of 3.67 ± 0.37 kg (standard deviation) were divided randomly in two groups. The first one was shaved two days before the beginning of the trial and the second one remained unshaved. During 24 consecutive hours, rectal temperature (23 rabbit does/treatment), feed and water intake (10 rabbit does/treatment) were recorded every 4 hours. In the same period the farm temperature and the relative humidity were recorded. The farm was an open-air building in which temperature-humidity index (THI) was higher than 28.9 (severe heat stress) from 10:00 to 18:00 h, which fit very close with the sun light period. In this period rabbit does rectal temperature evolved in parallel to farm temperature/THI, and almost no eating or drinking activity was detected. The maximum farm temperature (or THI value) was reached around 14:00 h, that is the same time in which rabbits showed their highest rectal temperature (increased from 39.1 to 39.6°C; $P < 0.001$). From 18:00 h onwards farm temperature/THI and rectal temperature decreased and rabbit does begun their feed and water intake period. The lowest farm temperature/THI and rectal temperature were observed during night that is the natural period for rabbit eating. Shaved rabbit does showed a lower rectal temperature than no shaved animals (39.3 vs. 39.4°C; $P = 0.045$) and a higher feed intake (10.0 vs. 7.87 g/kg^{0.75}/4 h; $P = 0.019$), especially at the beginning of the eating period ($P = 0.043$). Water intake varied in parallel to feed intake and was not influenced by shaving (21.6 ml/kg^{0.75}/4h, on average). In conclusion, rabbit heat stress hours fit with the highest rectal temperature and the lowest feed and water intake. Further and more practical strategies must be investigated to decrease heat stress in our environmental conditions.

EFFECT OF CAGE DENSITY ON PERFORMANCE OF FATTENING RABBITS UNDER HEAT STRESS

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Three hundred cross-breed rabbits of New Zealand, California, Butterfly, Dutch and Satin, weaned at 30 days and weighing 535 ± 8 g (standard error) were assigned at random to four treatments: 6, 12, 18 and 24 rabbits/m² (3, 6, 9 and 12 rabbits/cage, respectively, each cage of 0.5 m²) resulting 10 cages/treatment. During the experimental period (from weaning to 2.2 kg body weight) individual live weight, cage feed intake, the incidence of diarrhoea, ringworm and injured rabbits were recorded. The maximal temperature-humidity index ranged from 31 to 35 indicating a temporal severe heat stress. At the end of the experimental period 10, 20, 30 and 30 rabbits from the treatments with densities of 6, 12, 18 and 24 rabbits/m², respectively, were slaughtered and carcass performance recorded. Average daily gain and feed intake from weaning to the end of experimental period decreased by 0.31 ± 0.070 and 1.20 ± 0.25 g, respectively, per each unit that the density increased at the beginning of the experiment ($P=0.001$). The length of the fattening period increased by 0.91 ± 0.16 d ($P=0.001$) per each unit of increment of density. However, rabbit production (expressed in kg/m²) increased linearly and quadratically with the density ($P<0.008$). Cage density did not affect feeding efficiency, that was on average 0.214 g/g ($P=0.37$). Animals housed at the highest density compared to the average of those caged at lower density tended to show a higher incidence of ringworm (68.9 vs. 39.4%; $P=0.075$), a higher injured animals (16.8 vs. 3.03%; $P=0.12$) and a higher mortality (20.5 vs. 9.63; $P=0.043$). Density did not modify dressing out percentage and chilled carcass weight. Increasing density reduced linearly dorsal length ($P=0.001$) and reduced linearly and quadratically drip loss percentage ($P=0.097$ and 0.018, respectively). Based on these results, under our heat stress conditions it is recommended to avoid densities higher than 18 rabbits/m².

A JOINT PROJECT TO SYNTHESIZE NEW LINES OF RABBITS IN EGYPT AND SAUDI ARABIA: EMPHASIS FOR RESULTS AND PROSPECTS

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A selection programme for meat rabbits is being carried out in three Egyptian and one Saudi Arabian research centres, each of them having the task to develop lines of rabbits in their local conditions. This is the largest programme executed in countries of hot climate with comprehensive efforts to develop new lines of meat rabbits, where heat stress is considered as one of the most important limiting factors to raise meat rabbits. The set of lines in the programme are four replicates of the Spanish V line; two local breeds (Sinai Gabali and Saudi Gabali); and five synthetics originated from crossing V line with local breeds. The local breeds used to create the synthetic lines were Black Baladi, Red Baladi and Sinai Gabali in Egypt and Saudi Gabali in Saudi Arabia. The V line was replicated in four different locations, three in Egypt (Alexandria, Animal Production Research Institute (APRI), and Moshtohor) and one in Saudi Arabia (Al-Qassim University). The synthetic Alexandria line is 87.5% from V line and 12.5% from Black Baladi; the synthetic APRI line is 50% from line V and 50% from Red Baladi; the synthetic Moshtohor line is 50% from V line and 50% from Sinai Gabali; the synthetic Saudi 2 is 75% from V line and 25% from Saudi Gabali and the Saudi 3 is 25% from V line and 75% from Saudi Gabali. The lines and breeds are being selected by a BLUP methodology, under different local conditions, following different criteria of selection, depending on the Centres, aiming to develop maternal or paternal lines, giving special consideration of selection for milk production and growth after weaning, in addition to litter size. Data of the last year for V line in four replicates, Sinai and Saudi Gabali and the five synthetics were only used for describing the performances obtained and to get a general idea about the expected productivity. Performances obtained verified that the most stable and convenient trait in all synthetic lines influenced by line V is referring to the prolificacy, which has been around 9.0 total born, 8.5 born alive and 7.2 number weaned per litter in the majority of the locations. Post-weaning daily gains were also convenient and ranged from 34 g/d to 18 g/d in different locations studied. Outstanding performances in some synthetics seemed superior, in some cases, to local breed and the V line originating them.

PRODUCTIVITY OF RABBIT DOES OF A WHITE POPULATION IN ALGERIA

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Reproductive performance of 172 rabbit does of the “White strain” (638 litters) were registered in the Djelba rabbitry in Algeria between July 2005 and February 2007. The does were issued from commercial French hybrid rabbits (Hyplus, Grimaud Frères) imported in Algeria in 1985-86 and bred without external introduction since that time. Does were generally mated 8-14 days after kindling and eliminated after 3 infertile mating. Young were weaned when 30-35 days old. On average litter size at birth was 7.14 ± 2.42 total born, of which 6.67 ± 2.76 born alive. Litter size at weaning was 5.75 ± 2.42 . Stillbirth proportion was 7.34% of total kits born, and birth to weaning mortality was 15.8% of kits born alive. Prolificacy was similar to that observed for does of the local population, but kit mortality was lower. The average does live weight at mating was 15% higher than that of the local population (3.3 vs. 2.9 kg). Effects of parity and of season were also analysed. The does of the White population have a moderated prolificacy, similar to that of the local population maintained on closed population since much longer. On the other hand, because of a weak interval between two litters, the numerical productivity (weaned/doe/year) seems clearly higher than that noted for the local population. It will be advisable to check this important point for the future of the strain, while working under better controlled conditions of recording (for example including females not having never weaned but having occupied the places of reproduction in the breeding) and in situations of breeding different from those of the unit of breeding of the cooperative which profits from a strong technical farming because of its role in the maintenance and the diffusion of the White Strain. Finally it will be interesting to compare the performances of reproduction of this White Strain with those of the Synthetic Strain in the course of creation in Algeria.

AUTHOR INDEX

AUTHOR INDEX

- Abanikannda O.T.F. 447
 Abdel-Ghany A. 64
 Abdel-Kafy E.M. 179, 180
 Abdel-Khalek A.M. 181, 231, 409
 Abecia L. 184
 Abou Khadiga G. 35
 Abo-Zeid A. 203
 Abu O.A. 447
 Adamude H. 361
 Adelantado C. 227
 Adeleke T.O. 120
 Adepoju L.O. 182
 Aderinola O.A. 182, 225
 Adua M.M. 361
 Afifi E.A. 54, 473
 Agnoletti F. 269, 270, 271, 272, 274, 278,
 303, 304, 306
 Ahmed M.H. 241, 242
 Ajayi B.A. 466
 Akinlade J.A. 182
 Akpo Y. 36, 398
 Alberghini L. 393
 Al-Dobaib S.N. 37
 Ali W.A.H. 179
 Alicata M.L. 100
 Allain D. 43, 73
 Allalout S. 448
 Alonso F.M.U. 412, 461, 462
 Aloy N. 296, 297, 298
 Al-Saef A.M. 3, 37, 56
 Al-Sobayil K.A. 56
 Amara A. 386
 Ambriz D. 104
 Angelozzi G. 359
 Aramburú Jr J.S. 228
 Araújo C.C.B. 228
 Araújo I.G. 228, 235
 Archetti I. 339
 Argente M.J. 38, 39, 47, 52, 70, 119
 Arias-Álvarez M. 97
 Arsenos G. 216
 Astillero J.R. 229, 230
 Ayorinde K.L. 453, 454
 Azoiz A.A.A. 179
 Babatunde G.M. 225
 Bacchin C. 270, 271, 274, 278, 303, 304
 Badagliacca P. 308
 Badiola I. 296, 297, 298
 Baele M. 290
 Balcells J. 184
 Balogh K. 276
 Bano L. 269, 270, 271, 272, 274, 278
 Barge P. 195, 218, 244, 340
 Baricco G. 195, 218
 Barna T. 286, 311
 Barrio M. 123
 Baselga M. 35, 37, 54, 58, 59, 68, 71, 72,
 115, 473
 Battistacci L. 349
 Baylos M. 183
 Bayourthe C. 212
 Bázár Gy. 385
 Becerra J.J. 123
 Becerril-Pérez C.M. 469
 Belenguer A. 184
 Ben Hamouda M. 185, 386
 Ben Larbi M. 448
 Ben Rayana A. 185, 386
 Berchiche M. 217, 459, 474
 Bergaoui R. 185, 186, 386
 Bertazzoli A. 449
 Bianchi M. 387, 402, 404
 Bianconi G. 350
 Bielanski P. 347, 388, 450
 Biró-Németh E. 63, 76, 112, 113, 118, 128,
 344, 346, 351, 352, 396, 402
 Biscotto A. 393
 Blanch A. 227
 Blas E. 68, 187, 192
 Blasco A. 38, 39, 47, 52, 53, 57, 62, 69, 70
 Bodin L. 40, 130
 Bodnar K. 451, 458
 Boisot P. 207
 Boiti C. 98, 99, 102, 129
 Bolet G. 40, 46, 129
 Bónai A. 188, 189, 213
 Bonanno A. 100
 Bonazza V. 362

- Bonnet M. 73
Bordin P. 390
Borhami B.E. 203
Bostvironnois C. 295
Bószé Zs. 131
Bouvier A.C. 101
Bovera F. 190, 389
Brecchia G. 98, 99, 102, 129
Brivio R. 273, 288, 339
Bruant G. 308
Brum H.S. 235
Brun J.M. 65, 66
Bruno-Soares A. 206
Buonavoglia C. 288
Busani L. 272
- Cagiola M. 191
Callanan J.J. 310
Calvo M.A. 227
Campo M.M. 348
Canchola E. 104
Candotti P. 356
Cano P. 119
Cao Liang 74
Capucci L. 247
Carabaño R. 135, 183, 297, 400, 413
Cardile H. 363
Cardinali R. 102, 191, 341, 349
Carraro L. 239
Carrilho M.C. 348
Carro M.D. 184
Carvalho W. 205
Castellini C. 81, 102, 191, 341, 349, 403
Castillo M. 227
Castro-Solla L. 206
Catalá A.E. 68
Cauquil L. 197, 294
Cavani C. 387, 402, 404
Celestinos M. 103
Cerioli M. 273, 288, 339
Cervera C. 187, 192
Cesari N. 193
Cesari V. 193
Chamorro S. 135, 183
Chao-mei Li 279
Charrier S. 196
Chen Baojiang 41, 50, 237
Chen P. 194
Cherubini R. 195, 218
Chicco R. 340
Chiericato G.M. 125, 355, 391
Chlebec I. 122
Chrastinová L. 232, 233, 234
Chrenková M. 233, 234
Climent A. 57
Čobanová K. 233, 234
Cocchi M. 269, 270, 271, 272, 274, 278
Coelho A.C. 275
Coelho A.M. 275
Coelho C.C.G.M. 205
Colin M. 196, 276
Coman C. 277
Coman T. 277
Combes S. 197, 294, 394
Comin D. 390
Contiero B. 75
Contreras J.L. 104
Contreras-Ferrat L.G. 104
Cook J. 43
Cornez B. 300, 301
Corpa J.M. 305, 310
Corrent E. 238, 240
Cortell C. 105, 106
Cossu M.E. 452
Coudert P. 36, 215
Coureaud G. 317
Cremonini M.A. 387
Cuadrado M. 97, 341
Cuca-García J.M. 469
Cumini M.L. 452
Cutrignelli M.I. 389
- da Silva L.P. 228, 235
Daader A.H. 107
D'Agata M. 408, 467
Dairo F.A.S. 198
Dal Bosco A. 102, 191, 341, 349, 403
Dall'Aglio C. 98
Dalle Zotte A. 125, 220, 342, 391, 392, 408
Davoust C. 238, 240
de Blas J.C. 135, 183, 229, 230
De Gussem M. 290, 309
De Jong I.C. 343
De La Fuente L.F. 302

- De Poi E. 195, 218
de Quadros A.R.B. 235
de Rochambeau H. 73
de Toledo G.S.P. 228, 235
Delezie E. 399
Deotto S. 269
Deretz-Picoulet S. 43, 73
Deyun Wang 289
Di Grigoli A. 100
Di Meo C. 190
Di Provvido A. 308
Díaz J.V. 297
Díaz Z.S. 412, 461, 462
Dihigo L.E. 199
Djago A.Y. 36, 215, 398
Djellal F. 459
Dokoupilová A. 77
Domínguez M. 199
Donkó T. 108
Dovas C. 285
Drigo I. 269, 270, 271, 272, 274, 278
Duchateau L. 293
Ducrocq V. 48
Duperray J. 207
D'Urso S. 389
- Eady S.J. 42, 49
Eiben Cs. 200, 201, 202, 414, 415
Eissen J. 208
El-Adawy M.M. 203, 241, 242
El-Battawy K.A. 109
El-Medany Sh.A. 181, 231, 409
El-Raffa A.M. 473
El-Zarei M.F. 56
Erdélyi M. 204
Esparbié J. 40
Esquifino A. 119
Etcheluz L. 452
Euler A.C.C. 205
- Fábíán J. 415
Fábíán K. 286, 287, 311
Faix Š. 232
Falcão e Cunha L. 206
Falieres J. 40
Fallas-López M. 126
Fang Wang 279, 289
- Farinu G.O. 225
Farkas J. 63
Farougou S. 398
Farsang A. 285, 287, 311
Fayeye T.R. 453, 454
Fébel H. 188, 189
Fedrizzi G. 401
Fernandes S. 357
Ferreira P. 235
Ferreira W.M. 205, 226
Ferro T. 269, 271, 303
Fetter B. 354
Finzi A. 280, 282, 350, 403, 456
Fiorenza G. 276
Flores D.O. 55, 222
Folch J.M. 39, 47, 70
Fondevila M. 184
Fontanesi L. 43, 44, 45
Fontes M.C. 275
Forti K. 191
Fortomaris P. 216, 285
Fortun-Lamothe L. 129, 294, 317
Foubert C. 207
Fragkiadaki Eir. 281
Fragkiadakis M. 239, 358, 400
Freire J.P. 206
Fučíková A. 243
Furuta L. 226
- Gacem M. 46
Gado H.M. 203
Galeati G. 98
Gallocchio L. 390
García E. 114
García J. 135, 297, 298, 457, 471, 472
García M.L. 37, 38, 39, 47, 52, 54, 58, 59, 70, 119, 473
García Muñoz J.G. 126
García-García R.M. 97
García-Ispuerto I. 71, 72
García-Rebollar P. 135, 400, 413
García-Ruiz A.I. 135, 208, 229, 230
García-Tomás M. 71, 72, 110, 111
Garreau H. 40, 42, 48, 49
Gasco L. 244
Gatica R. 103
Gerencsér Zs. 51, 112, 113, 118, 128, 204,

- 342, 344, 351, 353, 396
Giaccone V. 393
Giammarco M. 359
Gidenne T. 197, 209, 212, 294
Gigaud V. 394
Gippert T. 200, 201, 202
Gobbetti A. 98, 99
Goby J.P. 209
Góczy E. 131
Gódor-Surmann K. 200, 201, 202
Gómez Gallegos G. 455
González J. 296, 297, 298
González-Redondo P. 282, 456
Grilli G. 193, 273, 288, 339, 405
Gu Zilin 41, 50, 237
Gualterio L. 282
Guan Li-hui 78
Guedes C.M. 410, 411
Guelfi G. 99
Guérin P. 127
Guillén O. 457, 471, 472
Gulinati A. 116, 117
Gullón J. 123
Guo K. 244
Guo Wanhua 50
Guolo A. 269, 270, 271, 304
Gutiérrez C.A. 412
Guttierez G. 196
Guyonvarch A. 207
Gyorgyi V. 64
Gyovai M. 402
Gyovai P. 51
- Haddad B. 448
Haesebrouck F. 290, 293
Hannachi R. 474
Harel J. 308
Hassan N. 64
Hauffe H.C. 44
Haviarová M. 232
He Dongchang 74
Heffron A.L. 306
Heinzl E. 362, 363
Hermans K. 293
Hernández P. 367, 395, 407
Hernández Y. 199
Herradón P.G. 123
- Herrler A. 62, 70
Hiripi L. 131
Hoda A.S. 179, 180
Hoop R. 283
Horn P. 76, 188, 189, 213
Horvath J. 451, 458
Houeix J. 284
Hoy St. 345, 360
Huang Yuting 41, 50
Huo Yanming 237
Hurtaud J. 40, 49
Huybens N. 284
Huyghebaert G. 399
- Ibáñez-Escriche N. 38, 52, 53
Iraqi M.M. 54, 473
Ishola M. 466
- Jackson R.H. 55
Jacquinet C. 101
Jakubec V. 77
Jekkel G. 346, 396
Jiabin Xue 279, 289
Jiménez G. 227
Jing Cui 41
Jobim C.C. 226
Joly T. 127
Juin H. 48, 130
Juncos A. 192
Jurcik R. 234
- Kaddech A. 386
Kadi S.A. 459
Kametler L. 188
Kammoun M. 186
Kermauner A. 210, 211
Khalil M.H. 3, 37, 54, 56, 473
Khalil M.S. 203
Kimsé M. 212
Kline B. 354
Komolafe S.O. 466
Kongwang He 279
Koptopoulos G. 216, 285
Korczak B. 283
Kovács F. 188, 189, 213
Kovács M. 188, 189, 213, 220
Kövér Gy. 385

- Kowalska D. 214, 347, 388, 397, 450
 Kpodékon T.M. 36, 215, 398
 Kritas S.K. 216, 285
 Kulcsár G. 286, 287, 312
 Kustos K. 200, 201, 202

 Laborda P. 57, 114
 Lachica M. 184
 Lafuente R. 348
 Lagunas B.S. 412
 Lakabi-Ioualitene D. 217
 Lambertini L. 359
 Lamothe E. 130
 Larzul C. 48
 Lasisi F. 463
 Lauková A. 232, 233, 234
 Launay C. 238
 Lavara R. 58, 59, 115
 Lavazza A. 247, 273, 288, 339, 356
 Lavrenčič A. 210, 211
 Lebas F. 46, 196, 217, 240, 474
 Legarra A. 49
 Lenarduzzi M. 61, 75
 Leroux C. 238
 Letizia A. 308
 Li Cun-xin 78
 Li F.C. 194
 Li Sufen 41, 50
 Li Wen-hai 78
 Li Yanping 74
 Liang Quanzhong 74
 Licois D. 284
 Lilli L. 98
 Limet A. 295
 Lipinska U. 293
 Liu Hanzhong 460
 Liu Yajuan 41, 50, 237
 Llobat L. 60
 Llorente A. 135
 Locsmándi L. 76
 Longsheng Yang 279, 289
 López M. 348
 López-Bejar M. 71, 72, 111
 Lorenzo P.L. 97, 124
 Lounaouci-Ouyed G. 217
 Lukács H. 213
 Lukefahr S.D. 55, 222, 419

 Luzi F. 291, 292, 362, 363
 Ma Xu-ping 78
 Macchioni P. 280
 Mach K. 77
 Macri S. 401
 Madamidola G. 466
 Maertens L. 188, 309, 399
 Mainil J. 284
 Majolini D. 239, 358
 Majzlík I. 77
 Makranszki L. 312
 Malpaux B. 130
 Maniero C. 390
 Mantovani R. 61, 75
 Maranesi M. 98, 99
 Marasciulo V. 273
 Marco M. 124
 Marco-Jiménez F. 115
 Marcon B. 271, 274
 Margüenda I. 400, 413
 Maria B.G. 226
 Marien M. 290, 309
 Mariscal-Aguayo V. 126
 Marlier D. 284
 Marongiu M.L. 116, 117
 Marono S. 190
 Marounek M. 221, 236, 243, 307
 Martella V. 288
 Martínez E. 187, 192, 227
 Martínez M. 199
 Martínez-Hernández P.A. 126
 Martino P.A. 291, 292
 Masoero G. 195, 218, 219, 244, 340
 Mastrantonio P. 272
 Matics Zs. 76, 108, 112, 113, 118, 128, 188,
 189, 204, 220, 342, 344, 351, 352, 396
 Matos C. 205
 Maurício R. 205
 Mazza F. 100
 Mazzolini E. 269, 270
 Mazzone G. 359
 Mazzone P. 191
 McEwan N.R. 221
 Meineri G. 219
 Melchior R. 235
 Mendoza B.J. 412, 461, 462
 Menotta S. 401

- Menoyo D. 135, 183
Mercati F. 98
Merchán M. 39, 47, 70
Metzger Sz. 51, 76, 220, 342, 402
Meulemans L. 293
Mézes M. 157, 204
Mezzadri M. 61, 75
Michelland R. 294
Milandri C. 393
Milcent N. 130
Milisits G. 213, 346, 396
Millán P. 97, 124
Mioni R. 390
Mitjavila T. 110
Mocé E. 114
Mocé M.L. 57, 62, 114
Mojto J. 232, 233, 234
Monclús R. 317
Monroy S.H. 412
Monteils V. 212, 294
Monteiro A.C. 226
Morel Saives A. 295
Moscati L. 191, 349
Motawei M.I. 56
Moura A.S.A.M.T. 357
Mourão J.L. 275, 406, 410, 411
Mourvaki E. 349, 403
Moya V.J. 227
Mozzoni C. 467
Mudunuru U. 222
Muelas R. 38, 39, 47, 52, 70, 119
Mugnai C. 191, 341, 349, 403
Murgiano L. 98
- Nada S.A. 181, 231, 409
Nagy I. 51, 63, 76, 112, 113, 118, 128, 344, 346, 351, 352, 353
Nagy Z. 76
Negretti P. 280, 282, 350, 456
Nelson S.D. 222
Newbold C.J. 221
Nguyen Thi K.D. 223, 224
Nguyen Van T. 223, 224
Nicodemus N. 97, 124, 135, 183, 229, 230, 400, 413
Nizza A. 190
Nizza S. 389
- Nodas A. 199
Nofal R. 35, 64
Ntafis V. 281
Núñez N. 413
- Obiyan R.I. 447
Odedire J.A. 120
Odeyinka S.M. 120
Ogunjimi L.A.O. 463
Ojebiyi O.O. 182, 225
Ola S.I. 121
Oliveira A.F.G. 226
Olvera J. 104
Ondruška L'. 67, 122, 233
Onifade A.A. 447
Orova Z. 113, 344, 351
Ortega B.E. 462
Oseni S.O. 463, 464, 465, 466
Ouerdiane K. 186
Oulmouden A. 43
Outor-Monteiro D. 406
Ouyed A. 65, 66
Oyedele O.J. 120
Oyegbade M.O. 121
- Paci G. 408, 467
Pankowski P. 450
Paraskevskou E. 281
Parkányi V. 67, 122
Pascual J.J. 68, 187, 192, 227
Pascual M. 69
Pecchioli E. 43, 44
Peiretti P.G. 219
Peiró R. 39, 47, 62, 70
Peña A.I. 123
Pena-Arnaud B. 73
Penadés J.R. 305
Pereda N. 124
Pérez de Ayala P. 208
Pérez de Rozas A.M. 296, 297, 298
Pérez-Bonilla A. 208
Petneházi Ö. 108
Petracci M. 387, 402, 404
Petrási Zs. 76, 108
Petridou E. 216, 285
Picard E. 238
Piccirillo A. 405

- Piccolo G. 389
 Pietras M. 347
 Piles M. 71, 72, 110, 111
 Pinheiro V. 275, 406, 410, 411
 Pinloche E. 221
 Pinto M.L. 275
 Pio A. 411
 Pisoni A.M. 193
 Pla M. 69, 395, 407
 Plachá I. 232, 233, 234
 Plakokefalos E. 281
 Podmaniczky B. 201, 202
 Poppi L. 405
 Pósa R. 189, 213
 Pradella G. 303, 304, 306
 Preziuso G. 408, 467
 Pridmore A. 306
 Prieto C. 123
 Prigent A.Y. 196
 Prinz Z. 112, 113, 118, 204, 342, 344, 352, 353, 396
 Pritt S. 354
 Pro-Martínez A. 469
- Quintela L.A. 123
- Radnai I. 51, 63, 76, 108, 112, 113, 118, 220, 344, 353, 396, 402
 Rafat S.A. 73
 Rafay J. 67, 122, 232
 Rafel O. 71, 72, 111
 Rafiu T.A. 182
 Raharjo Y.C. 468
 Ramon J. 71, 72, 111
 Rampin F. 405
 Rangel-Santos R. 126
 Rebollar P.G. 97, 124, 191, 341, 413
 Reimert H. 343
 Ren Keliang 74
 Renzi M. 299
 Repa I. 108
 Retore M. 228, 235
 Revez J. 299
 Revuelta L. 97
 Ribera J.G. 104
 Ricci C. 363
 Richard A. 300, 301
- Richez P. 300, 301
 Riovanto R. 392
 Rivaroli S. 449
 Rizzi C. 125, 355, 391, 392
 Robinson T.J. 43, 44
 Rödel H.G. 317
 Ródenas L. 68, 187, 192
 Rodrigues J. 275
 Rodríguez-De Lara R. 126
 Rolla C. 452
 Romero C. 229, 230
 Rommers J.M. 343
 Romvári R. 76, 385
 Rosell J.M. 297, 302
 Rossi M. 299
 Rota Nodari S. 356
 Rotolo L. 244
 Russo C. 408, 467
 Russo V. 43, 44, 45
 Rutili D. 191
- Sabatakou O. 281
 Sabino I. 206
 Saeed A.M. 180
 Saggiorato M. 303, 304, 401
 Sainz A. 183
 Sala G. 218, 219
 Saleh K. 35
 Saleil G. 40, 48
 Salem A.Z.M. 203
 Saliba C. 196
 Salvetti P. 127
 Samkol P. 419
 Sánchez J. 110
 Santacreu M.A. 38, 39, 47, 52, 57, 62, 70, 114
 Sartori A. 61, 75
 Sartori J.R. 357
 Savarino G. 244
 Savón L. 199
 Scacchia M. 308
 Scandurra S. 303, 304, 401
 Scapinello C. 226
 Schaal B. 317
 Schiavon E. 405
 Schiavone A. 244
 Schmidt J. 414, 415
 Schwarz B.F. 124

- Scicutella N. 191
Scotti E. 45
Segura P. 310
Selim N.A. 181, 231, 409
Selva L. 305, 310
Sevilla L. 400
Seydi M. 398
Sierra F. 199
Siloto E.V. 357
Silva J.A. 406
Silva S.R. 406, 410, 411
Simjee S. 306
Simonová M. 232, 233, 234
Siqueira E.R. 357
Sitjar M. 298
Siyanbola O. 466
Skřivanová E. 221, 307
Skřivanová V. 243, 307
Sorensen D. 53
Sosa-Montes E. 469
Sousa L. 205
Spigaglia P. 272
Stahel A. 283
Stanko R.L. 55
Strompfová V. 232, 233, 234
Suárez-López J.R. 469
Sun Lina 41, 50
Szabó A. 76
Szabóová R. 232, 233, 234
Szalo M. 284
Szendrő É. 470
Szendrő Zs. 51, 63, 76, 108, 112, 113, 118,
128, 188, 189, 204, 213, 220, 342, 344,
351, 352, 353, 385, 402, 470
- Tanimomo E. 36
Tazzoli M. 43, 44, 45
Teillet B. 196
Teixeira E. de A. 205
Theau-Clément M. 40, 129, 130
Thébault R.G. 73
Tian Shu-fei 78
Tittarelli C. 273, 288, 339
Tonelli A. 308
Tornyos G. 188, 189, 213
Torres C. 58, 59
Torres-Hernández G. 469
- Toschi I. 193
Tossou C.M. 215
Tóth T. 414, 415
Trocino A. 239, 358
Troislouches G. 238
Tudela F. 48
Tůmová E. 243
Turcu D. 277
Tusell Ll. 71, 72, 111
Tzika E. 216
- Vadillo S. 400
Vancraeynest D. 290, 300, 301, 309
Vašíček D. 67
Vasilková Z. 232, 233, 234
Vega M.D. 123
Velázquez O.V. 412, 461, 462
Vella N. 190
Venâncio C. 410
Venturi L. 387
Verga M. 362, 363
Viana D. 305, 310
Vicente J.S. 58, 59, 60, 115
Vignola G. 359
Villalobos O. 457, 471, 472
Villamide M.J. 135
Villena P. 413
Virág Gy. 131, 276, 286, 287, 311, 312,
414, 415
Viudes de Castro M.P. 105, 106
Vives N. 298
Vlase E. 277
Volek Z. 236
Vostrý L. 77
- Wabi K. 398
Wagner C. 360
Wang Fang 313
Wang Hai-yun 78
Wang Lei 41
Wang Xin 313
Wang Zhiheng 237
Weirich C. 360
Weissman D. 238
Weizhong Xu 279, 289
Wood J. 354
Worgan H.J. 221

- Wu Shu-qin 78
Wu Xinsheng 74
Wu Zhan-fu 78
- Xiccato G. 239, 358
Xylouri E. 281
- Yakubu A. 361
Youssao A.K.I. 36, 215
Youssef Y.K. 473
Yuanliang Hu 289
Yun Shi-feng 313
- Zai Ping 74
Zaki B. 241, 242
Zamparini C. 403
- Zanoni R.G. 299
Zeferino C.P. 357
Zeidan A.S.B. 107
Zerani M. 98, 99
Zerrouki N. 46, 240, 474
Zeweil H.S. 241, 242
Zhang Guolei 237
Zhangg Lijun 74
Zhao Chao 41, 50
Zhao Zhujun 237
Zhou Shenghua 74
Zita L. 243
Zoccarato I. 244
Zomeño C. 407
Zsédely E. 415
Zucca D. 362, 363



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