

**ABSTRACTS OF THE “XXXV SYMPOSIUM DE CUNICULTURA DE ASESCU 2010”
SEGOVIA, SPAIN, MAY 27-28th, 2010**

The annual Congress of the Spanish Association of Cuniculture (ASESCU) was held in Segovia (Castilla y León) on 27-28th May of 2010. The 35th edition was devoted to analyse the actual situation of the rabbit sector. It was presented an overview of challenges for the future of the Spanish rabbit farms, a review of the competitive factors that affect the rabbit production, and the management systems to ensure food safety in rabbit farms. Moreover, the Technology Platform for Sustainable Agriculture, and a summary of the actions for the promotion of the rabbit meat consumption carried out by the Spanish Rabbit Interprofessional (INTERCUN) were presented in 2 different conferences. Furthermore, a total of 20 communications were presented in 5 working sessions. Pathology session analysed a vaccine candidate against RHD, blood lymphocyte populations in multiparous does, the efficacy of Timilcosin, and serological test of blood samples collected in filter paper. Reproduction session studied a new method for artificial insemination, the effect of sperm DNA fragmentation on fecundation ratio, and the effect of development on seminal parameters in bucks. Management and Welfare session included references about the need to carrying technical-economic management in the farms and the link between cage height and welfare. Nutrition session analysed feeding costs, the effect of birth weight and rearing diet on the reproduction of does, the effect of feeding restriction on the productive and pathological parameters of fattening rabbits, the use of barley and lucerne hay on performance of growing rabbits, the effect of dietary short-chain organic acids on performance of growing rabbits, and the influence of a liquid alimentary supplement on fattening rabbits. Morphophysiology and Body Composition session studied intestinal barrier mechanisms and bone content of wild rabbit carcass.

PATHOLOGY**DEVELOPMENT OF A SUBUNIT
VACCINE CANDIDATE AGAINST
RABBIT HEMORRHAGIC DISEASE**

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Currently available vaccines against the Rabbit Hemorrhagic Disease Virus are based on the virus from the maceration of livers of previously infected with the virulent virus rabbits, with the logical ethic and safety questioning. From the gene that codifies the VP60 protein, from the Spanish isolation AST/89, the cloning and expression of a VLP, that contains this protein, in yeast (*Pichia pastoris*) were performed and its physic-chemical characterization, followed by the development of a purification process and the final formulation of a vaccine candidate. The induced immune response

in short and long term in experimental animals against different REHDV strains, including the antigenic subtype REHDVa has been evaluated, demonstrating the efficacy of the candidate. A confrontation with 100 DL50 of the virulent virus demonstrated that all the immunized animals survived the challenge. Different schemes of immunization, dose and routes of administration have been evaluated.

EVOLUTION OF THE PERIPHERAL BLOOD LYMPHOCYTE POPULATIONS IN MULTIPAROUS RABBIT DOES UNDER TWO REPRODUCTIVE RHYTHMS (WEANING AT 28 AND 42 DAYS POST-PARTUM)

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The emergence of a novel pathology that was previously unknown (epizootic rabbit enteropathy) is causing the change in the protocols of weaning in commercial rabbitries. The traditional protocols of weaning at 28 d postpartum (dpp) are being changed by longer periods. This late weaning is an empirical practice for to reduce the mortality during fattening, probably because of the protective role of milk and the development of the immune system of the young rabbits. The main objective of this study was to compare the evolution of peripheral blood lymphocyte populations in multiparous rabbit does and their pups under 2 reproductive rhythms at the end of their productive life. A total of 22 commercial adult female rabbits and 44 kits, weaned at 28 and 42 dpp, were studied. Samples of peripheral blood were taken in different critical moments and several lymphocytic populations were evaluated with flow cytometry. Additionally, the perirenal fat thickness of does was also measured to evaluate the body condition. In general, rabbit does with weaning at 28 dpp had higher number of different lymphocytic populations in peripheral blood during the studied period. These results were independent of their body condition, contrary to what happened in the animals with weaning at 42

dpp. Some correlations between the quantity of lymphocytes in the does and in the weaned pups were observed. The young rabbits weaned at 28 dpp had higher number of CD4⁺ lymphocytes in the weaning date than those weaned at 42 dpp.

STUDY OF THE SENSITIVITY TO TILMICOSIN OF DIFFERENT RABBIT STRAINS OF PASTEURELLA MULTOCIDA BETWEEN 2002 AND 2008

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Tilmicosin has been used for several years in rabbits to treat respiratory outbreaks due to *Pasteurella multocida*, and for this reason a study was conducted to determine the evolution of the sensitivity to this antibiotic. During the year 2002 and 2008 samples from different respiratory outbreaks in France were collected in which *P. multocida* was isolated. Globally 1902 strains were isolated and later the sensitivity study to tilmicosin was performed. Data confirmed that only basal resistance is detected and there is no increase in the resistance rate during the years.

PULMONARY DISPOSITION STUDY OF TILMICOSIN (PULMOTIL AC, ELANCO) AFTER REPEAT ORAL BOLUS ADMINISTRATION TO RABBITS

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Two groups of 4 non-infected New Zealand rabbits, received tilmicosin (TIM) by oral gavage at 12.5 mg/kg of body weight, daily, for 2 and 5 consecutive days, respectively, while 3 groups of 4 rabbits received the same dose of TIM for 7 d. Two hours after the last dose, all rabbits from groups

treated for 2, 5 and 7 d were sacrificed. Other 2 groups treated for 7 d were sacrificed during the 1st d and the 3rd d of withdrawal. At each time of sacrifice, plasma samples, alveolar macrophages (PAM) obtained by bronchoalveolar lavage and lung tissue were collected from each animal. TIM concentrations in PAM were calculated based on the macrophage cell volume. Mean timlicosin concentrations in lung tissue and PAM were high in TIM-treated animals, already at the second day of treatment, exceeding concurrent plasma levels by 7 and 400-fold, respectively. Among the different days of sacrifice, the highest concentrations were found at the 3rd d-treated animals. TIM concentrations declined in plasma and lung tissue to significantly lower level already at the first day of withdrawal, while PAM concentrations remained high also after 3 d of withdrawal. In conclusion, after daily dosing TIM showed prominent accumulation in lung tissue and PAM, already from the second day, with by far lower concentrations in plasma, as expected for a macrolide compound.

THE SEROLOGICAL TEST OF BLOOD SAMPLES OBTAINED ON FILTER PAPER

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It has been developed a technique for obtaining whole blood on filter paper for serological investigation of diseases of rabbits: RHD; Encephalitozoon, Chlamydia spp and Myxomatosis. This paper proposes a simple alternative to sampling for the determination of antibodies in rabbits that in addition does not require much care in sending to the laboratory. We have evaluated 94 samples of serum from rabbits arrived at the laboratory for the diagnosis of the entities mentioned above. The results of serological testing of samples obtained by venipuncture whole blood and filter paper were compared. The methods used included: Indirect immunofluorescence (IIF) to detect IgG, carbon immunoassay (CIA) and hemagglutination inhibition (HI) to detect total antibodies. The overall sensitivity, specificity and positive and negative predictive values obtained in this work are satisfactory and enable us to say that the taking and transportation of blood samples on filter paper is a useful and accurate for seroepidemiological studies in rabbits.

REPRODUCTION

COGAL, A NEW METHOD OF AI IN RABBIT FARMING

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Artificial insemination in rabbit farming appeared as a management technique in the 90's. During these years it has been evolving on a positive manner in favour of the productive results and in relation to the application of insemination doses. One of the greatest advances has been the replacement of the use of intramuscular GnRH by intravaginal one, resulting in a number of benefits in terms of health, management of hormonal products, labour and welfare of rabbits. In this paper it is explained the implementation of the pilot studies at industrial farms, leading to a new method of artificial insemination in rabbit farming.

SPERM DNA FRAGMENTATION IN SAMPLES FROM *O. CUNICULUS* USING HALOMAX®

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Semen quality is calculated by the assessment of concentration, motility and morphology in semen samples. These parameters do not inform about the state of the chromatin, which could be tested by measuring DNA fragmentation (SDF). High SDF values are related to low fertilization rate and low success of artificial insemination (AI). The present work determines SDF values in 81 rabbits, in order to establish the normal rate of SDF in this species. We use, for testing SDF, the commercial kit Halomax® (HALOTECH DNA SL, Madrid), that allows a fast, and easy way to test DNA fragmentation. Several scientific papers demonstrated that SDF increases after ejaculation, and that is why we study SDF at several time points (0, 24 and 48 h after ejaculation). We tested 81

rabbits from TARPAL centre. Mean of SDF values at t_0 was $9.68 \pm 9.48\%$, at 24 h was $14.31 \pm 12.06\%$ and at 48 h was $21.96 \pm 21.03\%$. The 75% of the animals present a SDF value lower than 10% at t_0 , but only 18% present SDF lower than 10% at t_{24} and t_{48} . Measurement of DNA fragmentation is recommended to select high quality semen doses.

SPERM DNA FRAGMENTATION ASSESSMENT IN *O. CUNICULUS* AND ITS RELATIONSHIP WITH FERTILIZATION RATE AND LITTER SIZE

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Semen quality is determined by the assessment of concentration, motility and morphology in semen samples. These parameters are unable to determine the state of the chromatin. Sperm DNA fragmentation index (SDF) measured with Halomax[®] allow evaluating the state of the chromatin in an easy and fast way. In humans, bulls and boar it has been demonstrated that SDF is related to fertilization rate and litter size. In the present study we determine if SDF is related to fertilization rate and litter size in rabbits. We also suggest a SDF cut-point to select males of high semen quality. Twenty-three rabbits were tested for SDF at 3 time points after ejaculation: 0, 24 and 48 h. Animals were classified in high SDF (SDF-a>10%) and low SDF (SDF-b<10%). Females were inseminated to compare fertilization rate and litter size of both groups. Fertilization rate and litter size was higher in those with low SDF (fertilization rate: 77 vs. 60%; litter size: 72 vs. 46). SDF values over 10% diminish fertilization rate and litter size in rabbits.

EFFECT OF DEVELOPMENT UNTIL REARING ON SEMINAL PARAMETERS IN BUCKS

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The aim of the work is to analyse the effect of birth weight (BW) and rearing development on the number of ejaculates and main seminal traits during training period on bucks selected for growth rate over fattening period (VC). Seminal analyses were performed from 227 ejaculates from 90 bucks born in summer. The animals were organized to range main variability of the BW (low: <60 g; medium: 60-67 g; high: >67 g), the weight at the end of rearing (low: <4720 g; medium: 4720-5400 g; high: >5400 g), as the growth rate during lactation (0-28 d: low: <24 g/d and high: >24 g/d), growth (28-63 d: low: <59 g/d and high: >59 g/d) and rearing (63-147 d: low: <26 g/d and high: >26 g/d). Bucks with high weight at the end of rearing had lower number of ejaculates during their training period than bucks with low weight (2.4 vs. 3.2 respectively; $P < 0.05$), as well as, higher percentage of abnormal spermatozoa than medium and low weight bucks (49.0 vs. 37.1 and 38.1% respectively; $P < 0.05$). The interaction results between BW and weight at the end of rearing showed that bucks with lower BW and high weight at the end of rearing had the lowest number of ejaculates and the highest percentage of abnormalities ($P < 0.05$). These results indicate that early development (gestation, lactation and rearing) could have a relevant effect in the future reproductive characteristics during training period of bucks selected for growth rate.

MANAGEMENT AND WELFARE

BDCUNI: TECHNICAL MANAGEMENT RESULTS IN RABBIT PRODUCTION DURING 2008 AND 2009

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Technical management indexes during 2008 and 2009 in Spanish rabbit farms using bdcuni (database of technical management in Spanish commercial rabbitries, www.ivia.es/bdcuni) are shown in the present work. Indexes obtained were occupation rate (120 and 125%), apparent fertility (81.8 and 82.8%), real fertility (77.3 and 78.0%), number of kindlings per doe and year (7.32 and 7.19), kindling interval (49.9 and 51.2 d), mortality

during lactation (11.4 and 12.2%), mortality during the fattening period (6.6 and 6.2%), averaged slaughter weight (2.113 and 2.101 kg per rabbit), replacement rate (118 and 109%), litter size at weaning per doe and year (56.6 and 55.6), does mortality per month (7.8% both years), produced rabbits per doe and year (53.7 and 51.8), total born alive per kindling (8.74 and 8.81), kilograms produced per insemination (12.0 and 11.8) and global feed conversion ratio (3.58 and 3.59).

RABBIT POSTURE AND BEHAVIOUR TO DETERMINE CAGE HEIGHT IN RELATIONSHIP WITH ANIMAL WELFARE

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Ten rabbits (4 bucks and 6 does) have been introduced individually in a cage 1.0×0.65 and 1.0 m height to test their behaviour in relationship with the height reached assuming different postures. A web camera was utilised, shooting a photogram each minute, to check the posture of the animals during all the circadian cycle. The behaviour of each rabbit was video recorded for 1 wk and about 45000 photograms per animal were checked. The trial showed clearly that most of the time (99.52% of the total occurrences) the rabbits remained at an height lower than 40 cm, that is the standard height of commercial cages. Results show that rabbits don't need higher cages to get a better welfare. The attitude of rabbits to raise on the hind legs to reach heights exceeding 40 cm was analysed too. Though this was very uncommon, thanks to the great number of observations it was possible to demonstrate also a significant difference when comparing the diurnal with the nocturnal activity. The maximum difference was 11.2% of the occurrences about midnight versus 23.8% about eight o'clock ($P<0.01$).

NUTRITION

EFFECT OF BIRTH WEIGHT AND REARING DIET ON THE DEVELOPMENT AND REPRODUCTION OF RABBIT DOES

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In order to evaluate the effect of birth weight (BW: <52, 52-57, 57-62 and >62 g) and the rearing diet (control vs. fibrous), 355 crossbreed rabbit does (V×A; Universitat Politècnica de València) were used. The animals were sexed, weighted and identified at the birth day. Nine weeks after, young females were transferred to a commercial farm receiving one of the experimental diets from this moment to the first partum. BW differences were maintained until the 1st artificial insemination (AI). Thus, females with a BW>57 g had a greater perirenal fat thickness at 1st AI (+0.18 mm; $P<0.05$). Only those females with BW<52 g reached to first partum with a lower live weight (-270 g; $P<0.001$). Females with BW<57 g showed a larger number of total born (TB) at the 2nd partum (+1.24 kits; $P<0.05$). The use of a rearing fibrous diet did not impair the adequate development of young females until 1st AI, and although a reduction of TB at 1st partum was observed (-1.0 kits; $P<0.05$) this disadvantage disappeared in the next cycle. In conclusion, females with a BW<52-57 g arrive to 1st partum with a lower live weight and can show worse reproductive traits. On the other hand, fibrous diets could be a possible restriction choice for rearing.

EFFECT OF FEEDING MANAGEMENT AND FOOD ON PRODUCTION COSTS

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One hundred sixty two litters from 6th to 9th parturition of 88 rabbit does were distributed on 4 groups, varying on feed from 17 to 56 d of age and weaning time. Litters were divided at weaning, half were feeding *ad libitum* and half restricted until 56 d of life. Feed intake of does and litters were recorded from insemination to the end of fattening period (60 d) and feeding cost of each fattened rabbit was calculated. The use of a specific feed for young rabbits, the delay on weaning time and the

feed restriction during fattening period improve the viability of rabbits, but only a great reduction of mortality (50%) had a reduction in feeding cost of 30%.

IMPACT OF FEED RESTRICTION ON FATTENING PERFORMANCES AND YOUNG RABBIT SURVIVAL IN TWO CONSECUTIVE FATTENING PERIODS

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This work consisted of 2 consecutive studies and aimed to test the effect of a feed restriction (feeders were withdrawn from 17:30 to 09:30 h) during the first 14 d after weaning (35 d of age) on fattening performances and rabbit mortality. In both studies, feed restriction reduced ($P<0.001$) weight gain and feed intake from 35 to 49 d. In the following 14 d, restricted rabbits had a compensatory growth but, at 63 d of age, weighed less than 2 kg and their mean weight was lower ($P<0.005$) than that of *ad libitum* fed rabbits. In the second study, restricted rabbits reached a body weight of 2 kg when slaughter was postponed at 70 d. In none of the studies, feed restriction allowed to improve feed conversion. Mean mortality rate was 2.08% in the first study but amounted to 15.6% in the second one. In the latter study, feed restriction reduced mortality rate (from 25.6 to 6.34%; $P=0.017$). However, in this study, the higher diarrhea incidence did not only increase mortality rate compared to the first study but also rose number of morbid animals, leading thereby to a larger heterogeneity of productive results and to a real feed conversion ratio (3.62; calculated by taking into account feed intake of rabbits that died afterwards) that was much higher than that determined by solely considering animals that were alive at the end (2.88).

EFFECT OF TYPE OF GRINDING OF BARLEY AND LUCERNE HAY ON PERFORMANCE AND DIGESTION OF GROWING RABBITS

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This work consisted of three consecutive fattening periods (from weaning: 35 d, to slaughter: 63 d) in which 4 treatments arranged in a factorial structure 2×2 with grinding (coarse, 4.5 mm vs. fine, 1.5 mm) and ingredient (barley vs. lucerne hay) were tested. Fine grinding of barley only implied a decrease of weight gain (from 43.7 to 42.1 g/d; $P<0.05$) when also lucerne hay was ground at 1.5 mm. Mortality rate was significantly influenced ($P<0.0001$) by the fattening period, being on average 5.9, 7.0 and 16.0% for the first, second and third periods. However, none of the treatments did affect mortality rate. Apparent ileal digestibility coefficients of dry matter and starch increased by 19.1 ($P=0.037$) and 5.33% ($P=0.065$), respectively, in rabbits fed a diet containing coarse lucerne hay when barley was finely ground. Apparent faecal digestibility coefficients were not influenced either by type of grinding of barley or that of lucerne hay and measured on average 0.596, 0.370, 0.687 and 0.609 for dry matter, neutral detergent fibre, crude protein and energy, respectively and the average digestible energy content amounted to 10.8 MJ/kg dry matter.

EFFECT OF DIETARY INCLUSION OF SHORT-CHAIN ORGANIC ACIDS ON RABBIT GROWTH, FATTENING MORTALITY AND DEVELOPMENT OF GUT ASSOCIATED LYMPHOID TISSUE

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In this work, 3 homogeneous groups of 40 rabbits were assigned to 3 different diets consisting of a basal diet, this same diet added 150 ppm of zinc bacitracin and 120 ppm of colistin and the basal diet with 0.4% of a premix of short-chain organic acids and 120 ppm of colistin. At 35 d of age, all rabbits were given a 2 mL bolus containing 10^9 cfu of *Clostridium perfringens* and *Escherichia coli*. Neither dietary inclusion of antibiotics nor that of organic acids improved fattening performances. Feed intake was 102 g/d, on average. Animals receiving the basal diet had the highest mortality rate compared to the rest of treatments (62.0 vs.

0.0%; $P=0.005$). Villi measured, on average, 597 μm for rabbits fed the basal diet but grew 108 and 201 μm , respectively, for rabbits receiving bacitracin and organic acids from 35 to 56 d. Mean area of lymphoid follicles of Peyer's patch was not greater after the infection for rabbits fed the diet containing organic acids (59500 μm^2 on average) whereas follicles became larger in the other groups (follicle area increased, respectively, 55.7 and 57.0% for rabbits receiving the basal and the bacitracin diets).

INFLUENCE OF A LIQUID ALIMENTARY SUPPLEMENT CONTAINING MINERALS, VITAMINS AND AMINO ACIDS ON THE MORTALITY AND THE GROWTH OF GROWING FATTENING RABBITS

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Few experimentations have deal with the distribution of nutriments through drinking water for rabbits. Particularly, few researches have studied the effects of such a way of electrolyte distribution. For these reasons, the consequences of the distribution of a liquid alimentary supplement containing minerals, vitamins and amino acids, the Nutrireidrovit on the mortality and the growth of growing fattening rabbits have been studied. Globally, the Nutrireidrovit distribution has positive effects by decreasing the mortality in growing – fattening and by increasing the growth during the first part of fattening. Nevertheless, the mortality decrease is mainly observed when the mortality is up than 5 % and the growth is improved only during the first part of growing - fattening. Consequently, the use of the product is advised in farms with mortality higher than 5 % or after weaning to boost the growth.

MORPHOFISIOLOGY AND BODY COMPOSITION

EVOLUTION WITH AGE OF GUT BARRIER MECHANISMS.

1. DIGESTIVE TRACT AND CAECAL MICROBIOTA

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One hundred and six rabbits from 20 litters were weaned at 26 d of age weighing 429±57 g. One rabbit per litter were slaughtered at 26, 31, 38, 45 and 52 d of age. The weight of the digestive tract and caecum (% body weight) increased from 26 to 38 d ($P<0.001$). Development of caecum was higher from 26 to 31 d of age compared to total digestive tract (5.2 to 7.1% vs. 20.8 to 23.3%, respectively), whereas the evolution from 31 to 38 d was similar, showing maximum values at 38 d (8.5 and 28.1%, respectively). From 38 to 52 d of age weight of total digestive tract and caecum decreased and remained stable from 45 d onwards (7.6 and 24.3%, respectively at 52 d). The similarity rate (SR) of caecal microbiota was higher among animals of the same age (varied between 89.1 and 95.3%) than among animals differing in age (varied between 82.3 and 92.5%). The variability in caecal microbiota composition was lower at 45 and 52 d of age (showing a SR of 92.5% between them) compared to younger animals (26, 31 and 38 d, showing a SR between 82.3 and 86.7 among them). The greater differences in SR were detected between suckling rabbits and rabbits just after weaning (31 and 38 d of age).

EVOLUTION WITH AGE OF GUT BARRIER MECHANISMS. 2. MUCOSA MORPHOLOGY AND IMMUNE SYSTEM

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One hundred rabbits from 20 litters were weaned at 26 d of age weighing 429±57 g. One rabbit per litter were slaughtered at 26, 31, 38, 45 and 52 d of age. Samples of intestinal mucosa and appendix were processed to determine mucosa morphology (jejunum, n from 16 to 20 per age)

and the expression of several cytokines (ileum and appendix, n=6 per age). Villi length and crypt depth in the jejunum were reduced ($P<0.05$) between 26 and 31 d and 26 and 31 d, respectively. However, we observed an increase in both traits from 31 to 38 d. From 38 to 52 d of age, villi length did not change, showing similar values that those observed at 26 d, however the crypt depth increased ($P<0.05$). The expression of cytokines were higher in the vermiform appendix than in the ileum, corresponding the highest expression levels to IL-10, IL-8 and TNF- α with values of threshold cycle (Ct) of 19.8, 20.6 and 20.7, respectively. The expression of IFN- γ in the appendix significantly increased with age ($P<0.05$). In the ileum, the expression of IL-2 was significantly increased with age ($P<0.05$) and the expression of INF- γ show a tendency ($P=0.06$) to increase.

($P<0.001$) was achieved. No sexual dimorphism was found for the bone content, nor correlation between carcass weight and bone content of the carcass of the wild rabbit. When compared to figures published for meat breeds and lines at the usual age of slaughtering in Spain, the bone content of the wild rabbit carcass is moderate.

CARCASS BONE CONTENT IN WILD RABBITS HUNTED IN ANDALUSIA (SPAIN)

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With the aim to characterise the carcass bone content of the wild rabbit (*Oryctolagus cuniculus algirus*) obtained from hunting, 53 specimens (unskinned, eviscerated rabbits) bought in markets of Seville (Spain) were analysed. The specimens weighed 767.8 g. The reference carcass, obtained by flaying the specimens, weighed 551.2 g, and after carrying out the technological division according to the method proposed by the World Rabbit Science Association, 14.3% of fore leg, 38.0% of hind part, 32.1% of loin, and 11.4% of thoracic cage were obtained. Bone percentage was 16.3% in the fore leg, 13.4% in the hind part, 9.4% in the loin, 22.5% in the thoracic cage, and 13.0% in the reference carcass. Bone percentage of the hind leg gave a reliable prediction of the bone percentage of the carcass, because $R^2=0.737$