

ABSTRACTS OF THE "XXXVII SYMPOSIUM DE CUNICULTURA DE ASESCU"**BARBASTRO, SPAIN, 24-25 MAY, 2012**

The annual Congress of the Spanish Association of Cuniculture (ASESCU) was held in Barbastro (province of Huesca, autonomous community of Aragón) on 24-25th May of 2012. The 37th edition was mainly devoted to analyse the present situation of the rabbit sector, as well as some new aspects in relation to diseases of the rabbit. It was presented an overview of the situation of the rabbit farming in Aragón, a commercial analysis of the rabbit farming sector in Spain, a main paper on the role of rural development as a complement to rabbit farming, and an analysis of the production cost of rabbit meat. Main papers related to parasite control and mixomatosis were also presented in 2 different conferences. Moreover 2 round tables were held on a new variant of rabbit haemorrhagic disease virus and a Sectorial Plan for the Spanish rabbit sector, respectively. Furthermore, a total of 18 communications were presented both in working sessions of oral communications and posters. Nutrition session analysed the influence of fibre type on its digestibility, the characterisation of intestinal microbiota in rabbits after weaning, the utilisation of distillers dried grains with solubles as well as of beet pulp in diets for fattening rabbits, the effect of dietary restrictions on the growth of fattening rabbits, and the effect of a prebiotic, a probiotic or a symbiotic in diets for fattening rabbits. Technico-economic management session studied the evolution of technical results of the Spanish farms during 2008-2011, as well as technical and economical implications of reproductive rhythms and weaning age for the profitability of farms. Genetics session evaluated the effect of divergent selection for residual variance of litter size on the embryo development. Ethology session analysed sex influence in rabbit mutual olfactory relationships. Reproduction session studied prediction of body fat deposits from perirenal fat measurements using ultrasound. Meat quality session analysed the effect of a supplementation with vegetable DHA on the growing and fattening performances and meat quality of the rabbit. Pathology section studied the effect of selection for reproduction or longevity on blood lymphocyte populations of does under conventional and heat stress conditions, the origin of staphylococcosis in rabbits, the epidemiology of staphylococcosis, pasteurellosis and bordetellosis in Portugal, and several works related to a new variant of rabbit haemorrhagic disease virus.

NUTRITION**INFLUENCE OF FIBRE TYPE ON ILEAL AND FAECAL DIGESTIBILITY OF FIBRE****ABAD R., GÓMEZ-CONDE M.S., CARABAÑO R., GARCÍA J.**

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The effect of type of fibre on the ileal and faecal digestibility of the total dietary fibre (TDF), insoluble dietary fibre (aNDFom-cp) and soluble fibre (SF, expressed as TDF-aNDFom-cp) was studied in adult cannulated rabbit does. A control diet (DA) containing 10.3% of SF included dehydrated alfalfa as the main source of fibre. Another diet (B-AP) was formulated by replacing half of dehydrated alfalfa with a mixture of beet and apple pulp

(75/25) resulting in 14.6% of SF. A third diet (OH) was obtained by substituting half of dehydrated alfalfa with a mix of oat hull and a soybean protein concentrate and contained 8.43% of SF. All diets contained similar levels of TDF (41.9%). Thirty three adult rabbits (11/diet) were used to determine the ileal and faecal digestibility. The TDF and aNDFom-cp digestibility (ileal and faecal) were higher ($P<0.001$) in the B-AP diet respect to OH and DA diets. Experimental diets did not affect the faecal digestibility of SF, although, the B-AP diet showed the highest ($P<0.001$) SF ileal digestibility. The SF was highly fermentable (84%) independently of the fibre source. The mucin correction to determine the TDF and SF digestibility was important (mainly in the ileum) because it represented an average difference of 5.5 percentage units in TDF and 20.8 in SF. There was a positive effect of the SF in the concentration of mucins. The mucins were highly fermentable in the caecum (88%).

CHARACTERIZATION OF INTESTINAL MICROBIOTA IN YOUNG RABBITS AFTER WEANING

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This study described the development of the rabbit microbiota in the caecum and soft faeces (CC), and in the ileum (IL) from weaning, performed at 26 d, to 52 d of age. The intestinal flora consisted mainly in the *phylum Firmicutes*, *Bacteroidetes* and *Verrucomicrobia* both at CC and IL, while in IL it was a high proportion also important *Proteobacteria*. At 26 d *Firmicutes* and *Bacteroidetes* represented a high proportion of CC microbiota (47 and 53% respectively). As age increased *Bacteroidetes* ($P < 0.001$) was replaced by *Firmicutes* ($P < 0.001$) than at 52 d was 85% of the flora majority. In the IL there was not a clear evolution with age of any *phylum*. Within the *phylum Firmicutes*, the most abundant clones belonged to the order *Clostridiales* NID-identified their families-both in CC ($P = 0.002$) and IL ($P = 0.001$). On the other hand, within the *Bacteroidetes phylum*, family *Bacteroidaceae* ($P < 0.001$) was the most important in the CC representing 24% at 26 d. In the ileum, the most important family was *Porphyromonadaceae* ($P = 0.015$) which appeared in a low proportion in early ages (5%), but increased from 45 to 52 d where represented 37%.

UTILIZATION OF DISTILLERS DRIED GRAINS WHIT SOLUBLES (DDGS) IN DIETS FOR FATTENING RABBITS. PRELIMINARY DATA

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One hundred and seventy-six weaned rabbits 28 d old were fed for 31 d, in 2 runs and assigned to 5 diets: control (CD, 0% DDGS), D1 (20% barley DDGS), D2 (20% corn DDGS), D3 (20% wheat DDGS) and D4 (40% corn DDGS), evaluating the response to production variables (final live weight (g), weight gain (g/d), consumption (g/d), conversion food and carcass yield (%)). Data analyzed using a linear model with fixed effects: run and diet, and the covariate: weight at weaning and were tested the effect of levels

(0, 20 and 40% DDGS corn) with orthogonal polynomial contrasts. Preliminary results of the study suggest that the inclusion of DDGS in rabbit feed tends to improve weight gain, food consumption, and especially the performance of the carcass yield. Finally, the limit of incorporation of corn DDGS in diets for fattening rabbits would be about at 40%.

RABBITS PERFORMANCE WITH DIETS BASED ON BEET PULP. PRELIMINARY DATA

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One hundred and twenty weaning rabbits were used in a fattening trial of 31 d and feeding with 5 isoenergetic, isoproteic and isofibrous diets including 0, 20 and 40% of 2 types of beet pulp (BP) with or without molasses. Live weight and food intake were recorded during the period and conversion rate was determined. At the end of fattening trial, 20 animals 59 d old were slaughtered to evaluate the carcass yield. Feed intake and weight gain were lower ($P < 0.01$) for BP diets, especially with diets including 40% BP, but conversion rates was lower ($P < 0.01$) only for the diet recording the lower intake, and carcass yield decreased ($P < 0.001$) with diets including 40% BP in relation to control diet without beet pulp.

EFFECT OF DIETARY RESTRICTIONS ON THE GROWTH OF RABBITS FROM 35 TO 70 DAYS OLD

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This study used 166 rabbits in order to evaluate the effect of feed restriction on growth rate. The rabbits were divided in 4 treatments according to the access time to feed (*ad libitum* (C), access to feed during 5 or 10 h a day (D5 and D10, respectively) or on alternate days (D24)). Rabbits were weighed weekly between 35 and 70 d of age and growth was studied using the linear fit of the growth curve and the test of mean comparison of the regression coefficients. The correlation coefficients in linear fit of the growth curves were higher than 0.97 with

the 4 treatments. The average regression coefficients of the treatments D5 and D24 differ significantly from the coefficients of diets C and D10.

THE EFFECT OF THE USE OF A PREBIOTIC, A PROBIOTIC OR SYMBIOTIC IN RABBIT DIETS AFTER WEANING

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The use of prebiotics, probiotics or both can contribute to maintaining and improving intestinal health and consequently reduce mortality and improve the zootechnical results and influence the diet digestibility and conditions of the caecal ecosystem. In order to evaluate the parameters referred by the addition of FOS (Fibrulose®), *Saccharomyces cerevisiae* (Levucell®) and both, were formulated 4 diets: the control diet (CTR) without additives, and PRE, PRO and PRE+PRO diets, identical to the first, but to which 25 g/kg of FOS, 0.6 g/kg of yeast and 25 and 0.6 g/kg of pre and probiotics, were respectively added. These diets were distributed *ad libitum* to an initial group of 192 (48×4) rabbits, weaned at 25 d, and housed in groups of 4 per cage according to their body weight. Three experimental periods were considered depending on the age of rabbits: P1 of 25-28 d, 28-35 d of PII and PIII of 35-45 d. The mortality was recorded every day. Faeces were collected during 5, 6, 7, and 8th days for digestibility determination. At the end of each period, one rabbit from each cage was slaughtered and caecal contents were collected for pH, DM and volatile acidity determination. The addition of prebiotic, probiotic or both did not lead to significant improvement in zootechnical parameters or on the mortality rate. The addition of prebiotic, probiotic or both did not affect the digestibility of the fractions considered although the digestibility of cellulose (ADF-ADL) was higher in diets with additives ($P=0.061$). The parameters of cecal fermentation activity indicate a significant decrease of the pH of cecal contents in all the periods. The lowest caecal content pH was obtained when rabbits were fed PRE or PRE+PRO. However, this decrease was not reflected on the volatile acidity: total, individual VFA molar proportion or in C3/C4 proportion. The addition of FOS and/or *Saccharomyces cerevisiae* had no effect on performances, on mortality rate, on digestibility of growing rabbits in the 20 d following weaning.

TECHNICAL AND ECONOMICAL MANAGEMENT

EVOLUTION OF TECHNICAL RESULTS 2008-2011 WITH THE BDCUNI SPANISH DATABASE

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Bdcuni (database of technical management in Spanish commercial rabbitries) is a free and confidential system which offers technical and economical management to Spanish rabbitries. The system works through the web page www.ivia.es/bdcuni and provides the technical indexes obtained in the own rabbitry, the whole database and the association of the rabbitry. In the present work, the mean technical indexes obtained from 2008 to 2011 are shown. Some of the technical indexes obtained in 2011 were 7.5 kindlings per female and year, 56.2 rabbits produced per female and year and a global feed conversion ratio of 3.63.

REPRODUCTIVE RHYTHMS AND WEANING AGE IN RABBIT FARMS. TECHNICAL AND ECONOMICAL IMPLICATIONS

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This work studies the impact of Reproductive Rhythm (RR) and Age at Weaning (WE) on economic and productive performance of a rabbit farm. RR is defined as the number of days from parity to next mating. The choice of RR and WE have consequences on growth and kit survival as well as on doe reproductive performance and replacement rate. In commercial farms, it usually can take the following values: 4, 11, 18, 25, 32 and 39 d. Therefore, the length of the production cycle (PC) (i.e. the sum of RR and length of gestation) can show the following values: 35, 42, 49, 56, 63 and 70 d. Results show a decrease from 6.71 to 4.13 kindling per female and year when RR took values of 4 and 39 d, respectively (-38% drop in production). Production costs increased from 1.62 to 1.97 €/kg when RR increased from 11 to 39 d (22% increase in costs). Overcrowding was effective at offsetting losses in number of births per female. High values of this parameter lead to an increased production of kits but depended on RR. The consequences of the choice of RR on productive results are not parallel to those on economic returns. Thus, the

production cost increases from 1.62 to 1.79 € per kg of alive animal for a RR values of 11 and 39 d, respectively (10% increase). In overcrowding conditions, the greatest production per female and year are reached with a RR of 11 d, while the best economic results are achieved with a RR of 18 d.

MEAT

EFFECTS OF A SUPPLEMENTATION IN VEGETABLE DHA ON THE GROWING-FATTENING PERFORMANCES AND THE MEAT QUALITY OF THE RABBIT

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This publication presents the results of an experimentation concerning the supplementation of rabbit feed with vegetable DHA. One thousand and fifty-four rabbits are split between 2 treatments corresponding to the distribution of a control diet and of a diet containing 0.2% of vegetable DHA. The supplementation with DHA decreases highly significantly the mortality during the finishing period but has no effect on the growth. Rabbits receiving the DHA diet have a meat with a high level of this fatty acid, till 275 mg/100 g for the back. The omega 6 level of the meat decreases too, improving consequently the omega 6/omega 3 ratio. These changes of the fatty acid level don't modify significantly the hedonic characteristics of the meat.

PATHOLOGY

EFFECT OF SELECTION FOR REPRODUCTION OR FOUNDATION FOR LONGEVITY ON BLOOD LYMPHOCYTE POPULATIONS OF RABBIT DOES UNDER CONVENTIONAL AND HEAT STRESS CONDITIONS

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This work is a continuation of the preliminary study ("Effect of heat stress on immune system of rabbit does from different genetic origin. Preliminary study") presented at the congress of ASESCU 2011, which is characterized how selection for reproduction (by comparing 2 generations –16th and 36th– of the V line selected for litter size at weaning) or foundation for reproductive longevity (the LP line), can affect the blood lymphocyte populations of reproductive rabbit does under normal and heat stress conditions from the first to the second parturition. These results indicate that, under conventional housing conditions, litter size selection at weaning for 20 generations may affect the immune system since the V36 animals had lower lymphocyte counts than V16 animals at a very critical time, e.g., the second parturition, whereas under heat stress conditions, the animals from a line founded by screening for reproductive longevity (the LP line) presented higher lymphocyte counts at this particular stage than those from V36. This scenario could contribute to the greater ability to confront infectious challenges and to confer animals a more robust nature.

DETECTION OF A NEW VARIANT OF RABBIT HAEMORRHAGIC DISEASE VIRUS IN WILD RABBITS IN SPAIN

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Rabbit haemorrhagic disease virus (RHDV) is a virulent calicivirus that causes high mortality both in domestic and wild rabbits. It appears to be a mutant form of a benign virus that existed in Europe long before the first outbreak. To date, however, phylogenetic data indicate that all the field strains of RHDV found in the Iberian Peninsula have a common ancestor closely related to pathogenic strains isolated during the first RHD outbreaks in this area. In order to study RHD epidemiology, an experimental wild rabbit population was monitored twice at week during 2010 and 2011 yr in order to find rabbits dead by the disease. Detection of viral RNA in liver tissue of carcasses was conducted by RT-PCR reaction and, when positive, PCR products were purified and sequenced. Across 2010, 12 rabbits were found dead by RHD, whereas 9 rabbits were found dead in 2011. The original RHDV field strain was identified in all dead rabbits found in 2010 and in 3 of 2011. However, a new strain was identified in 5 of remaining 6 carcasses found in this year. Phylogenetic

analysis showed that this new viral strain would be a member of the new variant of RHDV recently detected in France in 2010. This variant is relatively distant from already described pathogenic and non-pathogenic lagoviruses, and it has been reported to cause increased mortalities in French rabbitries. In Spain, several atypical RHD outbreaks have been described affecting rabbitries during 2011 and this variant has been proposed as the putative aetiological agent. This survey confirms that this new RHDV variant would be already present in Spain.

EPIDEMIOLOGY OF STAPHYLOCOCCOSIS IN PORTUGUESE RABBIT FARMS

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An epidemiological study was carried out between January of 2007 and April of 2011 in Portuguese rabbit farms to investigate farm characteristics related with staphylococcosis. In 7 farms (17.1%), one or more animals were positive to *Staphylococcus aureus*. Differences were observed between the farms. The majority of positive farms (85.7%) were from the North of Portugal. Five positive farms (71.4%) had less than 900 female's rabbits, and 2 positive (28.6%) had more than 900 females. In 7 positive farms, ammonia levels were less than 5 ppm and in 3 farms (57.1%) had humidity levels between 55 and 80%. This study provides preliminary data in staphylococcosis in farms. More extensive investigations are required with multiple farms and replicates to confirm our findings and assess the role of each of these factors.

CROSS-SECTIONAL STUDY OF PASTEURELLOSIS AND BORDETELLOSIS IN PORTUGUESE RABBITS

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Pasteurella multocida is the most common cause of respiratory problems in rabbits. *Bordetella bronchiseptica* can contribute to upper respiratory tract infections. A cross-sectional study was performed to investigate the epidemiology of pasteurellosis and bordetellosis in rabbit farms in North and Center of Portugal. In all, ammonia

levels were lower than 5 ppm and humidity were between 55 and 80%. In total, 41 farms were studied, 9 farms (22.0%) were positive for *Pasteurella* and 5 were positive for *Bordetella*, 4 of them (80%) were from the North. Two farms had concomitant infection with *Pasteurella* and *Bordetella*. Respiratory disease on farms is a reality in Portuguese rabbitry.

PARADIGMS AND NEW CHALLENGES IN THE DIAGNOSIS OF THE VIRAL HEMORRHAGIC DISEASE OF RABBITS

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The rabbit hemorrhagic disease (RHD) was described in the 80s and is still an emerging disease in the rabbit. Its "recent" description and the inability to isolate the causative virus (RHDv) using *in vitro* systems like cell cultures, have prevented explore in detail some aspects of the infection. While professionals involved in the modern rabbit industry are perfectly familiar with the RHD, due to the negative impact of the disease in the production of this domestic animal, it is important to investigate further about the basics of the biology and evolution of the causal agent, the virus-host relationships, and some aspects of the epidemiology of the disease, among others.

The inability to isolate *in vitro* the RHDv represents an important limitation in basic research of the disease, since there are no field strains that can be adequately characterized at the genetic and antigenic level. This also affects the diagnosis of the disease, since the methods used in field outbreaks to detect infection are always indirect, and seek the antigen or the virus's genetic material present in the tissues of affected animals. Typically, the diagnosis is made by PCR, which stands as the alternative for a diagnosis that otherwise could not be done with the sensitivity and specificity required in cases of lethal diseases such as RHD.

PCR can be used to amplify and detect RHDv genome for diagnostic purposes. PCR can also be used to amplify fragments of the genome to conduct more detailed studies of genetic sequence features. In a hypothetical example, after obtaining a positive result for PCR in the organs of rabbits affected by the infection, and once established consistency between this result and the clinical signs observed, it is possible to use the same samples to investigate other fragments of the viral genome, using another(s) PCR(s). In this case the second PCR is designed to determine the exact sequence of some genes, that provide information on their similarity with those of other strains previously described, or its possible origin.

In order to amplify the virus genome using PCR either to detect or to characterize it, it is essential that the PCR lacer or “hybridize” perfectly on the genome of the virus. There must be a perfect complementarity between the PCR and the template of genetic material; the greater the stability of the virus genome (low mutation rate over time), the higher the possibility of perfect match with the PCR. Something that has been evident in recent years, thanks to the availability of molecular biology tools, is that the genetic sequences of the circulating field viruses show a remarkable homogeneity when compared to each other. This may be due to the rapid transmission and high lethality of the virus in populations susceptible to infection, not allowing time for selective pressure of divergent clones of the virus generated after mutation. As a result of this uniformity, “classic” RHDv may be detected by PCR with a reduced risk of false negative results. However, the recent description of viruses that have a high antigenic and genetic divergence regarding classic strains represent a challenge for PCR based diagnostics. The question is whether existing PCR protocols will detect also these new strains. There is a possibility that the newly described variants of the virus, and those that may be identified in the future exhibit heterogeneity in the genome fragment used for detection by PCR. As a result the PCR could not identify them and go unnoticed.

A good example of this scenario is the French variant strain recently described (RHDvFra10). This variant belongs to a new genotype, as its genome differs substantially from the strains described so far. Because it is an emerging pathogenic strain that has also been detected in Italy causing outbreaks of the disease, it is necessary to detect and differentiate it from classical strains. Molecular characterization requires the adaptation of specific PCRs to identify it on a “variant” level. This will make it possible to determine the degree of similarity between strains circulating in different countries, and even “follow” their temporal and geographic evolution.

The RHDv, like many other human and animal viruses, has the natural ability to agglutinate erythrocytes when mixed together in a suspension. This phenomenon is known as haemagglutination (HA), which has been the basis of a presumptive diagnosis of RHD. Whenever samples come from animals died in the first hours after infection (<3 d pi), are preserved in good condition (cooling and immediate shipment), and the test is done properly, the HA may be a screening test that provides immediate results, helping the decision-making in case of an active outbreak of infection. However, the virus present in tissues of “chronically” infected animals (5-7 d pi) usually present degradation as a result of the clearance process by the animal’s immune system. It is possible that in these cases the outcome of HA test may be a false negative.

Furthermore, there is variability in HA results among laboratories, due to the use of test procedures that are not standardized. Variations in the incubation temperature or changes in the pH conditions of the solution alter the reading of the result, which is subjective and somewhat dependent on the experience of the person performing it.

The haemagglutinating capacity of the RHDv is determined by viral genome sequences, which encode proteins that bind erythrocyte surface. There have been identified RHDv strains that have mutated their genome and have lost this ability, without having changed much its antigenic composition. The loss of this ability is random and independent of the type of virus, as it has been described in classical strains, as well as in variants “a”. The presence of HA⁻ strains, and the inherent variability in the laboratory method, make the results of the HA should be interpreted with caution, and in any case be supplemented by clinical signs, necropsy findings and, if possible with PCR to confirm the diagnosis. During the presentation will detail these and other issues that illustrate the complexity of the diagnosis of RHD in a background of change.

GENETICS

EFFECT OF DIVERGENT SELECTION FOR RESIDUAL VARIANCE OF LITTER SIZE ON THE EMBRYO DEVELOPMENT

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The aim of this study is to evaluate the embryo development at 72 h *post-coitum* in 2 lines of rabbits divergently selected for residual variability in litter size. Females of the high line for residual variability in litter size show a higher percentage of embryos of early *morulae* (6.0%) than females of the low line (2.1%). These results suggest that selection to reduce residual variability in litter size may have an effect on the early embryo development.

ETHOLOGY

SEX INFLUENCE IN RABBIT MUTUAL OLFACTORY RELATIONSHIPS

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An investigation aimed to ascertain if sniffing behaviour, as a trait of social behaviour, is influenced by the rabbit's sex when they are kept in cages was carried out. Three replications, each time of 3 adult rabbits (Leprino of Viterbo breed) 7 mo old, were located, at the same time, in contiguous cages where they remained for 7 d. The cages were limited by a solid tin wall with small holes that impaired the animals to see each other. Bucks were located in the central cage, while 2 does were set at both sides. The bucks, sniffing at the walls, showed an olfactory preference for 1 of the does located at both sides. The behaviour tended to decrease slowly. The significance was $P < 0.01$ for the first 3 d, it lowered to $P < 0.05$ in the next 3 d and in the seventh it loosed significance ($P > 0.05$). The interest of bucks towards the does also showed a typical behaviour of frenetic scratching at the separation wall, contemporary with intense sniffing, which was observed only for the first 35 min of the first day. A prevalent sniffing of females at the central cage where the bucks were located was observed ($P < 0.01$) though not so marked as for males. It was significant at the beginning and it decreased across the trial. In conclusion, rabbits establish a sex-oriented and transitory olfactive relationship with the conspecifics located in contiguous cages. Because of the diffusion in the air of the olfactory stimuli, this behaviour appears to be no longer necessary when the animals have recognized each other by sniffing.

REPRODUCTION

PREDICTION OF RABBIT BODY FAT DEPOSITS FROM PERIRENAL FAT MEASUREMENTS OBTAINED WITH ULTRASOUND

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This study aimed to use images obtained by real time ultrasound (RTU) technique to predict body fat reserves in does with different body condition. After image analysis it was obtained measurements of perirenal fat thickness using kidney as a reference. Measurements were made *in vivo* in 21 adult rabbits does. After RTU measurements animals were slaughter and quantified the fat deposits. A statistic descriptive of data and correlation analysis between RTU measurement and fat deposits were performed. Fat deposits present a large variation, being higher (CV=123%) for pelvic fat. The best correlation coefficients were found between the RTU perirenal fat thickness measurement and fat and perirenal fat ($r=0.90$, $P < 0.01$) and total body fat (0.894, $P < 0.01$). The results of this study indicate that the perirenal fat thickness obtained by ultrasound can be considered for predicting body fat reserves of adult rabbits does.