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ABSTRACTS OF THE 47TH SYMPOSIUM ON CUNICULTURE, ASESCU

León, Spain, 31st May-1st June, 2023.

The 47th Congress of the Spanish Association of Cuniculture (ASESCU) was held in León (Castile and Leon, Spain) on the 31st of May and 1st of June. The event was organised by ASESCU and the Faculty of Veterinary Medicine at the University of León. Four main lectures were delivered. The first two of these lectures delved into the past, present and future perspectives of cuniculture in Castile and Leon. The third talk focused on the use of state-of-the-art technologies in animal production for precision livestock farming. Lastly, the fourth lecture provided an overview of the current status of Staphylococcus aureus studies and introduced novel tools for its control. Moreover, two round tables were conducted to examine the current state of sustainability in rabbitries, as well as the effects of implementation of the new antimicrobial laws. Throughout the congress, a total of 21 oral communications and two posters were presented in working sessions on genetics, sustainability, nutrition, reproduction, pathology, welfare and products. The event was attended by approximately 160 participants from different European and American countries. The abstracts of contributions are reported below.

MAIN PAPERS

PAST AND FUTURE OF RABBIT FARMING IN LEÓN. MY STUDIES IN VETERINARY MEDICINE IN LEÓN, BETWEEN THE COURSES 1973-74 AND 2022-2023 AND SOME LINKS WITH RABBIT PRODUCTION

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These retrospective notes, intended to be shared with readers and attendees at the 47th ASESCU symposium, cover my experiences related to rabbit farms, since I came to León in October 1973, to begin my studies in Veterinary Medicine, until now, when I am finishing them. I show data from some observations on 1714 visited farms, of which 198 were in Castile and León, from 1988 to 2018. They are the results of sampling rabbits for diseases and risk factors, which we studied in collaboration with professors from the Veterinary School of León and other centres. In 2009 I showed my gratitude to rabbit producers in a similar way, at the symposium in Seville. I hope these reflections serve as an introduction to suggestions for the future for people related to rabbit farming.

PRESENT AND FUTURE OF RABBIT FARMING IN CASTILLA Y LEÓN (BRIEF SURVIVAL MANUAL)

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In this short work, we intend to start from a "still photo", which is the current situation of rabbit farming in Castilla y León in Spain, and taking into account the legislative processes that are already affecting the rabbit sector and are expected to appear in the near future, to try to give some brushstrokes on the possible ways of survival and development for rabbit farms in the short-medium term.

PRECISION LIVESTOCK FARMING IN CAGE-FREE RABBIT REARING

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Precision livestock farming uses technology to monitor animals with sensors. Its practical implementation can contribute to improving animal care and the technical-economic management of animal production. Technology has a high potential and can be useful for the people

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working in the rabbit sector in different fields. The aim of this work is to analyse how precision tools can help in the transition towards low-impact, innovative and cage-free production systems and address opportunities in a cage-free scenario in intensive rabbit rearing.

STAPHYLOCOCCUS AUREUS IN RABBIT FARMING. NEW CHALLENGES, NEW TOOLS

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Infections caused by *Staphylococcus aureus* are a major problem in rabbit farming. The most common lesions associated with this bacterium are suppurative dermatitis, abscesses, foot rot and mastitis. Staphylococcosis in rabbit farms is related to strains mostly associated with the clonal complex (CC) 121 and, secondly, CC96. CC121-associated strains are considered more virulent than CC96-associated strains, with differences in infectivity observed in experimental infections and in the immune response of rabbits to both types of strain. Phylogenetic analysis has shown that rabbit strains belonging to the major CC originated from a jump from humans to rabbits, where the *dlt*B gene on the central chromosome was key, without the involvement of mobile genetic elements. One of the most significant problems in combating staphylococcosis is antibiotic resistance. Strains from rabbit farms that are resistant to methicillin (LA-MRSA) are multidrug-resistant and mostly belong to CC96. To continue investigating the evolution and characteristics of *S. aureus* strains and their relationship with rabbits and facilitate data analysis, a Power BI®-based application has been developed that allows data to be entered in several formats and from various sources and analysed in an intuitive and visual manner.

GENETICS

GENETIC TRENDS IN THREE RABBIT LINES SELECTED FOR FEED EFFICIENCY

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An improvement of efficiency in the use of feed will have positive consequence on both the economic balance of rabbitries and their environmental fingerprint. Aiming to generate animals with enhanced feed efficiency, we have conducted a selection experiment over 7 generations -34 batches- to improve feed efficiency in 3 rabbit lines. The ADGR, RFI and GRP lines were selected for growth under feed restriction, individual residual feed intake and cage-average (4 animals) residual feed intake, respectively. Although animals in lines ADGR and RFI were raised in groups (6 animals), as we used electronic feeders, an individual recording of the feed intake was possible. The traits studied were average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR=ADFI/ADG). Records in the GRP line were cage-average (n=873), while in RFI (n=2482) and ADGR (n=2343) lines they were individual records. The reported genetic trends are the regression coefficients of the within year-of-birth average predicted breeding values on the year-of-birth. Breeding value predictions were obtained with REML. The estimated genetic trends in RFI and ADGR lines were 0.03 ((g/d)/year) ($P>0.05$) and 1.20((g/d)/year) ($P<0.01$) for ADG; –1.79 ((g/d)/year) ($P<0.01$) and 0.34 ((g/d)/year)) ($P<0.05$) for ADFI; and –0.09 (((g/d)/(g/d))/year) ($P<0.01$) and –0.01 (((g/d)/(g/d))/year) ($P<0.05$) for FCR. In the GRP line, the genetic trends were 0.04 ((g/d)/year) ($P>0.05$), –1.67 ((g/d)/year) ($P<0.01$) and –0.07 (((g/d)/(g/d))/year) ($P<0.01$), for ADG, ADFI and FCR, respectively. It can be concluded that the three selection strategies implemented to improve feed efficiency of rabbits raised in groups are being effective.

A NEW LINE SELECTED FOR FEED EFFICIENCY. 1. SELECTION CRITERIA

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Different criteria are examined to define feed efficiency, in order to propose the most suitable for a selection line that acts as terminal male in the industry. The selection index by feed intake and growth rate, the feed conversion ratio, feed intake at constant growth, growth at constant feed intake, residual feed intake and the residual growth rate are compared. After examining their advantages and disadvantages and their relationships, it is concluded that growth at constant feed intake or residual growth rate are preferable to the others.

ASSESSMENT OF SPERM QUALITY IN RABBIT LINES DIVERGENTLY SELECTED FOR LITTER SIZE VARIABILITY

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The aim is to study the correlated response in sperm quality of two lines divergently selected for litter size variability: High line, selected to increase litter size variability and Low line, selected to decrease litter size variability. For this purpose, 136 semen samples from 34 young rabbits of both lines were analysed. Two semen samples per male were extracted every 7 d for 6 wk and the following sperm quality parameters were analysed: volume, gel, motility, concentration, total production, percentage of normal spermatozoa, percentage of drop, percentage of abnormal head, tail and midpiece spermatozoa, percentage of reacted acrosomes and percentage of live and dead spermatozoa. Statistical analyses were performed using Bayesian methodology. Both lines showed similar values for volume (0.85 mL for the Low line and 0.84 mL for the High line), motility (4.50 and 4.68), concentration (21.88 and 24.98 million spermatozoa), percentage of normal spermatozoa (85.66 and 88.09%) and percentage of reacted acrosomes (2.37 and 2.62%). Low line showed higher sperm viability (88.77% of live spermatozoa) than High line (79.55%; *P*=95%). In conclusion, selection for litter size variability does not seem to have produced a correlated response in semen quality in males starting their productive life, except for viability, which is higher in the line selected to homogenise litter size.

SUSTAINABILITY

CHARACTERISATION OF CUNICULTURE AS A SUSTAINABLE AGRIFOOD SYSTEM: SYSTEMATIC REVIEW

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The aim of this research was to analyse the main characteristics of rabbit farming as part of a sustainable food system, in order to provide descriptive information that helps promote the production, sale and consumption of rabbit meat in Mexico as an important source of protein of high nutritional quality. A systematic literature review was carried out in the Scopus database on rabbit meat production and sustainable food systems. The keywords "Rabbit production", "Rabbit farming", "Rabbit meat" and "sustainable food systems" were used in the information searches. The ones that obtained the best results were "sustainable food systems" (726), "Rabbit meat (214) and "Rabbit production" (68). Some 55% of the publications for "sustainable food systems" are concentrated in the United States (164), the United Kingdom (132) and Italy (101). For "Rabbit meat", 58% of the publications are concentrated in Italy (47), China (29), Poland (25) and Spain (24), while for "Rabbit production", 51% of the publications are found in Spain (35), France (10), Egypt (9) and Mexico (8). Italy and China present a greater number of publications related to rabbit meat, while Spain and France focus on production. Rabbit meat production is adapted to the characteristics of the rural environment in Mexico (resilient), all family members participate (inclusive), it has low production costs, high profitability (efficient) and can be used comprehensively (sustainable): meat (healthy), skin, viscera and excreta. Rabbit farming meets the desirable characteristics for sustainable food systems: it is inclusive, resilient, healthy, sustainable and efficient, and can contribute to achieving food security, so it is necessary to set out strategies to promote it.

NUTRITION

USE OF ORGANIC ACIDS IN POST-WEANING RABBIT: CHOICE OF ACID AND DOSE

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The aim of this study was to evaluate the use of organic acids (OA) in the water of post-weaning rabbit at different pH in the evolution of pH in the gut, gastric pepsin activity and animal performance. Six organic acids (acetic, formic, propionic, lactic, citric and butyric) were tested at 3 concentrations (pH 3, 4 and 5). In addition, a negative control (non-acidified water) was included. The study was conducted with 228 weaned rabbits (28 d old) divided into

2 batches and lasted 7 d. In each batch, animals were divided into the 19 experimental treatments and housed in group cages (6 animals per cage, treatment and batch). Animals were fed an unmedicated commercial feed. At 31 and 35 d of age, 3 animals per day and treatment were slaughtered in each batch and we measured the pH in the fundus, antrum, duodenum, jejunum, ileum and cecum, as well as gastric pepsin enzyme activity. Daily water and feed intake per cage and individual live weight were recorded to calculate average daily gain. According to the results, the inclusion of OA in the drinking water of at pH 3 reduced water and feed intake, limiting growth. OA reduced the pH in the ileum and caecum, promoting gastric pepsin activity at pH 4. According to our results, formic, acetic and citric acid at pH 4 presented the highest potential for use in rabbits post-weaning.

TRAINING OF RABBIT DOES DURING REARING: EFFECT ON THE BODY RESERVES' FLEXIBILITY AND LONG-TERM REPRODUCTION

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This work evaluated the effect of a rearing training strategy for young rabbit does, based on 0 to 3 feed restriction schemes, on their body reserves flexibility and long-term reproduction. Each restriction was 6 d of progressive reduction from *ad libitum* to zero and 3 d of progressive recovery until *ad libitum* feeding. At 63 d of age, 120 rabbit females were divided into 4 strategies: AL, fed *ad libitum*; 1R, fed *ad libitum* with one restriction scheme from 92 to 101 d of age; 2R, fed *ad libitum* with two restriction schemes from 70 to 79 and 114 to 123 d; 3R, fed *ad libitum* with three restriction schemes from 70 to 79, 92 to 101 and 114 to 123 d. Females were artificially inseminated (AI) at 137 d of age and at 11 days postpartum thereafter. Live weight (LW), perirenal fat thickness (PFT) and feed intake of females were controlled until the 2nd parturition. Alive and total litter size at birth was controlled until the 9th reproductive cycle. A few days before the first AI, does were challenged with isoproterenol to determine their lipolytic potential. Young rabbit does from the R strategies showed clear losses of LW and PFT during the application of restriction schemes but recovered the AL group values some weeks after refeeding. At first AI, R females had lower basal blood concentration of non-esterified fatty acids compared to AL females (on av. –13.9 mEq NEFA/L; P=0.002). There was a linear increase in total born and live born over 9 reproductive cycles with the number of restrictions applied during rearing (+0.47 and +0.42 per restriction, respectively; P<0.05). The restrictions applied during rearing did not affect body reserves' flexibility but improved the prolificacy of the females.

EFFECT OF LEVEL OF DIETARY CALCIUM ON GROWTH TRAITS OF GROWING RABBITS

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Four diets were formulated (varying only the percentage of calcium with the inclusion of calcium carbonate): the first one without added calcium carbonate (0.45% Ca), and three others with 0.525, 0.600 and 1.000% Ca and 10.3 MJ digestible energy/kg. The diets were fed *ad libitum* to a total of 236 rabbits, weaned and housed in individual cages from 30 to 64 d of age (59 rabbits/treatment). Increasing calcium level linearly reduced growth rate and feed efficiency from 30 to 64 d of age (P=0.036). In addition, increasing calcium tended to reduce feed intake (P=0.086) and increased the sum of mortality and morbidity (P=0.095), without affecting the mortality rate. The increase in calcium level impaired mineral retention efficiency and increased urinary mineral excretion (P<0,001), but did not change the strength and elasticity characteristics of the tibia (P=0.35).

EFFECT OF THE INCLUSION OF SEAWEED PRODUCTS ON FAECAL DIGESTIBILITY AND OTHER DIGESTIVE TRAITS IN GROWING RABBITS

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A control diet was formulated and another four diets obtained adding to control diet 1.025% of dehydrated *Saccharina latissima*, or aqueous extracts of *Saccharina latissima, Himanthalia elongata* and *Ulva* spp., respectively. Two experiments (100 rabbits/exp) were carried out to determine faecal digestibility, volatile fatty

acid concentration and other digestive parameters. Rabbits from the control group showed a lower dry matter/energy and protein digestibility values than the mean of the groups fed with seaweed or the two groups fed with *S. latissima* ($0.015<P\leq0.12$). The ileal proportion of butyrate of the groups fed *S. latissima* extract and *H. elongata* tended to be higher than that of the group fed *Ulva* spp. ($P=0.094$).

RELATIONSHIPS BETWEEN GROWTH AND FEED EFFICIENCY WITH FEEDING BEHAVIOUR TRAITS AND INDICATORS OF SOCIAL STATUS OF GROWING RABBITS

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Understanding the potential mechanisms by which an animal exerts its effect on the productive behaviour of its cage mates is of great interest for management and welfare, as well as for selection. The aim of this study was to use information from a novel automated feeder for rabbits to: i) identify specific feeding behaviour traits that could serve as indicators of an individual's ranking within the social hierarchy of the cage; ii) assess the relationship between growth and feed efficiency with the above traits, as well as those describing the individual consumption pattern. At 39 d of life, rabbits from 15 batches were placed in 6-rabbit cages and fed *ad libitum* to get used to the electronic feeder. From 42 to 60 d, one group of 1086 rabbits was fed *ad libitum* while other group of 1134 rabbits was fed on restriction by limiting the feeding time to the period between 18:00 and 8:00 h the following day. The variables defining how resources are distributed among cage mates can be good indicators of the animal's dominance/subordination position within the cage, as they are correlated with the animal's priority for access to food. The most efficient animals are the ones that eat fewer times, have less amount of food and spend less time in the feeder trough. They also appear to be the subordinates, as evidenced by the fact that they do not have priority access to food and have the smallest share of resources.

REPRODUCTION

EFFECT OF RABBIT RECOMBINANT NERVE GROWTH FACTOR MICROENCAPSULATED WITH CHITOSAN ON RABBIT SEMEN PARAMETERS

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Nerve growth factor (βNGF) has its main role in the nervous system, but is also involved in the reproductive system. βNGF and its receptors have been detected in the female and male rabbit reproductive tracts, as well as in rabbit sperm. Previous studies indicate that it is involved in ovulation induction and recently, artificial insemination (AI) was performed with addition of chitosan encapsulated recombinant βNGF (rrbNGFch) in the seminal doses, inducing analogous results to those obtained by GnRH intramuscular (i.m) administration to the female. Chitosan is a molecule commonly used in biomedical fields for the microencapsulation of molecules. In this work, the main objective was to investigate the effect of chitosan or rrbNGFch spheres on *in vitro* semen quality parameters in rabbit spermatozoa. For this purpose, semen diluted with commercial diluent was incubated at 37 °C for 2 h with chitosan spheres, rrbNGFch or diluent alone. Viability, motility and capacitation status were analysed at addition time and after 2 h of addition. The empty chitosan microspheres group maintained sperm quality parameters and capacitation state, suggesting that the spheres are safe for spermatozoa. When spermatozoa were incubated with rrbNGFch, no effects on motility and viability were observed, but the percentage of spermatozoa with acrosome reaction at 0 h increased compared to the control group. In conclusion, the use of chitosan spheres did not cause negative effects on semen doses, suggesting that it is suitable for use in AI. Encapsulation of rrbNGF with chitosan maintains semen quality parameters, leading to changes in the capacitation status of rabbit spermatozoa.

ASSESSMENT OF BIOSTIMULATION METHODS BASED ON CHEMICAL COMMUNICATION IN BUCK REPRODUCTION. PRELIMINARY STUDY

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Biostimulation is an animal management practice that helps improve reproductive parameters by modulating animal sensory systems. Chemical signals, mostly known as pheromones, have a great potential on this regard. This study was conducted to determine the influence of short-term male rabbit exposure to different biological secretions, potentially pheromone-mediated, on reproductive parameters of males. Groups of 18 males/each were exposed to 1) female urine, 2) vaginal fluid, and 3) phenoxyethanol as a source of pheromones, just before semen collection. A control group was exposed to distilled water. The following semen analysis were performed for each group: semen volume, spermatozoa concentration and characterisation of motile, progressive, fast progressive, medium progressive, non-progressive, immotile and live sperm. We found higher semen volume in the control group than in the group exposed to female urine. However, the percentage of motile, progressive, fast progressive and mid-progressive spermatozoa was higher in any of the experimental groups than in the control group, and the percentage of immotile and non-progressive spermatozoa was generally higher in the control group than in the rest of the experimental groups. This indicates that sperm motility increases when animals are exposed to specific biological fluids containing pheromones. In conclusion, our results support the importance of pheromones in reproduction, particularly in rabbits, and encourage future studies that seek to implement the use of pheromones in rabbit husbandry to improve certain reproductive and productive parameters in farmed rabbits.

EFFECTS OF COLLECTION FREQUENCY AND ADDITION OF ALARELIN TO THE SEMINAL DOSE ON SPERM QUALITY. PRELIMINARY STUDY

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Artificial insemination (AI) in rabbits has evolved significantly in recent decades and has become common practice in rabbit breeding. As collection frequency is one of the main factors affecting semen quality, and since it is necessary to induce ovulation when performing AI, the aim of this study was to determine whether there are differences in vitality, abnormal acrosomes and shapes and sperm motility of semen doses depending on collection frequency and addition of alarelin to the diluent. Forty male Hyplus PS39 rabbits were included. Every Thursday, for 10 wk, 4 heterospermic samples were obtained and classified according to the diluent (with or without hormone) and the frequency of collection (once or twice a week). Samples were assessed at 0, 12, 24 and 30 h. Subjective motility, percentage of live sperm, abnormal sperm and sperm with abnormal acrosomes were assessed. Data were analysed using a general linear repeated measures model. Significant differences were found in sperm motility, percentage of live sperm and percentage of abnormal acrosomes for the frequency of collection. No statistically significant differences were found in any of the parameters analysed for the extender, nor were significant interactions between the extender and collection frequency. In conclusion, collection frequency influences the quality of the semen dose. However, the use of alarelin to induce ovulation in rabbits, by vaginal administration included in the seminal dose, would have no effect.

GROWTH OF CROSSBRED FEMALES AND ITS EFFECT ON REPRODUCTION

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The aim of this work was to determine the growth pattern of the crossbred female descendants of grandfather LP with grandmother V of the Valencian lineage, depending on the season in which they are born, and how their own birth weight or lactating growth affect the growth curve and litter size at their first parturition. A total of 692 crossbred rabbits were controlled until 226 days of age. We verified that rabbit does born in the warm season weighed significantly more when they reach adulthood than those born in the cold season (4.660 and 4.463 kg, respectively; $P<0.05$). This difference may be due more to the session when they were reared when they were born in the warm season. It was observed that crossbred females that were born in a larger litter and with a higher weaning weight reached an adult weight significantly earlier. Regarding the litter size of crossbred females at first parturition, it was significantly observed that the larger the litter size at the female birth, the smaller the

litter size at their first parturition (–0.103 kits at their first parturition per kit more in the litter when the female was born). However, with values close to significance, the more the rabbit doe weighs at birth, the greater the litter size at its first parturition (for every 22 g more at birth, half a kit more in its first parturition; *P*=0.06).

PATHOLOGY

CURRENT DISTRIBUTION OF THE MAIN CLONES OF S. AUREUS IN COMMERCIAL RABBITRIES IN SPAIN

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In commercial rabbit farming, *Staphylococcus aureus* is a health challenge due to the wide range of pathologies it causes in rabbits of all ages. For this reason, the Animal Pathology and Health group of the CEU Cardenal Herrera University has started a project whose main objective is to identify the new *S. aureus* clones responsible for the current outbreaks and to define the genetic elements that have potentially allowed them to increase their virulence. So far, 316 farms in Spain have been sampled and 878 samples have been obtained, of which 74.4% were positive for *S. aureus*. Ninety-three different *S. aureus* genotypes have been found in the samples, of which 75 had not been previously isolated. This could reinforce the hypothesis that strains are changing and certain genotypes are no longer present on farms while others are beginning to settle. However, at the clonal complex level, 76% of them belong to CC121 and CC96, which are the lineages traditionally described in rabbit breeding in Spain.

GENOTYPIC AND PHENOTYPIC STUDY ON THE ANTIMICROBIAL SUSCEPTIBILITY OF *STAPHYLOCOCCUS AUREUS* IN RABBITS

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Staphylococcus aureus is a bacteria that can develop resistance to multiple antibiotics, making it a significant public health problem. Antimicrobial drugs were selected for antibiotic susceptibility studies with the aim of broadening our knowledge on the level of resistances of this molecules in rabbitries and for public health concerns. Different phenotypes were obtained, depending on the families of antibiotics studied, from a total of 86 strains. The families with the highest percentage of resistances were fluoroquinolones and tetracyclines, while sulphonamides had the lowest percentage. In addition, the genetic study identified a set of 29 resistance genes that have an effect not only antibiotics but also heavy metals and disinfectant compounds such as quaternary ammoniums. These genes were classified into families, resulting in a total of 10 antibiotic families. Tetracyclines, phosphonates and macrolides showed the highest percentage of resistance among the families, while glycopeptides, lincosamides and sulphonamides had the lowest. Phenotypic and genotypic studies of antibiotic resistance were found to be positively correlated for most antibiotic groups, except for lincosamides. The results showed that the presence of resistance genes was strongly associated with their phenotypic manifestation. However, certain strains presented resistance genes but did not manifest them, which may be related to the elimination of the plasmid that harbours these genes. These results emphasise the importance of considering both the presence of resistance genes and their phenotypic manifestation when evaluating antibiotic resistance in clinical samples.

GENOMIC COMPARATIVE ANALYSIS OF *STAPHYLOCOCCUS AUREUS* CLONES IN RABBIT FARMING: CC121 *VERSUS* CC96

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Staphylococcus aureus is a bacterium that can cause multiple diseases in rabbits and negatively affect their productive capacity. Furthermore, this bacterium is important as a model organism for genomic and molecular studies due to its ability to acquire and transfer antibiotic resistance and virulence genes. Two of the most frequent and widely distributed *S. aureus* clonal complexes in rabbit farming in our country are CC121 and CC96. CC121 has been associated with more severe infections in vivo and in vitro, while CC96 with less severe infections. Genomic

differences between CC121 and CC96 are due to mutations and the acquisition of mobile genetic elements. This study aims to establish a preliminary *in silico* view of the genomic differences between the two most representative clonal complexes of *S. aureus* that affect rabbit farming. In the study, 71 *S. aureus* sequences were analysed, of which 50 were associated with CC121 and 21 with CC96. The pangenomic analysis of the selected strains showed that 56.8% of the genes were present in all the strains, while 19.27% of the genes determine the differences between strains, as occurs in the profiles of virulence and resistance to antibiotics. The phylogeny of both clonal complexes is not fully differentiated, since the ST96 of CC96 are evolutionarily closer to certain strains of CC121 than to CC96. Finally, the presence of certain virulence factors has been observed depending on the clonal complex analysed. Therefore, this preliminary study highlights the importance of genomic analysis in the understanding of bacterial pathogenesis and the development of effective treatment strategies.

DEVELOPMENT OF AN ELISA FOR THE DETECTION OF ANTI-MYXOMA VIRUS ANTIBODIES BASED ON RECOMBINANT VIRAL PROTEINS

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Myxoma virus (MYXV) is the causative agent of myxomatosis, a disease that affects the European rabbit (*Oryctolagus cuniculus*). Upon its deliberate introduction in Europe in the 1950s, MYXV has had a devastating impact on native rabbit populations. Over years of coevolution with its host, wild rabbits have become a reservoir for the virus whereby myxomatosis is currently considered an endemic disease in Europe. Despite there being several vaccines currently available, there are annual myxomatosis outbreaks affecting both wild and farm rabbits. Routine serological monitoring procedures to indicate disease prevalence in wild rabbit populations and thorough surveillance of the serological status after vaccination on rabbit farms are essential to help establish suitable control measures. Here, we describe the development of serological tests that could be used for the detection of anti-MYXV antibodies based on recombinant proteins. To this end, several candidate proteins were selected to explore their antigenic properties in indirect enzyme-linked immunosorbent assays (ELISA) for the detection of specific antibodies in rabbit sera. Several positive and negative rabbit sera were used to standardise the indirect ELISAs based on the rM022L and rM115L recombinant proteins, and their comparison with a commercial ELISA. The preliminary results show that they can be a useful alternative for the serological evaluation of vaccinated farm animals, as well as in seroepidemiology studies of myxomatosis.

STUDY OF *ESCHERICHIA COLI* VIRULENCE FACTORS ASSOCIATED WITH DIGESTIVE DISORDERS IN RABBIT REARING

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Digestive disorders cause serious economic losses in rabbit farms. Among the bacteria, enteropathogenic *Escherichia coli* (EPEC) which is encoded by the *eae* gene, is one of the most important agents in these processes. Other virulence factors encoded by the *afr2*, *ral*, *lifA* and *paa* genes associated with EPEC strains in rabbits are known. The aim of this study was to investigate the relationship between these *E. coli* virulence factors and the O103 somatic antigen and their possible combinations. For this purpose, 113 strains of *E. coli* isolated from digestive samples of rabbits were selected, differentiated into three groups: 39 strains of EPEC *E. coli* and 39 strains of non-EPEC *E. coli* from rabbits with digestive symptoms and 35 strains (27 non- EPEC and 8 EPEC) isolated from rabbit samples without obvious signs of enteric disease. All EPEC strains were also positive for at least one other virulence factor. The positive profile for *liftA*, *ral* and *paa* was the most frequently detected (n=33) while the strains positive for *afr2* were scarce (n=6). The O103 serotype was found in 12 of the strains, all of them EPEC. The non-enteropathogenic strains (non-EPEC) from both groups of animals, with and without disease (n=39 and n=27) were negative for the rest of the virulence factors investigated as well as for the O103 serotype. We can conclude that the *eae* gene is the best genetic marker used to identify virulent *E. coli* strains in rabbits.

ANTE-MORTEM AND NON-INVASIVE DIAGNOSIS OF RHDV IN ASYMPTOMATIC RABBITS USING qPCR AND FTA CARDS

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The diagnosis of rabbit haemorrhagic disease is based on the detection of the virus (RHDV) in post-mortem samples, mainly in the liver. The aim of this study was to carry out

the detection of RHDV ante-mortem, validating the use of quantitative polymerase chain reaction (qPCR) and fast technology for analysis of nucleic acids (FTA) cards in faecal samples. For this purpose, 9 New Zealand White rabbits infected with a strain of RHDV2 were selected. At 15 d post-infection, none of the animals showed clinical signs and samples of liver and intestinal contents were obtained from all the animals. All the samples, both fresh and those inoculated on FTA cards, were analysed by RT-qPCR. There was 100% agreement between the results for the fresh samples and the FTA cards. Positivity was 100 and 66.67% for the liver and faecal samples, respectively. These results emphasise the fact that the liver is the major target for the ante-mortem diagnosis of this disease and demonstrate the feasibility of using faecal samples for ante-mortem and non-invasive diagnosis on a collective level. This methodology could contribute to defining the infection pressure of a farm and, indirectly, it could help to assess the risk of unvaccinated animals becoming infected. In conclusion, faecal samples could contribute to improving the diagnosis and control of rabbit haemorrhagic disease. However, more studies are necessary to confirm its use and real application on the farm.

WELFARE

STRESS ASSESSMENT BY INFRARED THERMOGRAPHY AFTER SPERM EXTRACTION IN RABBITS

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The present research learned the evolution of the body temperature measured by IRT of 20 male rabbits, after exposure to an acute stress stimulus based on a routine management process such as sperm extraction. Temperature was measured in the eyeball and nose at 15 moments per animal and anatomical region distributed over 90 min. The first extraction was performed after measured basal temperature at minute 0 and the second extraction was after minute 30. The experiment lasted 6 wk. Basal temperature in the eyeball and nose was 36.20±0.15°C and 36.20±0.15°C, respectively. After the first extraction, the temperature increased at minute 1 both in the eyeball (36.49±0.15°C) and in the nose (34.20±0.28°C); after the second extraction, the maximum value was at minute 35 (36.46±0.15°C) in the eyeball and 31 (34.95±0.27°C) in the nose. In both cases, stabilisation took place at minute 50. In conclusion, IRT could be an adequate method to evaluate the thermal evolution of the body surface in animals exposed to an acute stress stimulus. Likewise, both anatomical regions were propitious locations to carry out the assessment.

ENRICHMENT OF FATTENING RABBIT CAGES WITH TREE BRANCH: PRODUCTIVE AND HUMAN-ANIMAL INTERACTION RESULTS

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This study evaluated the effect of enriching cages with a tree branch attached to the side wall on mortality, productivity and behaviour of fattening rabbits. The study was conducted on a commercial farm at two times of the year, using a total of 448 rabbits weaned at 35 d of age, housed in mixed groups of eight littermates since lactation. No significant differences were observed in mortality, liveweight, feed intake and feed conversion efficiency between the two types of cages, although there was a lower daily weight gain in the period 56 to 63 d in the enriched cages compared to the conventional ones (37.1 *vs.* 40.2 g/d, respectively). In the human-approach test, no improvement was observed with the inclusion of tree branch in the cages, although it was observed with the advancement of the age of the fattening rabbits, since the percentage of animals inside the cage that approached the hand increased, being on average 51% at 63 d and 35% at 42 d. In summer, both productive parameters and the behavioural test showed more unfavourable results, which were associated with environmental and sanitary conditions. On the other hand, sex did not affect the production parameters studied. Overall, the results of this work do not show a benefit of including a tree branch in the fattening cages, although no significant detrimental effects were detected. Larger scale studies are required before recommending the inclusion of gnawing material in Spain, mainly in view of future scenarios where slaughter age, number of animals per group and health risk could increase.

PRODUCTS

COMPARISON OF THE ACCEPTANCE AND PREFERENCE OF FRESH RABBIT, CHICKEN AND PORK SAUSAGES BY YOUNG CONSUMERS

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The acceptance of organoleptic characteristics (hedonic scale: 1 to 9 points) and order of preference (1st to 3rd position) of fresh rabbit, pork and chicken sausages was compared by means of an untrained panel of 70 young consumers. The evaluation of smell and appearance by the panellists did not differ between types of sausages. The colour and elasticity of rabbit and chicken sausages were rated at the same level, and better than those of pork. The juiciness was valued better in the chicken sausage, followed by the rabbit and lastly in the pork sausages. Young people valued the texture, flavour and overall acceptance of chicken sausage better than pork sausage, with rabbit sausage being intermediate, with no difference on the one hand with the chicken sausage and on the other with the pork sausage. The texture, flavour, elasticity, juiciness and the global acceptance of the sausages were better valued by men than women. Chicken sausages were preferred in first place, with a difference over pork, and rabbit sausages being in the middle position without difference from each of the others. In conclusion, rabbit sausages were valued well, at the level of chicken and better than pork sausages, showing potential to expand rabbit meat consumption in young people.