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# Abstracts of the "GIORNATE DI CONIGLICOLTURA ASIC 2007" Forlì, Italy, 26-27 September, 2007

The second edition of the Italian Rabbit Days, product of the collaboration between ASIC (Italian Rabbit Scientific Association), Avitalia (Italian Poultry and Rabbit Producers Association), ASPA (Animal Production Scientific Association), and the Forlì Fair was mainly focused on "Meat safety and consumer protection" and "Animal welfare during rearing".

The first day of the congress included two main lectures: "Strategies for the reduction of antibiotic utilization during rearing", by L. Maertens and "Meat inspection and consumer's tutelage", by G. Grilli *et al*. Moreover, two sessions of oral communications on Pathology, Nutrition and Physiology were held.

During the second day it were presented three main lectures: "Welfare criteria in rabbit housing", by St. Hoy and M. Verga, "The effect of housing and management on rabbit welfare", by F. Luzi *et al*. and "An alternative rabbit keeping system aimed to welfare and sanity", by A. Finzi and P. Negretti. Moreover, it was held a session of oral communications on Genetic, Management and Meat Quality. A Poster Session was through the two d.

The congress was attended by about 70 participants, including researchers from Spain, Germany, Belgium, Greece and The Netherlands. A total of 5 main lectures, 19 oral communications and 13 posters were presented. Following are reported the abstracts of all contributions.

## MAIN PAPERS

### STRATEGIES FOR THE REDUCTION OF ANTIBIOTIC UTILIZATION DURING REARING

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There are several management methods which have proved to be successful in reducing the disease pressure in rabbit production. All-in all-out management combined with an effective cleaning, disinfection and prevention is a primary tool to reduce the disease risks and by consequence both preventive as curative antibiotic utilization. A second tool is the use of minimal disease level reproduction stock. As it is more and more the habit in pig production, healthy and even animals with a near SPF status reduce the disease risks and lead to high-health-status farms where antibiotics have rarely to be used. Enteric troubles are the most frequent disease problem and responsible for an excessive use of antibiotics. Apart from some qualitative feed characteristics, know for their impact on enteritis risks (especially the carbohydrate complex), also quantitative aspects in early fattening stage are important to reduce enteritis risks. Finally, although there is a lot of discussion concerning their effectiveness, pro(pre) biotics and some other additives have proven to have some potential in reducing enteric problems. However, the whole chain (reproduction stock producers, breeders, feed companies, slaughterhouse and even veterinarians) have to be convinced and forced in a global strategy for minimal antibiotic use. Otherwise both preventive and curative use of antibiotics remains a too easy and even not very expensive way to handle the disease control in rabbit production.

### MEAT INSPECTION AND CONSUMER'S TUTELAGE

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The field of the animal origin food, rabbit included, given its important social impact on the population, has always been submitted to very punctual checks. Already in the Royal Decree dated December 20th 1928, we can find in the art. No.59 a reference to the obligation of the sanitary surveillance carried out by the municipal veterinary on the meat of chicken, rabbit and game or wild fowl. At a European Community's level, since the beginning of the 80's, the National Regulations have issued and consequently acknowledged a certain number of directives, suggesting the rules to be respected regarding the free circulation (of the meat) in the territories of the European Union. The main purpose of this abstract is to present the 'meat surveillance' system adopted by our country providing an updating regarding the legislation and analysing all the aspects

prevailing upon the hygienic-sanitary qualities of the rabbit's meat for the customers' protection. We'll also mention the hygienic-sanitary rules concerning the animal's food (National Plan Animal Food) and the proper use of the medicine (Drug surveillance); we'll consider the procedures used until the slaughtering and the distribution of the final product ('Hygiene package' and National Food Plan) with specific reference to what has been decided regarding the rabbit's meat. We will also mention the updated information concerning the National Animal Food Plan and the National Food Plan regarding the rabbit.

## WELFARE CRITERIA IN HOUSING OF RABBITS

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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. Group housing of does with kits is possible if an individual electronic nest box recognition system is used. At the moment, the disadvantages (especially the labour-intensity, the production costs, difficulties of health control and implementation of new does into the group) do not allow the use of group housing on farm-level. The single housing of does with kits remains the main housing system also 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. wood sticks as gnawing material).

## THE EFFECT OF HOUSING AND MANAGEMENT ON RABBIT WELFARE

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In the first section of the present lecture, the issues related to housing and management, affecting the behaviour and welfare of growing rabbits, will be taken into consideration. Being social animals, keeping them in a single cage may lead to stress caused by social deprivation. So, nowad, growing rabbits are kept in groups with different environmental enrichments. In the second section, the main microbiological conditions of an intensive rabbit farm, affecting the health status of animals, will be analyzed. A reduction of animal suffering may be linked to better sanitary conditions of the animals and of the farm environment. The results showed that a good regular environmental control could be very useful to maintain a good health and welfare status. At the end, the Scientific Opinion of the Animal Health and Animal Welfare Panel of the European Food Safety Authority related to "The impact of the current housing and husbandry systems on the health and welfare of farmed domestic rabbits" will be considered.

## AN ALTERNATIVE RABBIT KEEPING SYSTEM AIMED TO WELFARE AND SANITY

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Results related with animal welfare and sanity in an alternative rabbit keeping system are described. The behaviour of rabbits does was recorded to study their preferences when housed in an alternative keeping system. The complete circadian cycle has been studied for many d before and after parturition. The behaviour strategy to avoid heat stress, properly utilising the different parts of the housing system, has been specifically considered. The behaviour of rabbits was compared when they could freely chose between the alternative system and range keeping. The way of controlling the sanitary conditions of the animals, avoiding any pharmacological treatment, is also described, with peculiar reference to the epidemic enteropathy.

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# ORAL COMMUNICATIONS

## *STAPHYLOCOCCUS AUREUS* AND BIOTYPES PREVALENCE IN RABBIT HERD BREEDING

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A cross sectional study with a two-stage sampling was undertaken to estimate *Staphylococcus aureus* prevalence in intensive rabbitries. Eleven herd were selected and 60 does were systematically sampled inside reproductive units. Nose, ear, abdomen and interdigital skin swabs were collected from 660 does and data about productive and health herd parameters were interviewed. In 6 herds a quantitative and qualitative evaluation of lesions referred to staphylococcosis was associated to individual sampling. Results evidenced an inside herd mean prevalence of 81% (95% CI 78% – 84%). No statistically significant association was found between *Staphylococcus aureus* or his biotypes prevalence with productive parameters and lesions; the possibility that test and culling may affect the latter association is considered, while an information biases was suggested

for the former lack of association. The possibility that the study design reduces power and affects results was also emphasized.

## SENSIBILITY EVALUATION OF SAMPLED ANATOMICAL SITE TO DETECT *STAPHYLOCOCCUS AUREUS* CARRIERS BY CULTURE METHOD

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Staphylococcosis can be considered a target of eradication in industrial rabbitries, as it is a widespread disease, it causes severe illness in the reproductive units and, at the present, there are no effective vaccines developed while antibiotic treatments should be avoided whenever possible. To eradicate the disease it is necessary to recognize contaminated or infected rabbits with high virulent *Staphylococcus aureus*. Being at the present no other systems available but the cultural method, an estimation of the sensibility of a screening system based on swabs scraping from four anatomical rabbits sites was investigated. Results pointed out that an association of ear, inter-digital and abdomen cute scraping can achieve a sensibility of 95% (85-100%, 95%CI). Bacterial contamination of samples can reduce sensibility but further studies are ongoing to understand the amount of such interference.

## *CLOSTRIDIUM PERFRINGENS* TOXINOTYPES ISOLATED FROM RABBIT

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*C. perfringens* is an anaerobic *Gram* positive bacterium, responsible for many pathologies both in human and animals. *C. perfringens* strains are classified into five toxinotypes (A-E) based on the production of four major toxins (α, β, ε, ι); each toxinotype is associated with a particular disease. A novel toxin (β2) has recently been identified, but its role in the pathogenesis of enterities/enterotoxaemia in animals is still in debate. Aim of this study was to investigate which toxinotype of *C. perfringens* is recovered in rabbit affected by entheropathy. In our study, 149/150 strains have showed *cpa* and in only one case *cpi* was found. 25% of *C. perfringens* toxintype A has harboured cpb2.

## IDENTIFICATION OF *CLOSTRIDIUM SPIROFORME* AND BINARY TOXIN GENE (SAS;SBS) IN STOOL SPECIMENS AND BACTERIAL ISOLATES WITH PCR

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Rabbit diarrhoea caused by toxigenic *Clostridium spiroforme* is responsible for significant losses in commercial rabbitries. Several phenotypic methods have been proposed for the identification of *C. spiroforme* but the biochemical tests are inappropriate for accurate species identification. The aim of this work is to design specific primers for identification of *C. spiroforme* and its binary toxin. The results indicate that our primers are specific for *C. spiroforme* and they are able to detect the bacteria with good sensitivity .

## ROLE OF *CLOSTRIDIUM DIFFICILE*-ASSOCIATED ENTERIC DISEASE IN ITALIAN COMMERCIAL RABBITRIES

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In order to investigate the role of *Clostridium difficile* (CD) in "rabbit enteritis complex", intestinal contents were collected from the small intestine and caecum of 317 rabbits with enteric lesions and 80 control rabbits. The animals were selected on the base of age and enteric lesions (caecal constipation or fluid-filled caecum). Intestinal contents were ethanol treated and cultured in a prereduced selective medium for CD. Isolates were identified by commercial biochemical panel kit and by the specific *C. difficile tpi* gene PCR. Each isolate was tested by multiplex PCR to reveal the presence of *cdtA* and *cdtB* genes encoding for toxin A and toxin B respectively. Intestinal contents were screened for *C. difficile* toxins A and B by using a commercial ELISA. *C. difficile* was recovered from the intestinal content of 10 rabbits. All the positive animals were older than 35 d. *C. difficile* was not isolated from control rabbits. Eight strains resulted positive for both *cdtA* and *cdtB* genes. One strain was $cdtA^-/cdtB^+$, and one strain was $cdtA^-/cdtB^-$. In 10 samples the presence of toxins by a commercial ELISA was detected. This study demonstrates that CD is occasionally involved in outbreaks of enteric diseases in Italian rabbitries.

## NEST'S MICROBIOLOGICAL PROPERTIES AND PATHOLOGY OF NEW-BORN RABBIT

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The research had the purpose to check the efficacy of two disinfectants of vegetable origin, employed in the

rabbit's nest. A total of 880 microbiological examinations into 60 nests at 1, 5, 12 and 19 d of life of new-born rabbits have been carried out. It has been proved the disinfectants' efficacy concerning *Staphylococcus* spp. and *Escherichia coli*. The mortality of new-born rabbits, up to 25 d of life, settled down between 4,5% and 6,5% in the treated samples against 12,5%-13,3% of the checked units.

## SEROLOGICAL RESEARCH INTO ENCEPHALITOZOON CUNICULI ON PET RABBITS. PRELIMINARY OBSERVATIONS

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During 18 months, 334 samples of pet rabbits serum have been collected and subjected, through CIA test, to an antibody research as regard *Encephalitozoon cuniculi*. 61,7% of samples shown a positive resulte, without any difference of age or sex of the examined animals. On the subjects with renal or nervous symptomatology, seroprevalence was of 74%. The research shows how this *microsporidium* is also largely wide-spread in pet rabbits living in Italy.

## BIOTYPING AND ANTIBIOTIC RESISTENCE OF *ESCHERICHIA COLI* STRAINS ISOLATED FROM ASYMPTOMATIC IMPORTED WILD HARES (*LEPUS EUROPAEUS)*

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A total of 44 *E. coli*, isolated from faeces of asymptomatic wild hares imported from Argentina (240 animals), Uruguay (120 animals) and Czech Republic (140 animal) are biotyped and checked for antibiotic sensitivity (nalidixic acid, aminosidin, apramycin, chloramphenicol, ciprofloxacin, colistin, sulph+trimethoprim, enrofloxacin, flumequine, gentamicin, neomycin, norfloxacin, streptomycin and the three sulphanilamide). Six different biotypes were identified: B18 (n. 7), B23 (n. 1), B28 (n. 5), B29 (n. 1), B30 (n.15) and B31 (n.15). Most of tested antibiotics resulted active (except for neomycin, active only on 3 strains). These preliminary findings need to be complete with serotype studies and identification of the gene coding for intimin and adesine, to give a fuller picture of the situation in these wild animals.

## ISOLATION AND ANTIMICROBIAL SUSCEPTIBILITY OF BACTERIAL PATHOGENS OF THE RABBIT

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The intensive rabbit breeding presents frequent bacterial diseases, able to damage the management. There are few drugs registered for rabbits and the repeated use of them leads to antibiotic resistance. From 01-01-2006 to 31-05-2007, 355 rabbits have been controlled through necroscopical, parasitological and bacteriological examinations. On the aerobic isolated bacteria the antimicrobial susceptibility test, following Kirby Bauer technique, has been applied. The results reveal that the period of breeding from 35 to 55 d of age is the most critical. In breeders, the most frequent lesions interest the gastroenteric apparatus, followed by the genitourinary, respiratory and cutaneous ones. Sucklings present frequent respiratory pathologic lesions. Bacteriological researches show the presence of *Escherichia coli* in 29,8% of animals, *Staphylococcus aureus* in 9,3% and *Pasteurella multocida* in 7,9%. The results of antimicrobial susceptibility tests show that a great part of bacteria isolated present many antibiotic resistances: an important problem not only in rabbit breeding but also for public health.

## STRATEGIES TO OPTIMIZE THE ANTIBIOTICS USE IN RABBIT BREEDING: PRUDENT USE

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There is a concern that using antibiotics in food producing animals may select for resistant pathogens. Antibiotics are, and will continue to be, necessary to ensure the health and welfare in food-producing animals. Antibiotics may be used to treat sick animals or to control or prevent disease outbreak. By reducing or eliminating specific animal pathogens, antibiotics used in food-producing animals improve food safety and quality. In the rabbit breeding, antibiotics should be used according the principles of judicious (or prudent) use guidelines. Within these principles lies the concept of precision therapy. The objective is to minimize resistance development in bacterial strains and to deliver appropriate veterinary treatment for animals by using the most appropriate antibiotic. Precision therapy involves an accurate diagnosis of an imminent or ongoing disease process, an implementation of a treatment protocol using a narrow (or limited spectrum) antibiotic, and a regular evaluation of the effectiveness of the treatment protocol. In the rabbit breeding an early intervention is an integral part of the precision therapy

approach and may prevent a disease outbreak or may prevent the need for further treatment.

## USE OF RABBIT HARD FAECES TO STUDY THE EFFECT OF CUT AND YEAR OF PRODUCTION ON ALFALFA ORGANIC MATTER DIGESTIBILITY

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Samples, in triplicate, of alfalfa from 3 cuts and 2 years of production were analysed for organic matter digestibility (OMd) by the DI[II] Ankom apparatus using rabbit hard faeces as inoculum. The first cut showed higher OMd (54.0 *vs* 52.3 and 51.8%, for I, II and III cut respectively, $P<0.01$) and the first year had higher OMd than the second year (53.1 *vs* 52.4%, $P<0.01$). Alfalfa OMd was correlated to protein (r=0,67) and lignin (r=−0,48) concentrations. The results suggest that this *in vitro* technique could be tested to be used in rabbit feed digestibility studies.

## EFFECT OF VEGETABLE AND FISH FAT QUALITY ON RABBIT FEED DIGESTIBILITY

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The present work aims to evaluate the effect of fish oil quality, oxidation level of fats and the presence of *trans* fatty acids and PAHs on the apparent digestibility coefficients from main nutrients of rabbit feed. Experimental feeds were formulated including 3% of fats, in 4 trials: 1. Palm fatty acid distillate (LT) and its hydrogenated palm fatty acid distillate (HT), 2. Two fish oils with different origin and quality (FO1 and FO2). 3. Two acid oils from chemical refining of olive oil and olive pomace oil, respectively with low (LP) and high (HP) PAHs content. 4. Vegetable oil, fresh (low oxidation; LO) or recycled after using in a commercial frying process (high oxidation HO). A total of 96 rabbits were used for measuring faecal apparent digestibility of nutrients. Neither the type of fish oil nor lipid oxidation had effects on digestibility values. HT diet showed lower EE and GE digestibility coefficients than LT diet, while HP diet showed general impairment of digestibility in comparison with diet LP. Additionally, EE digestibility varied depending on the added fat, averaging 82% when fish or vegetable oils and 73% when palm fatty acid distillate or acid oils. Finally, some digestibility values were lower in PAHs and lipid oxidation trials than in the other two (56.5 *vs* 59.7%, 57.4 *vs* 60.6%, 55.7 *vs* 61.7%, 28.9 *vs* 38.0% and 22.0 *vs* 30.4% for DM, OM, GE, NDF and ADF, respectively), probably as a consequence of in feed antibiotics affecting the caecal microbiota and fermentation.

## EFFECT OF THE DIETARY INCLUSION OF GLUTAMINE AND ARGININE ON THE MORTALITY AND INTESTINAL BARRIER IN WEANED RABBITS

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The aim of this work was to study the effect of the supplementation with glutamine and arginine on the intestinal barrier and mortality in rabbits weaned at 25 d, in a farm affected by epizootic rabbit enteropathy (ERE). A control diet (C) and two additional diets with similar composition to C but supplemented with 1% of glutamine (GLU) and 1% of glutamine and 0.5% of arginine (GLU+ARG) were formulated. A mortality trial was carried on 119 rabbits per diet, which were fed the experimental diets the first two weeks after weaning and thereafter received a commercial diet until 56 d of age. The morphology and N-aminopeptidase activity of the jejunum was studied in eight animals per diet at 35 d of age. The supplementation with glutamine or arginine did not affect either the morphology or the activity of N-aminopeptidases of the jejunum. The supplementation with glutamine reduced ($P<0.05$) the mortality both in the starter period (from 25 to 39 d of age) and during the whole fattening period, by 55% and 28% respectively.

## USE OF RABBIT HARD FAECES TO STUDY THE EFFECT OF COMPOST ORGANIC AMENDMENTS ON ALFALFA *IN VITRO* DIGESTIBILITY

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The effects of different phosphorus (75 kg $ha^{-1}$) fertilization treatment (municipal solid waste compost, olive pomace compost, mineral fertilizer) and an unfertilized treatment were compared on *in vitro* organic matter digestibility (OMd) of three cuts of alfalfa. The OMd was determined by the DI[II] Ankom apparatus using rabbit hard faeces as source of inoculum. The

first cut alfalfa showed the highest OMd (52.3 *vs* 51.1 and 51.4%, for 1st, 2nd and 3th cut, respectively). Olive pomace and mineral fertilizer treatments increased alfalfa OMd (52.4 and 52.8%) in comparison with control and waste compost treatments (50.2 and 51.0%). The results suggest that rabbit faeces is a potential inoculum for *in vitro* digestibility trials to be tested with *in vivo* digestibility trials.

## NEW INSIGHTS ON COAT COLOUR GENETICS IN RABBIT COMING FROM CANDIDATE GENE ANALYSIS

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We recently showed that mutations in the rabbit *MC1R* gene are associated with coat colours in different breeds. Here, we completed the characterization of the *MC1R* gene and further supported the association of the 30 bp deletion in this gene with the production of pheomelanic coat colours. Moreover, we identified 4 single nucleotide polymorphisms in the agouti (agouti signaling protein, *ASIP*) gene. These markers will be useful in association studies with coat colour in different rabbit breeds.

## A SURVEY ON RABBIT FARMS IN VENETO REGION: TECHNICAL DATA AND NITROGEN EXCRETION ESTIMATE

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A survey was performed on 48 rabbit closed-cycle farms located in Veneto Region to collect technical information and calculate nitrogen balance. Farms showed great variability in dimensions, management and productivity with on average about 1,200 reproducing does and 54,000 sold rabbits/year. The number of sold rabbits/doe/year was 42.8 on average (from 28.9 to 60.9). Data of reproductive efficiency were significantly correlated with the number of sold rabbits/doe/year, while independent from the number of reproducing does. The N excreted by the doe and its offspring was on average 7.40 kg/year and positively correlated with slaughter weight and number of sold rabbits/doe/year.

## ECONOMIC IMPACT OF LONG TERM POST-WEANING RHYTHM

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The animal welfare concerns have produced changes in European legislation for the livestock industry, and the most part of the research are focused to verify the animal welfare impact after the adoption of a low density of rabbits, different dimension of the cages, and different environmental conditions where to raise the rabbits. The aim of this paper was to verify the economic impact after the adoption a long term post-weaning rhythm. The results highlight a favorable impact on the economic performances of the enterprise and a low level of economic risk of activity.

## A SURVEY ON PRESLAUGHTER TRANSPORT MANAGEMENT OF RABBITS UNDER COMMERCIAL CONDITIONS

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A survey was conducted in order to assess the preslaughter conditions of rabbits in a commercial chain and to determine the effect of the season, journey time and lairage time on mortality, liveweight loss and slaughter yield. The study was carried out during a one-year period considering 975 rabbit flocks (average of 2,207 rabbits/flock) slaughtered towards a major Italian abattoir. The overall average incidence of mortality and liveweight loss were found to be 0.079 and 3.39%, respectively. During the winter, it was observed lower ($P \leq 0.01$) liveweight loss, while slaughter yield was higher ($P \leq 0.01$) during the summer. The shortest transport time (≤3 h) exhibited lower ($P \leq 0.01$) mortality rate and liveweight loss and higher ($P \leq 0.01$) slaughter yield in respect with medium (3-5 h) and long (>5 h) transport times. Rabbits laired for less than 2h also exhibited a significantly lower ($P \leq 0.01$) mortality rate and higher ($P \leq 0.01$) carcass yield compared to medium (2-4 h) and long (>4 h) lairing times. This survey has shown that the main preslaughter critical points are represented by long transport and lairage which can impair mortality rate and slaughtering yields.

## EFFECT OF PASTURE AVAILABILITY ON PERFORMANCE AND MEAT QUALITY OF GROWING RABBITS

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To verify the effect of pasture availability on performance and meat quality of fattening rabbits, 40 Leprino di Viterbo were assigned to two homogeneous (sex and weight) groups: Control, reared in standard cages

and Pen, provided of a grass pasture area. Productive performance and meat quality were strongly affected by housing system and in particular, the possibility of performing movement and grass ingestion produced lighter live weight and chilled carcass. Pen-housed rabbits showed lower daily weigh gain, feed efficiency index, mortality rate and higher percentages of gastro intestinal tract, hind leg, bone with lower fat and hind leg meat to bone ratio. Shear force of bone and *longissimus dorsi* were always higher in pen-housed rabbits. The pH and color parameters were also affected by rearing systems and pen-housed rabbits gave lower pH (initial and final) with more intense colored meat.

## POSTERS

### STUDY OF REPRODUCTION RHYTHMS ADAPTED TO RABBIT DOE PHYSIOLOGY. 1. BLOOD NEFA PROFILE AS AN INDICATOR OF BODY CONDITION AND RELATION WITH REPRODUCTION EFFICIENCY

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This research evaluated body condition and pregnancy rate of 120 hybrid rabbit does as affected by different reproduction rhythms: A.I. at 11 (I11=60) or 21 (I21=60) d after kindling and weaning at 35 d. Blood samplings were repeated at 12, 22 and 36 d *postpartum*. The extensive reproduction rhythm (I21 *vs* I11) increased the pregnancy rate as assessed at 15 d post A.I: 71.4 *vs* 82.7 % ($P$<0.001). NEFA mean concentration was lower in I21 than in I11 rabbit does (0.280 *vs* 0.314 mmol/L; $P$<0.05), reflecting a metabolism less directed towards catabolism of reserves. Moreover, NEFA values were consistent with a steadier mobilization of adipose depots during lactation in I21 than in I11 does (0.249-0.289-0.301 *vs* 0.284-0.339-0.318 mmol/L, respectively). The I21 reproductive rhythm fitted better the physiology of does and improved their reproduction efficiency, even if the impact of lactation stage at the moment of A.I. needs to be clarified.

### EFFECT OF DIETARY MICRO-ENCAPSULATED ORGANIC AND INORGANIC ACIDS AND ESSENTIAL OILS ON SERUM INNATE AND CAECAL FERMENTATION

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A total of 120 young weaned rabbits (27 d), were divided into three homogeneous groups and submitted to the following dietary treatments: Basal diet; Diet 2 (basal diet + 150 ppm Zinc Bacitracine); Diet 3 (basal diet + 0.4% FormaXol - mixture of microincapsulated formic acid, citric acid and essential oils). In the period 65 - 80 d of age, the antibiotic was removed in the Diet 2 whilst in the Diet 3 group, AciXol (blend of microencapsulated fumaric, citric, ortophosphoric, malic acids along with essential oils) at 0.1% was added in replacement of FormaXol. To analyse the serum immunity (lysozyme, haemolytic complement assay and serum bactericidal activity) haematic samples were made by cardiac puncture of 5 animals/group on different time (27, 35, 65 and 80 d old). At 65 and 80 d of age 5 animals/group were sacrificed to value the caecal fermentation. The serum bactericidal activity in the three diets showed a physiological trend, but the Lysozime and haemolytic complement in Basal diet and in Diet 2 showed an irregular trend probably to ascribe to sub clinical inflammation; in Diet 3 the lysozyme and the haemolytic complement values showed a physiological trend without any drastic change. Diet 3 showed a good fermentative activity explained with higher acetic acid concentration ($P$<0,05) and higher Volatile Fatty Acids produced. In conclusion, the blend of coated organic acids and essential oils integration allows to obtain a better serum innate and a good caecal fementation.

### INFLUENCE OF WEANING AGE AND FIBER DIETARY LEVEL ON PRODUCTIVE PERFORMANCE OF RABBIT

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A total of 632 New Zealand White rabbits reared at Dip. de Producción Animal (Università Politecnica di Madrid), were fed *ad libitum* two diets from 21 d of age: diet 1 (40% NDF and 9% starch) and diet 2 (32% NDF and 17.2% starch). Each group was divided in two subgroups in function of the weaning age: 28 and 42 d old. Animals were slaughtered at 56 d of age. Individual weights were recorded at 28, 42 and 56 d, mortality percentage and average daily gain were calculated for each group. Body weight at 56 d old ($P$<0.0001) and average daily gain ($P$<0.001) were lower in rabbits weaned at 28 d old. Diet 1fed rabbits showed lower

body weight ($P<0.001$) irrespective the age of weaning. The mortality was higher in rabbits weaned at 28 d ($P<0.01$), while diet did not affect this parameter. Our results, in conclusion, show that young rabbits weaned at 42 d and fed diets with fiber level of 32% and starch of 17.22% improved their growing performance.

## DIETARY INTEGRATION OF MICROENCAPSULATED ORGANIC ACIDS AND ESSENTIAL OILS ON THE CONTROL OF ENTERIC INFECTIONS IN RABBIT

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120 young weaned rabbits (28 d old), were divided in three groups and submitted to the following dietary treatments: basal diet; diet 2 (basal diet + 150 ppm Zinc Bacitracine); diet 3 (basal diet + 0.4% FormaXol - mixture of microencapsulated formic acid source, citric acid 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 during four weeks. Thirty d after infection, all rabbits were euthanized and intestinal swabs were collected from different tracts of small intestine, colon and caecum for investigation of aerobic and anaerobic bacteria; moreover a jejune portion was excised to analyze the villi height. The diets 2 and 3 group were harboured the similar percentage of *C. perfringens type A* and *E. coli O103*. From the rabbits fed diet 3, were harboured the highest percentage of no-pathogen bacteria. Moreover the results of villi height confirm this analyse, showing a better value of villi height with FormaXol integration than the basal diet, demonstrating a good efficacy to reduce the damage of both *Gram* $^-$ and *Gram* $^+$ pathogen bacteria in experimentally infected rabbits.

## DEVELOPMENT AND STANDARDIZATION OF A SEROLOGICAL ELISA TEST FOR MIXOMATOSIS

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The myxomavirus (MV) is the poxvirus that causes myxomatosis in the European rabbit, a disease responsible of severe losses to rabbit meat industry. The DNA of MV encodes for more than 100 proteins, most of which directly stimulate the immune system of the host. Protection induced by live vaccination is mainly based on cellular immunity but the detection of humoral antibodies is important for epidemiological studies and surveillance programs. We set up and used a competition ELISA based on the use of a monoclonal antibodies (MAb 1E5) that recognises an immunodominant protein of MV, named m71L. Such protein has been identified by immunoprecipitation with MAb 1E5 followed by analysis with mass spectrometry. The antigen was obtained from infected RK13 cell cultures. The ELISA test was initially used to detect antibodies both in vaccinated animals (titres at 1 month p.v. ranged between 1/80 and 1/640) and in convalescent animals (titres up to 1/20480). The validation of such method is not yet completed but preliminary results suggest a good sensitivity and a high specificity.

## OVARIAN ABSCESSES AND PURULENT SALPINGITIS OBSERVED IN COMMERCIAL RABBITRIES

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The increase of infertility rate in rabbit commercial farms represents a significant economic damage. Poor semen quality, nutritional deficiencies, adverse microclimatic conditions, artificial insemination mistakes and infective diseases should be suspected in ipofertility outbreaks. Infective diseases of rabbit reproductive system are supported by viruses (mixomatosis, RHD), bacteria (*Staphylococcus*, *Pasteurella*, Bordetella, *Pseudomonas*, *Salmonella*, *Listeria*, *Chlamydophila*, *Leptospira*, *Mycoplasma*), parasites (*Toxoplasma*). The present case report describes heavy reduction of fertility performances in commercial rabbitries due to an increase of periovarian abscesses and purulent salpingitis in does. Lesions were observed only in reproductive system (ovary, salpinx and sometimes uterus), therefore an ascending origin of pyogenic bacteria was suspected. Artificial insemination mistakes and semen contaminations have to be investigated to prevent such irreversible damages on does reproductive system.

## STUDY ABOUT *MEC*A GENE'S PREVALENCE IN *S. AUREUS* STRAINS ISOLATED IN RABBIT FARM: PRELIMINARY RESULTS

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The continuously high prevalence of methicillin resistant staphylococci (MRSA) throughout the

world, accompanied by the multiresistance pattern to antimicrobial agents is of relevant concern in public health. MRSA strains are associated with the presence of the penicillin binding protein (PBP)2a, encoded by *mec*A gene. Recently, MRSA was found in meat products. Initially, MRSA infections were a nosocomial problem; in the early 1980s cases of community-acquired MRSA have been described in persons without exposure to hospital or to other risk factors. In this paper we describe the detection of *mec*A gene by PCR in 73 strains of *S. aureus* isolated during staphylococcal infections in rabbit. Moreover the correlation between presence of *mec*A gene and resistance to oxacillin and cloxacillin was evaluated. Results indicate that all the strains don't harbour the *mec*A gene. Susceptibility to the antibiotics suggests that all the strains are sensitive to oxacillin and 72/73 to cloxacillin. Only 14% are penicillin resistant.

## SURVEY ON THE PREVALENCE OF THE BACTERIAL RESPIRATORY PATHOGENS IN INTENSIVE MANAGEMENT RABBIT FARMS IN CAMPANIA REGION

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The present study was undertaken with the aim to evaluate the health status of rabbits. In particular, bacterial respiratory diseases were investigated. 15 intensive rabbit farms (with a number of rabbit does more than 500) located in the Campania region were analysed. Each farm was twice sampled during the period November 2005/November 2006. In particular, 20 nasal swabs/farm were performed to check *Pasteurella multocida*, *Bordetella bronchiseptica*, *Staphylococcus aureus*. Moreover, the animals found dead were necropsied and analysed for *P. multocida*, *B. bronchiseptica*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* following the procedures suggested by Office of International Epizooties. *P. multocida* was isolated in 105/600 nasal swabs analysed. Furthermore, *B. bronchiseptica* and *S. aureus* were found in 8/600 and 82/600 nasal swabs respectively. Finally, out of the 22 carcasses analysed, *P. multocida* (n=12), *B. bronchiseptica* (n=3), *P. aeruginosa* (n=3), *K. pneumoniae* (n=1), *S. aureus* (n=9) were isolated. Our results show that the mortality rate due to specific respiratory pathogens (*Pasteurella multocida* e *Bordetella bronchiseptica*) are limited. In contrast, the remainder isolates represent a serious problem for the animals subjected to stress condition.

## EFFICACY OF TOLTRAZURIL AGAINST EIMERIA SPP. IN RABBITS

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The anticoccidial effect of toltrazuril against *Eimeria spp* in rabbits was tested. The study was carried out on five groups of rabbits after weaning. Every group was composed from eleven rabbits. The administration of drug was effected through the mouth for means of one syringe to the dose of 10 mg/Kg bw for 1 day (group B) and for 2 d (group C) and to the dose of 20 mg/Kg bw for 1 day (group D) and for 2 d (group E). The group A has been used like control. The groups C, D and E have stopped to release oocysts after 6 d from the administration of toltrazuril. The groups A and B have continued to release oocysts for some d until stopping to release oocysts later.

## AN OUTBREAK OF MYXOMATOSIS IN GREEK RABBIT FARMS DURING YEAR 2007

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Myxomatosis is considered as one of the most frequent endemic fatal viral diseases of rabbits in all over Europe except for Greece. Latest outbreak in our country was reported in 1973 by E. Stoforos. Even though Greek rabbit farms are totally dependent on purchasing parent stock from other European countries where myxomatosis is occurent, the disease was not clinically presented in Greek farms, until February 2007. No vaccination against myxoma virus was applied in Greek rabbit farms and epizootiologically the importation of the disease was inevitable, as no special preventive measurements were taken for the imported carrier animals. So, early in spring 2007, a myxomatosis outbreak was reported in two rabbit farms, situated in between a 150 Km distance that were sharing the same parent stock supplier, importing rabbits from a neighboring country. Mortality rate reached 80-90% and myxomatosis diagnosis was based on clinical signs and laboratory confirmation performed by PCR, virus isolation and histology. Eradication of the disease was applied in the affected farms by euthanizing the total rabbit population together with thoroughly applied hygienic measures. Prevention policy by vaccination was not encouraged by the local authorities in order to keep Greece Myxomatosis free status. On the other hand, veterinary services were alerted and quarantine

measures were strongly recommended to rabbit farmers every time that rabbit parent stock is imported.

## DOUBLE LACTATION IN RABBITS: EFFECT ON MILK PRODUCTION, FEED INTAKE AND PERFORMANCE OF LITTERS

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Does double lactation influence growth performance of litters? To answer this question, 42 multiparous rabbit does and their offspring were allocated to 3 groups, characterized by different frequency of lactation: free (group L), once a day (group S), double lactation (group D). The total milk production of group L (similar to that of group D) was higher ($P$<0,05) than that of group S (5,71 *vs*. 5,33 kg). The solid feed intake, from day 28 day onward, was higher ($P$<0,01) for D than L litters (41,2 *vs*. 33,6 g/d). At weaning, the weight of litters D and S was lower than that of group L (5150, 5042 and 5559 g, for D, S and L, respectively). Mortality rate did not differ between groups throughout the experimental period. These data, together with the scarce willingness of does to get into the nest box for the second lactation, suggest that the double lactation method could be useful only using hybrid characterized by high milk production. The better performance obtained with the free lactation confirm that the duration of controlled nursing could be carry out for a restricted period to prevent decrease of litter growth.

## OXIDATIVE STRESS IN SLOW GROWING RABBITS

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The aim of the study was to determine the oxidative stress in growing rabbits reared in standard condition. Blood samples were collected into EDTA-K tubes from the ear vein of 41 rabbits. Blood collections were scheduled at 57, 80 and 100 d of age. The markers of oxidative stress analysed, on red blood cells, were superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and thiobarbituric acid reactive substance (TBAR'S). The relationship between TBAR'S and enzyme activities was analysed by single regressions analysis. The parameters were analysed by ANOVA considering age as main categorical factor. The results showed high correlations between the parameters studied. The levels of antioxidant defenses, such as SOD and GPx activities, significantly decrease with ageing, while the levels of TBAR'S increase ($P$<0,01). The data indicate that a moderate situation of oxidative stress is age related.

## INFLUENCE OF DIETARY USE OF WHOLE LINSEED ON RABBIT MEAT QUALITY

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During the last period of growing (55-81d of age) 288 rabbits were fed a commercial diet (C) or diets containing 3, 6, or 9% whole linseed (L3, L6, L9, respectively). Whole sunflower was used as substituting ingredient for linseed (6, 3, 0% in L3, L6, and L9, respectively). Cooking loss, lipid content, fatty acid composition and TBARS were determined on both whole raw meat (*L. lumborum* and hindleg muscles) and minced meat (hamburgers). The use of linseed determined a lower content of total saturated fatty acid and a higher content of PUFA ($P$<0.01). The PUFA n-3 content of the meat increased significantly ($P$<0.01) from C toward L3, L6, and L9, mainly due to the increased content of α-linolenic acid, which also determined a reduction of the n-6/n-3 PUFA ratio. The hamburgers from L6 and L9 exhibited a higher TBARS in comparison with L3 and C. Finally, the cooking loss of hamburgers was lower in rabbits fed the control diet (C), intermediate in L3 and higher in L6 and L9 ($P$<0.01). However, the diet did not affect the cooking loss and TBARS of whole *L. lumborum*. Overall, the use of 3% linseed represents a good compromise to achieve both the enrichment of the meat with α-linolenic acid and maintain product quality.