

**ABSTRACTS OF THE 46<sup>TH</sup> SYMPOSIUM ON CUNICULTURE, ASESCU**PINEDA DE MAR, SPAIN, 1<sup>ST</sup>-2<sup>ND</sup> OCTOBER, 2022.

The 46<sup>th</sup> Congress of the Spanish Association of Cuniculture (ASESCU) was held in Pineda de Mar (Barcelona, Catalonia, Spain) from 1<sup>st</sup> to 2<sup>nd</sup> June 2022, hosted by the Institute of Agrifood Research and Technology (IRTA) and the Department of Climate Action, Food and Rural Agenda of the Government of Catalonia. Four main guest lectures were given. The first explained the trends in the meat products market and the implications for the rabbit sector. Another presented an overview of rabbit farming in Catalonia. Moreover, another talk addressed the question of how much a rabbit farm pollutes. Finally, a fourth talk was addressed to the prudent use of antibiotics in rabbit farming in a context of reducing the use of antimicrobials. A round table was held on the demands of European legislation and citizens in terms of rabbit housing and welfare, with the participation of experts from Belgium, Italy and Spain. In addition, a total of 26 communications were presented in working sessions (genetics, nutrition, reproduction, and pathology and welfare) as oral communications and posters. The meeting was attended by almost 140 participants from several European and American countries. Abstracts of the contributions presented are reported below.

**MAIN PAPERS****HOW MUCH POLLUTION COMES FROM A RABBIT FARM?**

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As with other human activities, livestock production involves the risk of several types of environmental impact that have to be minimised. In particular, global livestock production affects global nutrient cycles and atmospheric emissions. The rabbit production sector has a comparatively minor size related to other sectors. Nevertheless, it is necessary to improve the efficiency and reduce their impacts, contributing to a minor environmental damage. Additionally, rabbit production has some particularities that may position this sector as an alternative regarding the use of raw materials and emission mitigation, which may be more difficult in other sectors. This contribution summarises some of the main opportunities for the rabbit sector.

**PRUDENT USE OF ANTIBIOTICS IN RABBIT FARMING, A WELL-DONE TASK**

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In April 2017, rabbit farming veterinarians —aware of the problem of bacterial resistance to antibiotics— began contacting the AEMPS (Spanish Medicines Agency) to draw up a logical but effective plan that would reduce antibiotic pressure on farms. As a tool, the ARAC agreement (within the Reduce initiative) between the AEMPS and AVECU (Rabbit Farming Specialised Veterinarian Association) was launched. ARAC drafted the guidelines to reduce the use of antimicrobials in rabbit farming by 30% in two years and establish “Colistin zero” in medicated feed. Today, we have achieved a reduction in antibiotic use of more than 55%, expressed in mg of atbc/PCU, and we continue to decrease. With Regulation (EU) 2019/4 in force as of January 28th, 2022, we are now working with only one molecule in fattening and we have set new objectives for antibiotic use in line with the Green Deal. They consist of achieving a

maximum antibiotic pressure of 350 mg/PCU on medicated feed in 2025, as well as “Zero Enrofloxacin” by the same date. Not only the vets, but also INTERCÚN and all of the rabbit farmers are fully involved in this project. A project that is not free, but requires strong investments by rabbit farmers and assumes higher mortality figures at a time of very low profitability. Our goal is ambitious. It will again require a great effort from the sector and its technicians, as well as extra help from the AEMPS and MAPA (Ministry of Agriculture, Fisheries and Food). We have shown that “we know how to do our homework”; all forms of support from the Administration for our work are welcome.

## GENETICS

### SELECTION FOR ENVIRONMENTAL VARIABILITY OF LITTER SIZE. I: SURVIVAL CURVES

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A divergent selection experiment for litter size variability is being carried out in rabbits. The selection criterion is the phenotypic variance of litter size within the female, after correcting litter size for the effects of year-season, and physiological status. In previous studies, the line selected to decrease litter size variability (Low) has shown less sensitivity to disease and stress than the heterogenous line (High). The objective of this work is to compare the survival curves of the lines according to the order of parturition in which the female is eliminated or dies, as well as to study the causes of elimination. From the 4th parturition, the percentage of elimination or death of the Low line is lower than in the High line. The distribution of the Low line females according to the causes of elimination was: 39% by end of generation, 21% eliminated due to some pathology and 40% dead. The distribution was 30, 34 and 36%, respectively, for the High line. In conclusion, divergent selection for litter size variability seems to have modified the survival curves of the females. In addition, the elimination rate at the end of the generation is higher in Low line females than in High line females.

### SELECTION FOR ENVIRONMENTAL LITTER SIZE VARIABILITY. II: EMISSIVITY OF BODY TEMPERATURE

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A divergent selection experiment for litter size residual variability has been carried out in rabbits during 12 generations. Stress causes an increase in body temperature. Infrared thermography (IRT) has been shown to be a useful technique for identifying changes in body temperature emissivity. The aim of this work was to study the correlated response to selection for litter size residual variability in body temperature emissivity at mating. Mating can be considered a stressful stimulus for the doe. Temperature was measured in the eyeball by IRT before mating (basal temperature) and after 5, 30 and 60 min in does from homogenous and heterogenous lines. Both lines showed similar basal temperature (35.69°C in the homogenous line vs. 35.81°C in the heterogenous line). The homogenous line increased slightly in temperature at 5 min after a stressful stimulus (36.32°C), and this increase remained up to 60 min (36.55°C). We found no evidence of temperature differences at 5 min after mating between lines. However, the heterogenous line showed a higher temperature than the homogenous line at 30 min (+0.96°C,  $P=0.99$ ). At 60 min, temperature was similar between lines. The temperature evolution was different between lines, as the heterogenous line reached the peak temperature later than the homogenous line (30 vs. 5 min), and its peak was higher compared to the homogenous one (36.95 vs. 36.32°C). In conclusion, the does selected for reducing litter size variability presented a lower increase in temperature after a stressful stimulus, thus showing a lower response to stress and consequently better welfare.

### GENOMIC REGIONS OF MEAT RABBIT INVOLVED IN THE VARIATION OF ITS CAECAL MICROBIOTA

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The aim of this study was to identify the regions involved in genetic control of the composition of different microbial communities present in the rabbit caecum. The available material consisted of the genotypes (~200K single nucleotide polymorphisms; SNPs) and a set of microbial traits representative of the caecal microbiota of 412 rabbits. Two approaches were used to identify host genomic regions associated with microbial phenotypes: the implementation of a mixed regression model for each SNP (MIX-GWAS) and the simultaneous fit of all SNPs, assuming *a priori* that only 1% have an effect, with

BayesC. Initially, in order to evaluate the statistical power of both approaches, a simulated microbial phenotype generated under the structure of the real data was analysed for three heritability scenarios. Simulation results suggest the rather limited power of both approaches with the available data to detect the quantitative trait nucleotides (QTNs) associated with the variation of caecal microbiota. Although the probability of capturing a signal corresponding to a QTN is higher with the MIX-GWAS, the positive predictive value of this approach is one-third that of the BayesC. Only the results of the analysis of the real data with the MIX-GWAS method revealed a QTL region on chromosome 12 associated with the variation of an OTU belonging to the genus *Butyricimonas*. This result suggests that a variant in this chromosomal region could favour the presence of butyrate-producing bacteria in the rabbit caecum and contribute to maintaining the integrity of its mucosa. This region harbours *CD83* gen that codes for a membrane protein involved in the immune system, and this function makes this gen a serious candidate to explain the abundance of bacteria from *Butyricimonas* genus.

#### CHARACTERISATION OF FEEDING BEHAVIOUR IN RESTRICTED AND *AD LIBITUM*-FED ANIMALS

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The feeding behaviour of fattening rabbits housed in groups from 42 to 60 d of age was analysed through the use of electronic feeders that measure feed intake and consumption time for each animal. The animals belonged to two lines selected for feed efficiency during fattening, with two different selection criteria: ADGR, selected for daily growth under feed restriction, and RFI, selected for residual consumption (360 animals per line). The animals of the ADGR line, reared under time restriction (6:00 p.m. to 6:00 a.m.; approx. 80% of *ad libitum*) showed a higher rate of feed intake (+34%), and a higher level of competition between individuals, determined by a higher coefficient of variation of the occupation time and daily consumption, as well as by a greater feed intake during the hours of no restriction, than the animals of the RFI line, which were fed *ad libitum*.

#### FESC LINE. PARAMETERS AFTER 28 YEARS SINCE ITS CREATION

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Genetic lines results from the mating of animals of different breeds, whose main features are smaller populations in number and having a productive specialisation. In this project, we evaluate some productive parameters from the FESC genetic line, which came into being in 1994 at the Facultad de Estudios Superiores Cuautitlán (México) from where it acquires its name. Their current fertility rate is 88.7%, with 9 rabbits born alive and 8.65 rabbits reared to weaning, making them a great alternative for rabbit farmers.

## NUTRITION

#### EFFECT OF DIETARY LEVEL OF ARGININE ON GROWTH PERFORMANCE IN TWO RABBIT GENETIC LINES

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Five isonutritive diets were formulated except for the arginine content (6.5, 9.6, 11.1, 13.2 and 14.9 g/kg dry matter for diets P1, P2, P3, P4 and P5, respectively). A total of 345 weaned rabbits (28 d) of the LP and RLP lines were used. The animals were housed in individual cages and fed *ad libitum*. Mortality and morbidity were recorded daily and the weight and feed intake were checked at 49 and 63 d. Regardless of genetic line, the P1 diet produced a lower weight gain and feed intake than the rest of the diets from 28 to 49 d (-25 and -26%, respectively,  $P < 0.001$ ). This lower growth was only partially compensated from 49 to 63 d, as in the whole growing period the weight gain and feed intake were also lower with the P1 diet than with the rest of the diets (-11 and -15%, respectively,  $P < 0.001$ ). No differences were found between diets P2, P3, P4 and P5 in weight gain, feed intake and conversion rate. Thus, it can be concluded that the arginine requirements for maximum weight gain are covered with 9.6 g/kg dry matter (P2). On the other hand, the RLP line presented higher weight gain and feed intake (+34 and +29%, respectively,  $P < 0.001$ ) and a better conversion rate (-5%,  $P = 0.026$ ) than the LP line. However, the LP line presented lower mortality than the RLP line under a digestive disorders outbreak (20.6 vs. 41.5%,  $P < 0.001$ ).

### XYLANASE SUPPLEMENTATION IN GROWING RABBIT DIETS: EFFECT ON APPARENT DIGESTIBLE AND METABOLISABLE ENERGIES

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A trial was conducted to evaluate the efficacy of a xylanase in improving nutrient digestibility of fattening rabbit diets. Thirty-four animals were used. After weaning (at 31 d of age), each animal was housed in an individual metabolic cage. At 42 d of age, animals were assigned to the experimental diets (17 animals/treatment). Animals received the experimental diet *ad libitum* until 53 d of age. From 49 to 53 d of age, the total amount of faeces and urine produced per cage was collected separately on a daily basis. There were two dietary treatments: Treatment 1, negative control diet (NC) without xylanase, and Treatment 2, NC+xylanase providing 1500 endopentosanase units per kg feed. Body weight and feed intake were measured individually. Feed, individual faeces and urine were analysed for dry matter (DM), organic matter (OM) and gross energy (GE). The apparent digestibility coefficients of these nutrients were calculated. Additionally, digestible and metabolisable energy were calculated. The inclusion of xylanase increased ( $P<0.05$ ) DM, OM and GE digestibility compared with the NC and increased ( $P<0.05$ ) both digestible and metabolisable energy content in the feed.

### IN VITRO STUDY OF IN VITRO DIGESTIBILITY AND FERMENTABILITY OF 19 SEAWEED PRODUCTS IN RABBITS

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The *in vitro* gas production at 24 h of dehydrated *Saccharina latissima* and its aqueous extract were similar to that of beet pulp, while its aqueous extract from the hydrolysed algae produced even more gas than the pulp (112 vs. 68 mL/g dry matter; DM substrate.  $P<0.05$ ). The aqueous extract obtained after hydrolysis of *Laminaria ochroleuca*

also produced a similar amount of gas as the pulp, while the other algae/extract studied produced an amount of gas that did not differ from that of the straw (5 mL/g DM), except for the extract of hydrolysed *Ulva* spp. which had an intermediate value (30 mL/g DM). The proportion of butyrate was higher in dehydrated *Himantalia elongata* and the extract of hydrolysed *Laminaria ochroleuca* than in beet pulp ( $P<0.05$ ). No correlation was observed between digestibility and gas production *in vitro*.

### EFFECT OF FIBRE TYPE ON PERFORMANCE OF GROWING RABBITS

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Four feeds were formulated with different levels of fibre (neutral detergent fibre/soluble fibre, % dry matter): medicated Control and unmedicated control (T-1) (41.2/9.4), with higher level of soluble fibre (T-2: 39.9/11.8) or higher level of insoluble fibre (T-3: 43.5/8.6). The experiment was conducted in two farms (A: semi-controlled environment —24 litters/treatment—, and B: 2 controlled environment rooms —10 litters/room and treatment). In the fattening period on farm A, medication reduced mortality compared to the unmedicated control (6.8 vs. 10.7%), while both insoluble and soluble fibre increased mortality (18.9 and 15.3%;  $P<0.05$ ). The group fed a higher level of insoluble fibre showed worse values for growth rate (–12%) and feed conversion ratio (–19%) than the other three groups ( $P<0.05$ ). On farm B, a room×treatment interaction was observed for mortality during fattening ( $P=0.037$ ). In room 1, rabbits from Control and T-1 groups showed the lowest mortalities (1.25% on average), while the group with the highest level of soluble fibre showed the highest mortality (12.5%;  $P<0.05$ ), not different from that observed for the group with the highest level of insoluble fibre (10%). In contrast, in room 2, the group fed with the highest level of soluble fibre showed the lowest mortality, while the highest one was observed in those fed with the highest level of insoluble fibre (3.75 vs. 18.7%), obtaining intermediate values for the groups fed with the Control feed and T-1. The growth rate of the groups did not differ in the Control and T-1 groups, while it was reduced by 4% in the group with the highest level of soluble fibre and by 6% in the group with the highest level of insoluble fibre ( $P<0.05$ ).

### EFFECT OF XYLOOLIGOSACCHARIDES SUPPLEMENTATION IN DRINKING WATER AND FEED RESTRICTION ON FAECAL MINERAL DIGESTIBILITY AND MINERAL RETENTION EFFICIENCY IN GROWING RABBITS

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Four factorially structured treatments were used: 2 levels of xylooligosaccharides (XOS: 0, and 7.5 g/L) ×2 feeding systems (*ad libitum* and restricted from 32 to 51 d of age). The restricted group received a ration that started at 50% of that of the *ad libitum* group on the day after weaning and increased linearly until reaching the same 100% intake as that of the *ad libitum* animals at 51 d of age. A total of 106 rabbits weaned at 32 d of age were used and were not medicated. Faecal digestibility was determined between 39 and 43 d and between 57 and 60 d of age (8-9/treatment). No interactions were observed between XOS supplementation and feed restriction. In the overall period, XOS supplementation did not affect the mineral balance. During restriction, mineral digestibility increased by 19% ( $P<0.001$ ) in the restricted group. In the overall period, feed restriction reduced ( $P=0.018$ ) digestible mineral intake (8%) and mineral retention in the body (5%) and carcass (2%) by 8%, with even greater reductions in mineral losses in skin and viscera (17%) and faeces (24%). This meant that the retention efficiency of digestible minerals increased by 5%. These results indicate that feed restriction improves mineral balance during fattening by reducing mineral excretion to the environment.

### EFFECTS OF A FEED RESTRICTION ON THE DIGESTIBILITY IN RABBITS AT THE END OF THE FATTENING PERIOD

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In the present study, we intend to evaluate the effect of a moderate food restriction on the intake and digestibility of rabbits during a fattening period. The study was carried out in the rabbit farming sector of UTAD, Portugal. Twenty-four rabbits at 62 d of age (12 males and 12 females) of the New Zealand White×Californian breed were used, which were monitored until 86 d of age. Rabbits were housed individually and distributed according to gender, randomly between the two treatments; one with *ad libitum* feed (*ad libitum* group) and another with feed restricted

to 80% of that of the *ad libitum* group (restricted group). Between days 75 and 80 of age, intake was monitored and faeces collected, which were then analysed to calculate the digestibility of the diet components. The sex of the rabbits did not affect any digestibility parameters. Feed restriction significantly ( $P<0.05$ ) reduced feed intake (-17%) and dry matter excretion (-21%) and increased protein digestibility (from 71.7 to 76.1%). The digestibility of the fibrous fraction and fat were not affected. According to the results obtained and according to the working conditions, moderate food restriction reduced intake and protein digestibility was improved.

## REPRODUCTION

### REPRODUCTIVE PERFORMANCE OF RABBIT FEMALES FROM PATERNAL LINES: RECONCILING GROWTH RATE AND RESILIENCE

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For this trial, data from 197 does belonging to three paternal lines, R, RF and RLP, were used. The R line was selected for growth rate during the growing period for 36 generations; the RF line was founded through a high selection intensity of elite animals from the R line and the RLP line, which was obtained by backcrossing RF animals with the LP line. LP is a longevous-productive maternal line, characterised by high resilience. Body weight, perirenal fat thickness, ingestion and milk production of females from the first to third parturition were monitored. RF females were lighter than R and RLP females (on av. -5.0%;  $P<0.05$ ). In general, RLP rabbit females yielded more milk than R and RF throughout the trial (on av. +18.5%;  $P<0.001$ ). The results obtained allow us to conclude that the foundation of a paternal line with elite animals seems to favour early reproduction. However, the introduction of resilient genetics improves almost all the reproductive traits of the females.

### RENEWAL OF DOES AS A WHOLE GROUP AND USE OF MULTIPAROUS FEMALES AS OCCASIONAL REPLACEMENT: REPRODUCTIVE AND MORTALITY RESULTS

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To avoid empty cages and unproductive does, an annual replacement of 120% is recommended, which means the periodic incorporation of nulliparous does. In this work, the renewal of complete groups of reproducers using multiparous does as replacement is proposed, analysing the reproductive and mortality results in a commercial farm that implemented this management. The data correspond to 1854 mothers' cages distributed in four buildings during 2019-20. The groups were made up of nulliparous does and were kept without replacement until the fifth parturition to improve biosecurity. From the sixth parturition, the dead+discarded females were replaced with productive and healthy multiparous does from other groups that ended as such. The occupation rate in the newly constituted batches was 149.0%, with an average of 131.7% in the period analysed, which is well adjusted to the fertility of the farm (75.5%). A moderate prolificacy (8.57 kits/parturition) was accompanied by very low mortality at birth and in lactation: 0.94 and 8.12%, respectively. The mothers' mortality+culling was 5.23% in each cycle of 42 d. An average of 7.82 weaned rabbits/parturition were obtained. The proposed management provides low overall mortality and extraordinary longevity in mothers compared to conventional management.

#### OVULATION INDUCTION IN RABBIT DOES USING MICRO ENCAPSULATED RRBNGF

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Ovulation induction treatments in rabbits undergoing artificial insemination (AI) are under continuous study. The identification of nerve growth factor (βNGF) in rabbit seminal plasma suggested that it might be involved in this physiological process. However, the nervous stimulus due to the introduction of the cannula also collaborates significantly in this species. In this work, the role of recombinant rabbit NGF microencapsulated with chitosan and administered vaginally was studied. To achieve this goal, synchronised nulliparous rabbits with 20 IU of eCG 48 h before AI (Serigan. Ovejero, Spain) were used. To induce ovulation, the following treatments were used: recombinant βNGF encapsulated in Chitosan spheres that was introduced into the vagina

immediately before applying the seminal dose (Group NGFch0, n=10), or 30 min before insemination (Group NGFch30, n=10) with single use cannulas. These groups were compared with one treated with 20 µg gonadorelin (Gestavet, Hypra), i.m. (GnRH Group, n=10), and with another in which only the empty cannula was introduced (Group C, n=10). Fertility was 100, 60 and 90% for NGF\_Ch30, NGF\_Ch0, and GnRH, respectively ( $P<0.05$ ) and there were no differences in prolificacy ( $12\pm 0.6$  born alive and  $0.6\pm 0.2$  stillborn). The high concentrations of plasma progesterone determined on day 7 post-AI indicate that a high percentage of does that were only inseminated with the empty cannula also ovulated. In conclusion, the excellent fertility results obtained in this study cannot be attributed solely to the encapsulation of NGF with chitosan, as they cannot be dissociated from the nervous stimulus caused by the introduction of the cannula in these highly receptive females.

#### CHARACTERISATION OF EXOSOMES IN SEMINAL PLASMA OF FERTILE AND SUBFERTILE RABBIT BUCKS: PRELIMINARY RESULTS

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In this study, the methods to isolate and identify extracellular vesicles (EVs) including exosomes, from the seminal plasma (SP) of 3 rabbits of high (HSQ) and 3 of low (LSQ) seminal quality were developed. Ultracentrifugation and size exclusion chromatography analysis made it possible to isolate different SP-EVs concentrations ( $8.53 \times 10^{11} \pm 1.04 \times 10^{11}$  y  $1.84 \times 10^{12} \pm 1.75 \times 10^{11}$  particles/mL of SP;  $P=0.008$ ) and with a similar average size ( $143.9 \pm 11.9$  and  $115.5 \pm 2.4$  nm;  $P=0.7422$ ) in HSQ and LSQ males, respectively. Likewise, EVs were identified by electron microscopy and their marker proteins by Western blot. The concentration of SP-EV was positively correlated with the percentage of abnormal forms ( $r=0.94$ ;  $P<0.05$ ) and with the percentage of immotile spermatozoa ( $r=0.88$ ;  $P<0.05$ ). Particle size was not significantly correlated with any kinetic parameter. Further analyses are necessary to determine the type of micro RNAs that these particles carry and determine if they may be related to the fertilising capacity of rabbit ejaculates.

## ARTIFICIAL INSEMINATION AS A REPRODUCTIVE ALTERNATIVE FOR DOMINICAN RABBIT FARMING: TECHNICAL AND ECONOMIC EVALUATION IN RABBIT FARMS (*ORYCTOLAGUS CUNICULUS*), UNDER COMMERCIAL CONDITIONS

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Different reasons justify the application of artificial insemination (AI) in rabbits. All of them afford a series of advantages that not only improve the quality of the productions, but also the final cost of the production, as it is expected that their use will guarantee a decrease in it. The purpose of this research was to carry out the technical and economic assessment of the implementation of artificial insemination as a reproductive alternative in rabbit farms in the Dominican Republic. The study was conducted under a completely randomised design with three treatments (T1: natural mating; T2: AI without oestrus synchronisation and T3: AI with oestrus synchronisation) and 8 repetitions per treatment. A breeding rabbit, housed in an individual cage, was considered as the experimental unit. The variables evaluated were fertility and prolificity. The results indicate that the percentage of fertility favoured natural mating (T1) with 100% in relation to AI with and without heat induction, which obtained 69.12 and 88.88%, respectively. Although the prolificity values were higher in the inseminated females, these differences were not statistically significant ( $P < 0.05$ ). The marginal return analysis yielded similar results for the three technologies evaluated, allowing returns of RD\$ 1.32, 1.35 and 1.35 for T1, T2 and T3, respectively, for each coin invested.

## PATHOLOGY AND WELFARE

### EFFICACY OF DIFFERENT VACCINATION PLANS AGAINST MYXOMATOSIS

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Current vaccination plans against RHDV-2 and myxomatosis include the administration of monovalent or polyvalent vaccines. The aim of the present study was to evaluate the clinical efficacy of different vaccination plans against these diseases. For this purpose, New Zealand White rabbits ( $n=85$ ) were randomly distributed into 6 groups. Two groups were vaccinated subcutaneously,

both of them with a live attenuated vaccine against myxomatosis, and one of them with an inactivated vaccine against RHDV-2 in addition. Another group received the same live attenuated vaccine against myxomatosis administered intradermally, another group was given a trivalent recombinant live attenuated vaccine (RHD, RHDV-2 and myxomatosis), another one was vaccinated with an inactivated vaccine against RHDV2 and finally a last group was given a sterile saline solution. All the animals, except the control group vaccinated with the saline solution, were challenged with myxomatosis and clinical signs were monitored for 21 days. The antibody response produced in serum was periodically monitored by an indirect ELISA against myxomatosis. The results showed that the live attenuated vaccine that was administered protected most of the study animals regardless of the type of vaccination plan applied. Notably, when this vaccine was administered intradermally, none of the study animals developed symptoms of myxomatosis. The recombinant live attenuated vaccine protected 30-40% of the animals. The antibody response against myxomatosis produced by the intradermally administered vaccine stood out among the other vaccination plans tested. This produced 93.3% positive animals compared to the other vaccination plans, which presented between 33.3% and 60%. The present study helped to show that currently available vaccines have different efficacy and antibody production. This information is important for the design and monitoring of vaccination plans against myxomatosis in rabbits.

### EXPOSITION OF A RESEARCH PROJECT ON *STAPHYLOCOCCUS AUREUS* INFECTION: PAST, PRESENT AND FUTURE

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This exposition aims to disclose the project "*Staphylococcus aureus's molecular epidemiology and evolution in rabbits, with special attention to its virulence and methicillin resistance*". The project is funded by the Ministry of Science and Innovation (PID 2020-117897RB-I00), and has as objective to genetically characterise *S. aureus* isolates obtained in farms in the Iberian Peninsula (from animals, workers and veterinarians), to find new genes or mutations which could explain the increase in the virulence of outbreaks, and the trial of new therapies. This information obtained about the strains would allow us to correlate with their pathogenicity, as well as to

elaborate antibiograms to find out their resistances and fast-track the use of correct and efficient antibiotherapies. This information will be transmitted anonymously to an online platform with restricted access, with the aim of keeping every participant informed about this project for the distribution of strains throughout the autonomous communities and their antibiotic sensitivity testing results. This will help diminish the use of generic antibiotics, and consequently their resistance profile, as well as to establish a prevention protocol and prompt treatment, stimulating animal's health and lowering the current economic impact caused by *S. aureus* in rabbit farms. This project has been launched this year and is open to voluntary participation for the entire sector through its veterinarians.

### MOLECULAR EPIDEMIOLOGY AND EVOLUTION OF *STAPHYLOCOCCUS AUREUS*: PRELIMINARY RESULTS

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*Staphylococcus aureus* is a major pathogen in commercial rabbitries due to its economic impact. The increase in the number and virulence of staphylococcal cases in recent years concerns both vets and farmers. The Animal Pathology and Health group of the CEU Cardenal Herrera University leads a project funded by the MICINN (PID2020-117897RB-I00) whose main objective is to genetically characterise *S. aureus* isolates obtained from farms in the Iberian Peninsula to find new genes or mutations that may explain the increase in virulence. For this purpose, to date, samples have been taken from animals with different lesions compatible with *S. aureus* in 155 farms. Both previously described and new strains have been found among the isolates. This could justify a change in the virulence of outbreaks. However, this hypothesis needs to be confirmed by more detailed genetic studies, which will be carried out in the coming years. This article shows the results obtained so far.

### STUDY OF THE EVOLUTION OF THE ANTIBIOTIC SENSITIVITY OF THE MAIN BACTERIAL PATHOGENS IN RABBIT FARMING FROM 2019 TO 2021

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*Staphylococcus aureus*, *Pasteurella multocida*, *Escherichia coli* or *Salmonella* spp. cause diseases in rabbits that decrease productivity and quality of life. But the development of resistance makes them difficult to treat. The results of 4386 antibiograms obtained with the Kirby-Bauer technique from clinical samples of Iberian Peninsula's rabbit farms received at Exopol during the years 2019-2021 were evaluated. The evolution of antibiotic sensitivity over time was assessed using the Chi-square statistical test and 905 *P. multocida* antibiograms were performed. More than 80% of the strains were sensitive to most of the antibiotics tested. From the 1066 antibiograms of *S. aureus*, over 90% of the strains tested were sensitive to the combination of sulfamethoxazole-trimethoprim. However, less than 30% of the strains were sensitive to tetracycline, and less than 15% were sensitive to enrofloxacin. From *Salmonella* spp., 118 antibiograms were evaluated, obtaining a high percentage of strains sensitive to colistin, gentamicin and trimethoprim-sulfamethoxazole. Many cases of resistance to bacitracin, apramycin, erythromycin, spiramycin and valnemulin were presented. Some 2297 antibiograms were analysed for *E. coli* strains. Thirteen out of 18 antibiotics tested against *E. coli* did not exceed 50% of strains sensitive to them in 2021. For apramycin and neomycin, there is a significant decrease in sensitivity over time. There is a significant increase in the percentage of sensitive strains with respect to enrofloxacin time in *Salmonella* spp. and *E. coli*, which may be due to reduced use by the EMA antibiotic classification. The situation of *E. coli* is worrying and *Salmonella* spp. is similarly of growing concern. The rational use of antibiotics helps to reduce antibiotic resistance, and further studies should be carried out to assess its evolution and the indication of authorised drugs in Spain.

### EFFECT OF DIFFERENT MANAGEMENT TECHNIQUES AS AN ALTERNATIVE TO THE USE OF ANTIBIOTICS ON STRESS PARAMETERS IN FATTENING RABBITS

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The search for alternatives to antibiotic use in the fattening of meat rabbits is a necessity, as the abuse of antibiotics has led to the emergence of antimicrobial resistance.



These alternatives can be related to different alternatives, one of them being management techniques. In this work, 2 different management techniques have been evaluated with medicated (M) and unmedicated (SM) feeding, by the following techniques: group size (8 animals (I) and 32 animals (C)), and in turn the cages of 8 animals were divided by parentage (siblings (H) and non-siblings (NoH) in the same cage). A total of 1620 rabbits from the high longevity and reproduction genetic line (LP line) of the Polytechnic University of Valencia were used, distributed in 6 treatments: HM, HSM, NoHM, NoHSM, CM and CSM. For the evaluation of these techniques, blood parameters related to animal welfare were measured. These parameters are Immunoglobulins G (IgG) and M (IgM), Albumin, C-reactive protein (CRP), Glucose and Lactate dehydrogenase (LDH). Significant differences were only found for group size in reference to albumin and CRP, but all parameters were within the species standards. Therefore, it could be concluded from the results obtained that group size and parentage, as well as the absence of antibiotic in the feed, has no effect on the welfare parameters measured for a resilient genetic line such as LP.

#### EFFECT OF THE USE OF GNAWING HAY BLOCKS AND SEX-GROUP COMPOSITION ON GROWTH PERFORMANCE, USE OF SPACE AND SLAUGHTER RESULTS OF GROWING RABBITS HOUSED IN PARKS

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This study assessed the effect of the provision of gnawing hay blocks (A: absence, P: presence) and sex-group composition in the parks (XX: only females, YY: only males, XY: mixed-sex) on growth performance, slaughter results, and use of space in 288 growing rabbits reared in 18 parks (16 rabbits per park) from weaning to slaughter (31-73 d of age). The parks consisted of two communicating modules: a module intended for feeding (wire net floor; four nipple drinkers; one feeder; one gnawing hay block in P groups) and a module for resting (plastic slatted floor, two nipple drinkers). The presence of gnawing blocks increased daily weight gain (51.8 vs. 50.6 g/d;  $P<0.05$ ) and slaughter weight (2818 vs. 2763 g;  $P<0.05$ ). In the P group, a lower percentage of rabbits was observed in the resting area compared to the A group (49.5 vs. 53.0%;  $P<0.001$ ). Regarding the sex-group

composition, a better feed conversion ratio was recorded in XX and XY compared to YY parks ( $P<0.05$ ), whereas YY group showed a higher slaughter yield compared to XX ( $P<0.01$ ), with intermediate values in group XY. No signs of lesions were detected at the end of the trial. In conclusion, the use of a hay gnawing block could improve performance of growing rabbits, whereas the separate housing of females and males in group-housed rabbits does not offer significant advantages.

#### DIFFERENT TYPES OF VACCINES AGAINST RHDV-2 PRODUCE A DIFFERENT ONSET OF ANTIBODY RESPONSE

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Commercially available vaccines against RHDV2 are based on two types of technologies, recombinant inactivated or live attenuated antigens. The present study compared the antibody response produced by the two types of vaccines mentioned. For this purpose, New Zealand White rabbits ( $n=55$ ) were randomised into 4 groups. Two groups were vaccinated subcutaneously, both of them with an inactivated vaccine against RHDV2, and one of them with live attenuated vaccine against myxomatosis in addition. Another group received a live attenuated recombinant trivalent vaccine (RHD, RHDV-2 and myxomatosis) and finally another group was given a sterile saline solution. The antibody response produced in serum was periodically monitored by means of a competition ELISA against RHDV-2 until 18 d post-vaccination. The results showed that the inactivated vaccine administered simultaneously or not with the myxomatosis vaccine produced antibodies in almost all the animals studied (93.33-100%) from 7 d post-vaccination. The recombinant live attenuated vaccine produced an antibody response that was detected in a maximum of 33.33% of the animals throughout the study. The present study helped to show that the currently available vaccines present different dynamics of antibody production. This information is important for the design and monitoring of vaccination plans in rabbits.

#### EVALUATION OF THE EFFICACY OF ERAVAC® AGAINST A STRAIN OF RABBIT HAEMORRHAGIC DISEASE TYPE 2 (RHDV2) ISOLATED IN FRANCE IN 2020

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In 2010, a new variant of rabbit haemorrhagic disease (RHDV2) emerged in France and spread rapidly throughout the world. The aim of this study was to evaluate the efficacy of an inactivated RHDV2 vaccine against a recently isolated strain. For this purpose, 40 New Zealand White rabbits were selected and randomly distributed into two groups, one vaccinated and the other non-vaccinated. At 7 days post-vaccination, all the animals were infected intramuscularly with a strain of RHDV2 described as highly pathogenic and isolated in France in 2020. All the vaccinated animals survived the challenge and did not show any clinical signs, whereas the control group showed a 75% mortality rate. Therefore, ERAVAC<sup>®</sup> was shown to be effective in preventing outbreaks of currently circulating RHDV2 strains.

### BIOSAFETY IN RABBIT FARMING IN NORTHERN PORTUGAL: PRESENT AND FUTURE

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Biosecurity is a widely recognised concept in the animal production sector and, given its importance, it is an integral part of the strategy for the control and prevention of infectious diseases. Through the development of biosecurity programmes based on the epidemiology of different infectious pathologies, there has been a reduction in the mortality rate of rabbits, an increase in the health status and welfare conditions of the animals and increased profitability in the sector. During the period from September 2020 to May 2021, 28 rabbit farms in northern and central Portugal (20% of total farms in the country) were subject to a biosafety survey and reports of pathological diagnoses were drafted (2030). Only one producer (n=1) was aware of the health status of the nucleus of future rabbit does (3.6%). In 87.5% of farms, newly arrived rabbit does are not quarantined (n=24). Chemical and microbiological water control is in place in 28.6% of farms (n=8). Only 7.1% of rabbit farmers (n=2) have a record of people that access the farms. Thus, more efforts and investments must be directed to biosafety programmes in order to promote animal health and public health.

### BIOSAFETY AS A PREVENTIVE TOOL FOR COLIBACILLOSIS IN RABBITS

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*Escherichia coli* is a Gram-negative rod-shaped bacteria from the Enterobacteriaceae family. Although this organism can be an innocuous resident of the gastrointestinal tract of all mammals, it also has the capacity to become a highly adapted pathogen, capable of causing a range of diseases. Enteropathogenic *E. coli* (EPEC) is one of the main pathogenic *E. coli* pathotypes that causes substantial diarrhoea-associated morbidity and mortality in rabbits (colibacillosis) and has zoonotic potential. To evaluate the relationship between the occurrence of digestive pathologies with infectious ethology and the biosecurity conditions of rabbits' farms, an epidemiological survey about biosecurity was applied and reports of pathological diagnoses were analysed in 28 rabbit farms in northern and central Portugal (representing 20% of all rabbit breeds in the country). In the studied farms, the average occurrence of colibacillosis was the second highest (23.9%) after enterotoxaemia, with a standard deviation of 26.2%. In this study, the association between the facilities that control ventilation ( $P=0.034$ ), the farm holdings that are more than 100 metres away from public pathways ( $P=0.001$ ), as well as the presence of footbaths between a dirty and clean area ( $P=0.020$ ), and the presence of colibacillosis values below the mean is highlighted. The results obtained suggest that farmers should commit to and invest in biosecurity, in order to achieve better productive and economic parameters without compromising public health and animal health.