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Manuscript

Running head: feeding programmes for young rabbit does

Effects of rearing feeding programme on the performance and energy balance of young rabbit does

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ABSTRACT

A total of 228 young rabbit females aged 9 weeks were used to evaluate five rearing feeding programmes: CAL, fed *ad libitum* with a control diet [C: 11.0 MJ digestible energy (DE) and 114 g digestible protein (DP) per kg dry matter (DM)] until first parturition; CR, receiving the C diet restricted (140 g/d) from 12 weeks of age to first partum; F, fed *ad libitum* with a moderate energy fibrous diet [F: 8.7 MJ DE and 88 g DP per kg DM] until first partum; and finally, FC and FCF, fed with F diet *ad libitum* until 16 weeks of age, whereupon FC group received the C diet *ad libitum* until first partum, while FCF group received the C diet *ad libitum* until 20 weeks of age and then the F diet *ad libitum* until first partum. CAL group had a higher mortality rate compared to the other groups between 9 and 12 weeks of age (34 *vs.* 3%; P<0.05) and during the last 3 weeks of first pregnancy (14 *vs.* 3%; P<0.05). CAL and FC females presented higher BW and perirenal fat thickness (PFT) than CR females at week 20 (+0.41 kg and +0.6 mm; P<0.05), with F females showing medium values. The type of feeding procedure did not affect the fertility rate of young females at first AI. Differences in BW disappeared at parturition, when only CAL females presented a greater PFT than CR and FC females (+0.3 mm; P<0.05). In comparison to FCF, CAL females had smaller and thinner live born litters (-2.5 kits and -139 g, respectively; P<0.05), with CR, F and FC females showing medium values. The low number of kits born alive for CAL females was due to their lesser total number of kits born (-1.7 kits; P<0.05) and the greater mortality of their litters at birth (+13.9 %; P<0.05) compared to FCF. NEFA was higher in the blood of females fed C diet (CAL and CR) than in others at partum day (on average +0.15 mmol/L; P<0.05). In conclusion, the *ad libitum* use of diets for lactating rabbit does throughout the rearing period could lead young rabbit females to present a higher risk of early death and smaller litter size at first parturition. Feed restriction or earlier use of suitably fibrous diets led females to achieve the critical BW and fat mass at first mating to ensure reproduction.

Key words: rabbit females, rearing, pubertal development, body condition, metabolic status.

IMPLICATIONS

Obtaining well-developed rabbit females that produce a large number of healthy, marketable litters per mating over multiple parities is still one of the main priorities for rabbit production. This objective not only involves the use of adequate management programmes during reproduction, but also appropriate management of nutrition during pre- and post-pubertal growth to ensure better development of future reproductive females. The correct design of rearing programmes that take into account the young rabbit female's requirements and priorities, while ensuring both adequate pubertal development and future reproductive performance, is a pressing need.

INTRODUCTION

The negative effects of underfeeding on pubertal maturation have long been known in numerous species (Frisch, 1984). However, overfeeding during rearing period has been also related with lower reproductive performance in dairy heifers (Sejrsen *et al.*, 1982), pullets (Whitehead, 1988) and gilts (Klindt *et al.*, 1999). Young rabbit females fed *ad libitum* until first parturition usually suffer similar problems to those mentioned for other species.

For this reason, in the last decade some works assessed the possible impact of different management and feeding plans for rearing period on female development and reproduction: feed restriction (Rommers *et al.*, 2004), BW at weaning or at the first artificial insemination (AI) (Rommers *et al.*, 2001a, 2001b, 2002), or the use of fibrous diets (Xiccato *et al.*, 1999; Pascual *et al.*, 2002; Quevedo *et al.*, 2005). However, some of these works have shown an antagonism between proper development and improvement of reproductive response. The earlier the introduction of the restriction programme and the lower the energy supply, the higher the voluntary feed intake of primiparous does with improved milk yield or reduced body reserves mobilisation during first lactation (Nizza *et al.,* 1997; Xiccato *et al.*, 1999; Pascual *et al.*, 2002), but the later or the lower their pubertal maturation (Pascual *et al.*, 2002; Rommers *et al.*, 2004).

On the basis of this previous information, in this work we evaluated the effects on nulliparous rabbit does development of a diet for reproductive rabbit does provided during the rearing period both *ad libitum* and restricted, compared with three different feeding programmes based on the use of a moderate-energy fibrous diet designed for young rabbit females and provided: i) until first parturition, ii) until first mating and iii) until first parturition applying a flushing around first mating.

MATERIAL AND METHODS

The experimental procedure was approved by the animal welfare ethics committee of the Universitat Politècnica de València (UPV) and carried out following the European Union recommendations on care and protection of animals used for experimental purposes (2003) and the advice for applied nutrition research in rabbits according to the European Group on Rabbit Nutrition (Fernández-Carmona *et al.*, 2005).

Diets

Ingredients and chemical composition of the experimental pelleted diets used in this trial are summarised in Table 1. A control diet (C), similar to a commercial diet for reproductive rabbit does [11.0 MJ digestible energy (DE) and 114 g digestible protein (DP) per kg dry matter (DM)], was formulated following the recommendations of De Blas and Mateos (2010). In addition, a moderate energy diet with a high fibre content (F) was also formulated [8.7 MJ DE and 88 g DP per kg DM], including some minor ingredients and supplements to partially correct obvious deficiencies in amino acids and minerals.

(Table1)

Apparent digestibility coefficients of energy and CP were determined for each diet, using a total of 30 three-way crossbred rabbits, aged 42 days with an average BW of 1.32 (s.d. 0.07) kg according to Perez *et al.* (1995).

Chemical analysis of diets and faeces were performed following the AOAC (1999) methods for DM, ash, ether extract, CP, and crude fibre (934.01, 942.05, 920.39, 976.06 and 978.10, respectively). Ether extract was determined after acid hydrolysis.

NDF, ADF and ADL were analysed sequentially (Van Soest *et al.*, 1991) using a thermo-stable amylase (Thermamyl L120, Novo Nordisk, Gentofte, Denmark) pre-treatment and expressed exclusive of residual ash. Gross energy was determined by adiabatic bomb calorimetry (Gallenkamp Autobomb, Loughborough, UK) following the recommendation of EGRAN (2001).

Animals and experimental procedure

A total of 228 young rabbit does (line A from UPV, selected over 36 generations for litter size at weaning) were used from 9 weeks of age to first parturition. The animals were housed in a traditional building under controlled environmental conditions, with light alternating on a cycle of 16 h light and 8 h dark. The experiment was carried out from January to June 2007.

Until 9 weeks of age, young rabbit females were caged collectively, receiving the same commercial diet *ad libitum* (185 g crude fibre and 175 g CP per kg DM), and subsequently housed in individual cages with access to one of the experimental diets. Combining two diets and three different feeding schemes, five feeding programmes were formed (Figure 1). The CAL group included females which received the C diet *ad libitum* until first parturition. The CR group included females which received the C diet *ad libitum* until 12 weeks of age and then 140 g per day until first parturition, with a 7-day flushing period (C diet *ad libitum*) around AI. The F group included females which received the F diet *ad libitum* until first parturition. Finally, FC and FCF groups were females that received F diet *ad libitum* until 16 weeks of age, whereupon the FC group received the C diet *ad libitum* until first parturition, while FCF group received the C diet *ad libitum* until 20 weeks of age and then the F diet *ad libitum* until first parturition.

(Figure 1)

While animals from different experimental groups kept the same feeding programme, data were analysed and presented as a whole (CAL and CR until 12 weeks of age, F, FC and FCF until 16 weeks of age, and then FC and FCF until 20 weeks of age).

Does were artificially inseminated at the end of the 18th week of age. As of this date, successive AI were carried out every 21 days, as necessary. After the 28th day of pregnancy, maternal cages were provided with a nest equipped for the litter.

The traits measured for all does were BW and food intake at 9, 12, 16, 18 (AI), 20 and 23 (parturition) weeks of age, as well as perirenal fat thickness (PFT) by ultrasounds at 9, 12, 18, 20 and 23 weeks of age. Total and live litter size and weight at partum were also recorded. From 12 rabbit does per group, blood samples were collected at 9, 12, 18 and 23 weeks of age. On sampling day, feeders were closed at 07:00 h and blood samples were taken from the central ear artery into EDTA-containing tubes from 11:00 to 13:00 h. Blood samples were centrifuged immediately after sampling (3000×g, 4ºC and 10 minutes) and plasma was stored at –20ºC before being assayed for insulin, glucose, non-esterified fatty acids (NEFA), leptin, cortisol and tri-iodothyroxine (T3) concentrations. Controls at 9, 12, 16 and 20 weeks of age were done on Mondays, and those at 18 weeks of age (AI) on Friday.

Ultrasound measurements

The PFT of does was measured by ultrasound to evaluate body condition, as described by Pascual *et al.* (2000 and 2004). Images were obtained with an ultrasound unit (JustVision 200 'SSA-320A' real-time machine; Toshiba) equipped with image analyser software to determine distances. Estimated body energy content (EBE; MJ/kg) was determined at AI and parturition from BW and PFT data as described by Pascual *et al.* (2004).

Hormone and metabolite assays

Plasma insulin concentrations were determined by the double antibody/PEG technique using porcine insulin radioimmunoassay (RIA) kit (Linco Research Inc., St Charles, MO, USA). The antiserum was guinea pig anti-porcine insulin, while both labelled antigen and standards used purified recombinant human insulin. Leptin concentrations were determined by double antibody RIA using the multi-species leptin kit (Linco Research Inc.) as previously reported (Brecchia et al. 2006). Total T3 was assayed by RIA according to the procedure provided by the manufacturer (Immunotech, Marseille, France). The assay sensitivity was 0.13 ng/mL, and the major analogues of T3 did not interfere with the assay. Plasma cortisol was assayed by RIA, using the CORT kit (ICN Biomedicals Inc., Costa Mesa, CA, USA). CORT assay sensitivity was 0.15 ng/mL. Dilution and recovery tests done on insulin, leptin, T3 and corticosterone using five different samples of rabbit plasma showed linearity. Glucose was analysed by the glucose oxidase method using the Glucose Infinity kit from Sigma (Sigma Diagnostic Inc., St. Louis, MO, USA). NEFA concentrations were analysed using enzymatic colorimetric assay from Wako (Wako Chemicals GmbH, Neuss, Germany) as previously reported (Brecchia et al., 2006).

Statistical Analysis

The model used to analyse performance, hormonal and metabolic data of young rabbit does during rearing and first gestation was a mixed model (PROC MIXED by SAS, Statistical Analysis System, 2002), in a repeated measure design that took into account the variation between animals and covariation within them. Covariance structures were objectively compared using the most severe criteria (Schwarz Bayesian criterion), as suggested by Littell *et al.* (1998). The model included the feeding programme (CAL, CR F, FC and FCF), the week of age (9, 12, 18, 20 and 23 weeks; data for week 16 was also included for consumption and BW), and their interaction as fixed effects. Random terms in the model included a permanent effect of each animal (p) and the error term (e), both assumed to have an average of zero, and variance σ^2_p and σ^2_e.

Different contrasts were computed to test the significance of the differences between treatments while animals of different experimental groups received the same feeding programme at 12 weeks [(CAL+CR)/2 *vs.* (F+FC+FCF)/3], at 16 weeks [CAL *vs.* CR *vs.* (F+FC+FCF)/3], at 18 and 20 weeks [CAL *vs.* CR *vs.* F *vs.* (FC+FCF)/2] and parturition [CAL *vs.* CR *vs.* F *vs.* FC *vs.* FCF].

To analyse the litter data at first parturition, a fixed effects model (PROC GLM of SAS, 2002) was used that included only the feeding programme (CAL, CR F, FC and FCF). Data concerning mortality of females during the rearing and first pregnancy were analysed according to a nonparametric procedure (PROC NPAR1WAY of SAS, 2002), using a chi-square test for mean separation.

RESULTS

Animal Performance

A high mortality rate was observed in the CAL group (34%) between 9 and 12 weeks of age (Figure 2) compared to the F group (3%; *P*<0.05), probably due to an outbreak of epizootic rabbit enteropathy (ERE). Mortality was low and similar in groups under different feeding programmes from 12 to 20 weeks of age. However, the CAL group again presented a significantly higher mortality (14%; *P*<0.05) compared to the other groups (on average 3%) during the last 3 weeks of pregnancy.

(Figure 2)

Daily intake, BW and PFT of young rabbit does during rearing and pregnancy are presented in Table 2 and Figures 3 and 4. The BW and PFT at 9 weeks of age was 1.97±0.03 (standard error) kg and 6.9±0.1 mm, respectively. Females *ad libitum* fed with C diet (CAL) showed significantly higher DE and DP intake between 9 and 12 weeks of age (+89 kJ and +11 g per day, respectively; *P*<0.05) and BW at week 12 (+0.11 kg; *P*<0.05) than those with F diet. From 12 to 16 weeks, DE and DP intake were similar for CAL and F females (on average 841 kJ and 86 g per day, respectively), but significantly lower for those restricted (CR; 699 kJ and 72 g per day; *P*<0.05). Thus, BW at week 16 was significantly higher for CAL than for F group (3.69 and 3.47 kg, respectively; *P*<0.05), and higher for both than for CR (3.24 kg; *P*<0.05).

(Table 2)

From 16 to 20 weeks of age, DE and DP intake of F group was even higher (on average 792 kJ and 80 g per day, respectively) than that observed for CAL group (742 kJ and 76 g per day; *P*<0.05) and higher for both than for CR group (690 kJ and

71 g per kg per day; *P*<0.05). In fact, F females going on to *ad libitum* C diet at 16 weeks (FC) showed the highest intake values (on average 883 kJ and 91 g per day; *P*<0.05). In consequence, CAL and FC females at week 20 presented higher BW and PFT (4.34 kg and 7.3 mm, respectively) than CR females (3.93 and 6.7 mm; *P*<0.05), with F females showing medium values (4.14 kg and 7.1 mm).

The type of feeding programme did not affect the fertility rate of young females at first AI (85.2, 84.0, 89.7 and 85.0% for CAL, CR, F and FC females, respectively).

During the last 3 weeks of pregnancy, F and FC females presented higher DE and DP intake (on av. 630 kJ and 65 g per day, respectively) than CR and FCF females (on av. 586 kJ and 60 g per day; *P*<0.05), with CAL females showing the lowest intake values (537 kJ and 55 g per day; *P*<0.05). Thus, differences in BW between feeding programmes disappeared at parturition (Figure 3), while only CAL females presented a greater PFT than CR and FC females (6.4 *vs.* 6.1 mm, respectively; *P*<0.05).

(Figures 3 and 4)

Table 3 shows the effect of the feeding programme adopted during doe rearing on litter traits at the first parturition. In comparison to the CAL group, FCF females had larger (7.7 *vs.* 5.2 kits; *P*<0.05) and heavier live born litters (419 *vs.* 280 g; *P*<0.05), with CR, F and FC females showing medium values (on av. 6.1 kits and 349 g). The small number of kits born alive at first parturition to CAL females was due to their lower number of total kits born (6.6 *vs.* 8.3 kits *P*<0.05) and the greater mortality of their litters at birth (20.6 *vs.* 6.7 %; *P*<0.05) compared to FCF.

(Table 3)

Metabolic and hormonal parameters

The plasma profiles of insulin, glucose, NEFA, leptin, cortisol and T3 during rearing and first pregnancy in the different feeding programmes are shown in Figure 5. An increase in circulating insulin concentrations was observed with advancing age in all the groups, although it decreased at parturition (Figure 5a). CAL group animals presented lower mean plasma insulin concentration than F females at 18 weeks of age (–19.5 µUI/mL; $P<0.05$).

Both glucose and NEFA plasma concentrations showed the highest values at 9 weeks of age and dropped thereafter. Glucose concentration in plasma was opposite to insulin, being lower for CAL (–31.4 mg/dL; $P<0.05$) than for F females at 12 weeks of age (Figure 5b). At partition day, glucose was lower in CAL, CR and FCF than F and FC females (on av. –20.7 mg/dL; $P<0.05$). Although NEFA levels were similar for all the groups at 18 weeks of age (Figure 5c), females receiving the C diet (CAL, CR and FC) presented the highest NEFA values in plasma at parturition, only being significantly higher in CAL and CR compared to F females (on av. +0.18 mmol/L; $P<0.05$).

Leptin levels were similar for all groups at 12 weeks of age and at partum day (Figure 5d). An increase in plasma leptin concentration was observed at 18 weeks, especially in CR females (6.6 ng/mL; $P<0.05$), where plasma had higher leptin levels than CAL (5.1 ng/mL), and both F and FC females (on average 3.3 ng/mL; $P<0.05$). Plasma cortisol increased from 9 to 12 and 18 weeks of age, although it decreased at parturition (Figure 5e). No significant differences between feeding programmes on cortisol in plasma were observed throughout the experiment. Plasma concentrations of T3 at 12 weeks of age were similar for all the groups (Figure 5f). Females given C diet *ad libitum* at 18 weeks (CAL and FC) had higher levels of plasma T3 than CR females (on av. 0.75 mmol/L; P<0.05). However, CAL females showed higher T3 levels than FC females at parturition (–0.96 mmol/L; P<0.05).

(Figure 5)

DISCUSSION

No previous work evaluating the use of rearing diets described the high mortality rate observed in the present work from 9 to 12 weeks of age when young females were fed the control diet. This fact seems to be related to ERE incidence when no medicated diets are used. Under these conditions, insufficient level or inadequate quality of dietary fibre can increase the risk of digestive disorders in young rabbits (Gidenne, 1997; Gidenne and Garcia, 2006). In the current work, although higher soluble fibre was expected for the F diet (from alfalfa and beet pulp), both diets were designed to meet fibre recommendations to prevent digestive problems from 9 to 12 weeks of age (ADL>50, ADF>190 and NDF-ADF>80 g/kg). However, a recent review (Blas and Gidenne, 2010) highlighted that, even if requirements proposed to prevent digestive disorders are met, replacing starch with low or high digestible fibre reduces mortality rate, especially in the context of ERE.

Young rabbit female needs from 9 to 12 weeks of age (approx. 1.52 MJ per day, considering their mean live weight and daily gain; Xiccato and Trocino, 2010) were met with both C and F diets (1.74 and 1.54 MJ per day, respectively). Although the lower DE intake led females receiving the F diet to reach 12 weeks with a smaller BW, as in a previous work (Pascual *et al.*, 2002), the main metabolic and hormonal parameters here examined were not greatly affected. Rebollar *et al.* (2011) also found similar concentrations of leptin (2.8 ng/mL) and NEFA (0.22 mmol/L) in the blood of young females at 11 weeks of age when comparing *ad libitum* supplying of control and fibre-rich diets. However, when higher feed restriction is asserted (even below animal needs 1.03 MJ per day; Rommers *et al.*, 2004), the blood levels of glucose, leptin, insulin, and T3 of young females (from 6 to 12 weeks of age) were clearly reduced.

As a consequence of feed intake restriction from 12 to 18 weeks of age, CR females reached 18 weeks of age with a delay in their development, showing lower BW and PFT than those fed *ad libitum*. These results agree with those reported in previous works where feed restriction reduced BW as well as body fat and protein content of young rabbit females at first AI (Rommers et al., 2001, 2004), and even caused a delay in the effectiveness of this AI (Rebollar *et al.*, 2011). In the present work, CR females presented a slight reduction of T3 blood levels at 18 weeks of age together with an unexpected higher concentration of leptin compared to those with free access to the control diet. Several studies have shown that fasting reduces leptin, mainly synthesised and secreted by adipocytes, circulating in blood at levels proportional to body fat stores in humans (Weigle *et al.*, 1997), gilts (Barb *et al.*, 2001), ruminants (Chilliard *et al.*, 2000), and also in rabbits (Rommers *et al.*, 2004; Brecchia *et al.*, 2006; Rebollar *et al.*, 2011). However, the mechanisms whereby feeding restriction affects circulating leptin levels are still unclear, and different responses were observed depending on type and length of fasting and blood sampling protocols. In this respect, Brecchia *et al.* (2006) described higher leptin levels in the plasma of 48-h fasted than in 24-h fasted does. In any case, it might be considered that CR females were subject to a 4-day flushing period prior to AI, where animals had free access to the C diet, which could have conditioned the plasma metabolic profile for these days.

On the other hand, females with free access to the F diet were able to compensate for the lower nutritive dietary concentration with a greater feed intake from 12 to 18 weeks of age. Thus, they achieved DE and DP intakes similar to those of rabbits receiving the C diet *ad libitum* and, consequently, reduced their gaps in BW and PFT at 18 weeks of age. In fact, these differences disappeared when females of F group had free access to C diet as of 16 weeks of age. Pascual *et al.* (2002) described how young rabbit females fed with a low-energy diet (8 MJ DE/kg DM) as of 10 weeks of age presented, during late rearing, a greater DE intake than those fed 150 g per day of a standard diet (11 MJ DE/kg DM). The greater feed intake, however, did not compensate earlier differences in BW and these rabbits achieved first AI 10 days later. However, a later introduction of a low-energy diet at 13 weeks of age (Quevedo *et al.*, 2005) or the use of moderate-energy diets (9.5 MJ DE/kg DM; Xiccato *et al.*, 1999) enabled young rabbit females to achieve first mating at an adequate age and BW.

In this sense, although the use of a moderate low-energy diet during the rearing period led females to reach first mating with lower energy body reserves (Figure 6) and lower blood leptin levels than those fed with a conventional diet for reproductive does, no consequence on fertility at first AI was reported. It is well-known that nutrient restriction may delay the onset of puberty, leading to the hypothesis that a critical soma must be achieved before puberty can occur (Frisch, 1980). Furthermore, Arias-Álvarez *et al.* (2009) recently proposed that reaching the permissive leptin threshold should be necessary for pubertal reproductive activity, and may be associated with inhibition of reproduction if the critical soma is insufficient to trigger gestation (Moschos *et al.*, 2002). In fact, when the relationship between fertility and blood leptin levels of young rabbit females around first insemination is drawn (Figure 7), the hypothesis of a leptin threshold for initiation of puberty and reproductive success which is not improved by additional provision of this hormone seems to be confirmed. Consequently, these results reveal that in terms of *ad libitum* feeding during rearing, both feed restriction and earlier use of a moderate low-energy diet (8.7 MJ/kg DM) led females to achieve the critical BW and fat mass at first AI to ensure reproduction, in spite of their lower fatness and leptin content in blood.

(Figures 6 and 7)

After the first AI, although young females receiving the C diet *ad libitum* maintained a greater consumption than those restricted until 20 weeks of age, the fatness accumulated by CAL females throughout rearing allowed them to reduce their feed intake as pregnancy progressed, allowing CR females to diminish the differences in BW, PFT, and EBE observed up to this point with the CAL group during late pregnancy. In a previous work (Rommers *et al*., 2004), where development between young females fed *ad libitum* and early restricted (restriction: from 5 to 10 weeks of age; recovery: 10 to 17.5 weeks of age) was compared, although compensatory growth of the restricted group was also observed during pregnancy, the early differences achieved in BW of females were maintained throughout the 3 reproductive cycles controlled by the authors. In gilts, where feed restriction of young females has been studied extensively, most works (Sørensen *et al*., 1998; Klindt *et al.*, 1999 and 2001b) show that moderate feed restriction during the rearing period helps females avoid excessive fatness, while more intense restriction (earlier and/or stronger) leads to smaller development and sometimes even to lower reproductive performance. Therefore, these results seem to confirm the effectiveness of moderate restrictive feeding in preventing excessive fatness in young females, although the starting age and restriction level should be controlled to avoid an inadequate pre-pubertal body development.

A practical alternative to restriction could be the use of fibrous diets. Several works found in the literature showed that the use of fibrous diets during rearing led nulliparous rabbit females to a greater DE intake after first mating, independently of their previous growth rate during development. Even so, when low-energy fibrous diets are used (<8.5 MJ DE/kg DM), females are not able to compensate the previous developmental delay (Pascual *et al.*, 2002; Quevedo *et al.*, 2005). However, when females have the chance of receiving a moderate-energy fibrous diet (approx. 9 MJ DE/kg DM), they reach the first parturition with a development and BW similar to those of rabbit does fed *ad libitum* a diet for reproductive does (>10.5 MJ DE/kg DM), but with a lower fatness (Xiccato *et al.*, 1999; Rebollar *et al.*, 2011). In the present work, and independently of the fibrous feeding systems used (F, FC or FCF), females reached first parturition in an intermediate developmental situation to that observed in females fed with the C diet *ad libitum* or restricted. Similar results were also obtained by Rebollar *et al.* (2011), where the use of a fibrous diet (9.4 MJ DE/kg DM) from 11 weeks of age to first parturition led young rabbit females to reach the end of first pregnancy with body energy and protein content halfway between *ad libitum* and restricted administration of a control diet (11.6 MJ DE/kg DM). The use of a fibrous diet with 8.5 to 9.5 MJ DE/kg DM should therefore allow young rabbit females to reach first parturition in an adequate state of development, avoiding excessive fatness without the need for feeding restriction.

In fact, the possible negative effects of excessive fatness could be behind the problems detected around first parturition in the CAL group. Compared to the other feeding systems evaluated here, females fed the C diet *ad libitum* during rearing showed the lowest DE intake and the highest body energy mobilisation recorded during late pregnancy. In fact, the plasma of these females at partum day was characterised by higher NEFA and lower glucose levels. The aforementioned profile is frequently related to pregnancy toxaemia risk (Martenink and Herdt, 1988; Bezille, 1995; Rosell, 2000), and could explain the higher mortality in late pregnancy for the females of this group and the smaller size of their litters at first birth caused by both lower total litter size and higher mortality at birth. Rommers *et al.* (2002) also observed that heavier young females at first AI (more than 4 kg BW) had a higher percentage of stillborn at first parturition (13.4%) than smaller females (5%). In gilts, Klindt *et al.* (2001a and 2001b) related an excessive energy intake during rearing with a lower number of corpora lutea and live embryos per gilt, and also observed a tendency towards the reduction of litter size at first birth (–0.8 piglets born) and the increase of gilts removed until this time (+13%). In this sense, the highest prolificacy and the lowest mortality at birth were recorded for females given the F diet with a flushing of 4 weeks with the C diet applied around first mating. In a recent revision, Theau-Clement (2007) concluded that feed flushing after nutritive restriction could improve the reproduction performance, at least at the beginning of the reproductive career.

From the results of the present work it could be concluded that the *ad libitum* use of diets formulated to cover the needs of lactating rabbit does for the whole rearing period could lead young rabbit females to present a higher risk of early death and smaller litter size at first parturition. As an alternative, either feed restriction or earlier use of an adequate fibrous diet could lead females to achieve the critical BW and fat mass at first AI to ensure reproduction. However, under these feeding programmes for young females, the starting age and nutritive level of the fibrous diet should be controlled to avoid an inadequate pre-pubertal development.

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Table 1 *Ingredients and chemical composition of experimental diets*

	Diets	
	C	F
Ingredient (g/kg)		
Barley	312	78
Alfalfa hay	450	570
Sunflower meal	94	51
Soya meal	85	-
Sugar beet pulp	-	152
Cereal straw	-	100
Soya oil	30	10
HCl L-lysine, 780	2	3.9
DL-methionine, 990	-	0.85
L-threonine, 980	-	1.45
L-tryptophan, 980	1	1.5
L-Arginine, 990	-	4
Dicalcium phosphate	17	1.8
Monosodium phosphate	-	16.5
Salt	5	5
Vitamin-mineral mixture[1]	4	4
Chemical composition (g/kg DM)		
Dry Matter (DM, g/kg)	899	900
Ash	90	103
Starch	205	63
Ether Extract	52	29
Crude Protein	179	146
Neutral Detergent Fibre	358	476
Acid Detergent Fibre	277	394
Acid Detergent Lignin	59	88
Gross Energy (MJ/kg DM)	18.24	18.67
Digestible energy (DE; MJ/kg DM)	11.03	8.72
Digestible protein (DP; g/kg DM)	114	88
DP/DE (g/MJ)	10.3	10.1

[1] Per Kg of feed: Vitamin A: 8,375 IU; Vitamin D3: 750 IU; Vitamin E: 20 mg; Vitamin K3: 1 mg; Vitamin B1: 1 mg; Vitamin B2: 2 mg; Vitamin B6: 1 mg; Nicotinic acid: 20 mg; Choline chloride: 250 mg; Mg: 290 mg; Mn: 20 mg; Zn: 60 mg; I: 1.25 mg; Fe: 26 mg; Cu: 10 mg; Co: 0.7; Butyl hydroxylanysole+ethoxiquin: 4 mg.

Table 2 *Daily dry matter (g DM per kg metabolic weight (BW$^{0.75}$)), digestible energy (kJ DE per kg BW$^{0.75}$) and digestible protein (g DP per kg BW$^{0.75}$) intake of young rabbit does during rearing and first pregnancy (mean ± standard error)*

	Feeding programme[1]				
	CAL		F		
9-12 wk					
DM intake	82.44^{a} ±1.21		94.05^{b} ±0.94		
DE intake	909.3^{b} ±12.3		819.7^{a} ±9.5		
DP intake	93.86^{b} ±1.28		83.00^{a} ±0.99		
	CAL	CR	F		
12-16 wk					
DM intake	75.46^{b} ±1.62	63.34^{a} ±1.72	97.43^{c} ±0.91		
DE intake	832.3^{b} ±16.3	698.6^{a} ±12.3	849.0^{b} ±9.2		
DP intake	85.91^{b} ±1.7	72.11^{a} ±1.8	85.96^{b} ±0.96		
	CAL	CR	F	FC	
16-18 wk					
DM intake	68.58^{b} ±1.50	63.17^{a} ±1.59	90.54^{d} ±1.5	84.28^{c} ±1.03	
DE intake	756.4^{b} ±15.6	696.7^{a} ±16.5	789.0^{b} ±15.5	929.5^{c} ±10.6	
DP intake	78.07^{b} ±1.60	71.91^{a} ±1.69	79.88^{b} ±1.6	95.94^{c} ±1.09	
Early pregnancy; 18-20 wk					
DM intake	66.14^{b} ±1.5	61.78^{a} ±1.59	91.09^{d} ±1.48	75.61^{c} ±1.03	
DE intake	729.5^{b} ±15.6	681.3^{a} ±16.5	793.9^{c} ±15.3	833.9^{d} ±10.6	
DP intake	75.29^{b} ±1.6	70.33^{a} ±1.69	80.38^{c} ±1.57	86.08^{d} ±1.09	
	CAL	CR	F	FC	FCF
Late pregnancy; 20-23 wk					
DM intake	48.68^{a} ±1.68	53.13^{b} ±1.61	73.18^{d} ±1.5	56.42^{b} ±1.47	61.35^{c} ±1.55
DE intake	536.8^{a} ±17.2	585.9^{b} ±16.5	637.8^{c} ±15.3	622.3^{bc} ±15.0	586.5^{b} ±15.9
DP intake	55.41^{a} ±1.76	60.48^{bc} ±1.69	64.57^{c} ±1.57	64.23^{c} ±1.55	59.79^{ab} ±1.63

[1] Feeding programme: CAL group received the C diet *ad libitum* until 1st partum; CR group received the C diet *ad libitum* until 12 wk and then, 140 g per day until 1st partum; F group received the F diet *ad libitum* until 1st partum; FC and FCF group received F diet *ad libitum* until 16 wk and then, FC group received the C diet *ad libitum* until 1st partum and FCF group the C diet *ad libitum* until 20 wk and then the F diet *ad libitum* until 1st partum.

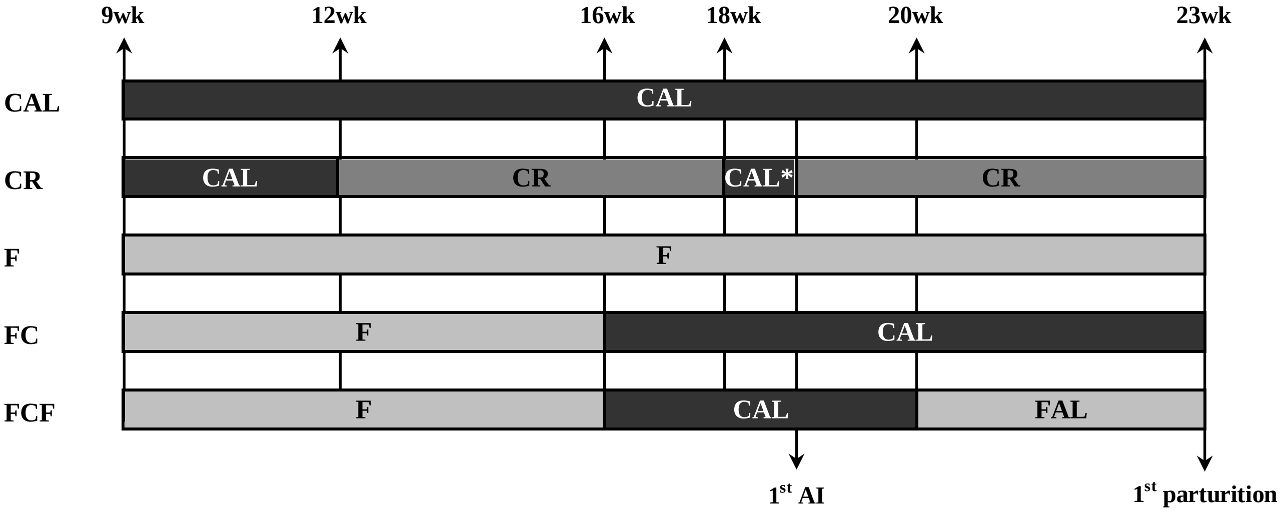
[a,b,c,d] Means within a row not sharing any superscript are significantly different at P<0.05.

Table 3 *Litter size and weight at first partum (mean ± standard error)*

	Feeding programme[1]				
	CALminuscula?	CR	F	FC	FCF
Litter size at partum					
Total	6.6^{a} ±0.6	6.8^{ab} ±0.6	6.9^{ab} ±0.06	7.0^{ab} ±0.6	8.3^{b} ±0.6
Alive	5.2^{a} ±0.7	6.2^{ab} ±0.7	6.0^{ab} ±0.6	6.1^{ab} ±0.6	7.7^{b} ±0.6
Mortality at birth	20.6^{c}	8.3^{ab}	12.1^{ab}	12.4^{b}	6.7^{a}
Litter weight at partum (g)					
Total	340^{a} ±25.4	391^{ab} ±25.4	406^{ab} ±23.6	368^{a} ±23.2	448^{b} ±24.5
Alive	280^{a} ±31.6	355^{abc} ±31.6	369^{bc} ±29.3	323^{ab} ±28.8	419^{b} ±30.4

[1] Feeding programme: Abbreviations as in Table 2.
[a,b] Means within a row not sharing any superscript are significantly different at *P*<0.05.

Figure 1 *Diagram of the different feeding programmes carried out during rearing and first pregnancy for the 5 experimental groups (CAL, CR, F, FC and FCF)*



*Flushing 4 days before insemination

CAL: C diet *ad libitum*; CR: C diet restricted at 140 g per day; F: F diet *ad libitum*

AI: Artificial Insemination

Figure 2 *Percentage of does dead during the rearing and first pregnancy (from 9 to 23 week of age) with the different feeding programmes (abbreviations as in Table 2). Bars within a period not sharing any superscript are significantly different at P<0.05. AI: Artificial Insemination*

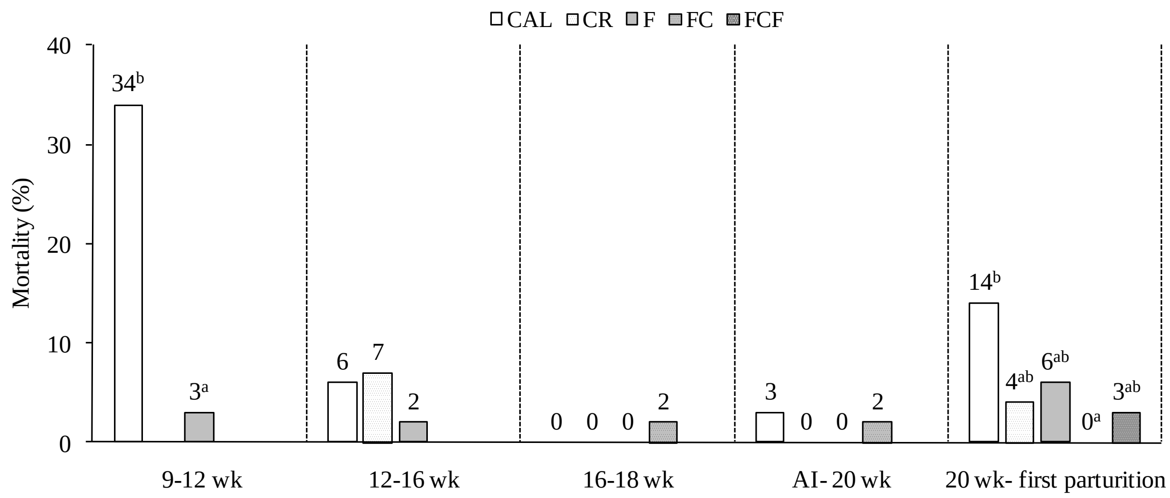


Figure 3 *Live weight evolution of young rabbit does during rearing and first pregnancy (9 to 23 wk of age) with the different feeding programmes (abbreviations as in Table 2). Data at 9 week of age are presented as a whole. Bars not sharing any superscript are significantly different at P<0.05*

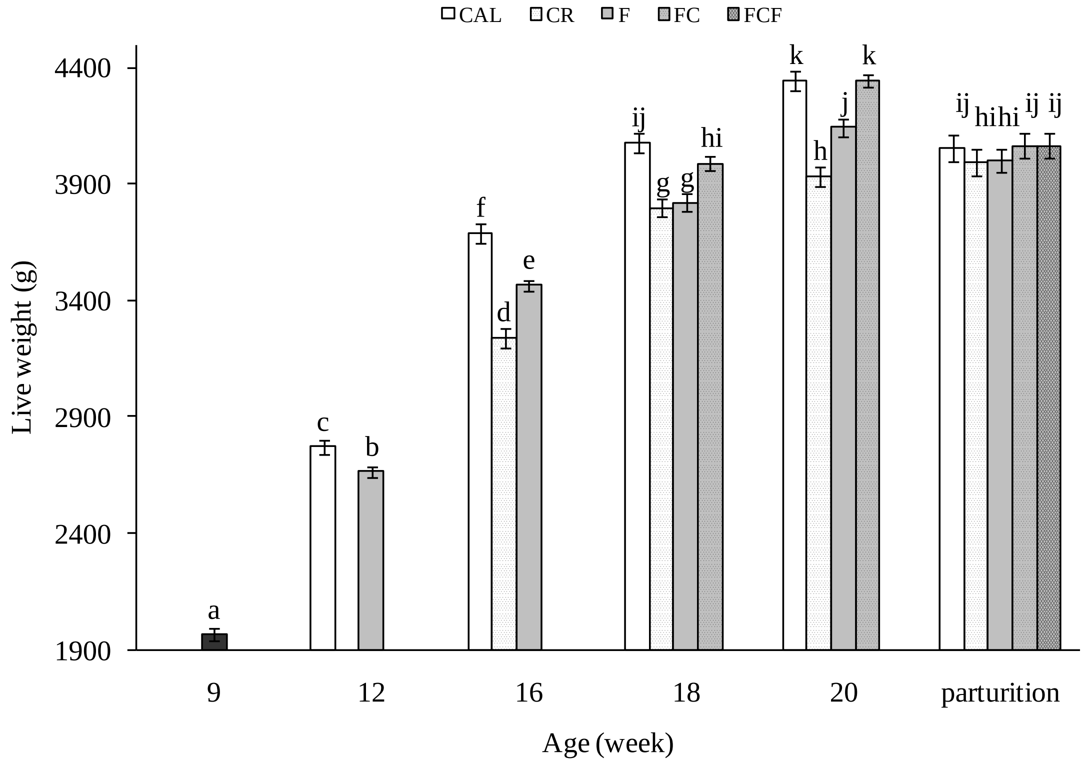


Figure 4 *Perirenal fat thickness evolution of young rabbit does during rearing and first pregnancy (9 to 23 wk of age) with the different feeding programmes (abbreviations as in Table 2). Data at 9 week of age are presented as a whole. Bars not sharing any superscript are significantly different at P<0.05*

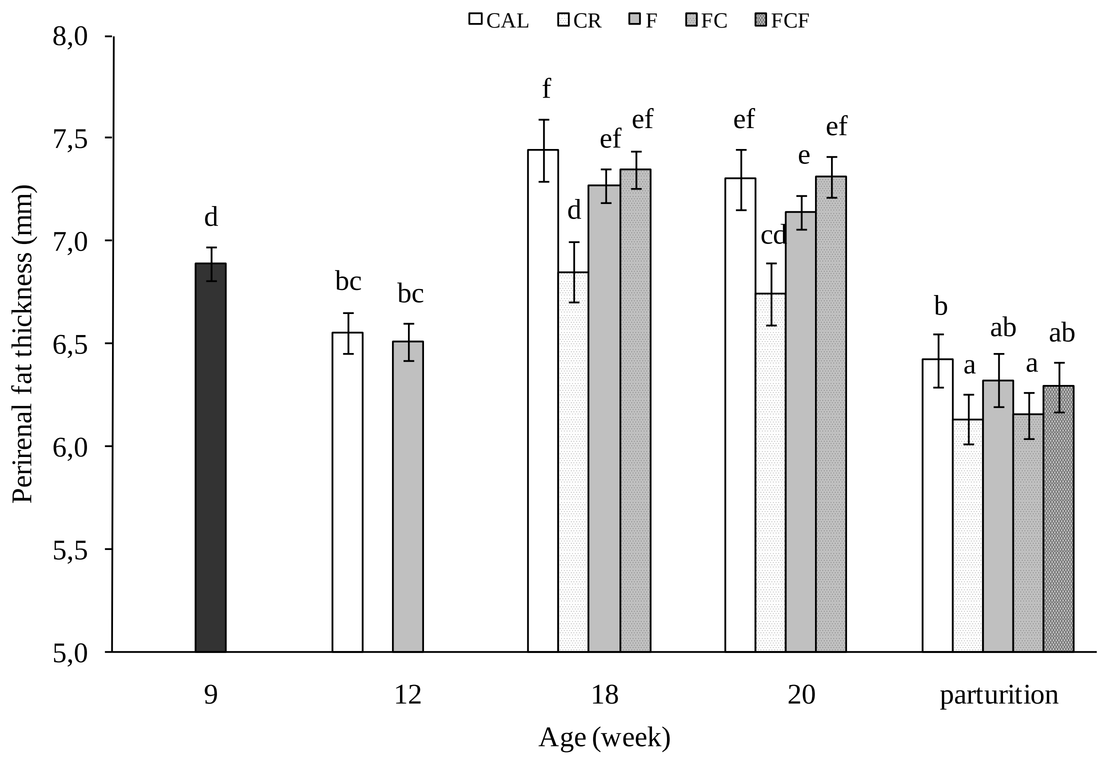


Figure 5 *Evolution of blood plasma (a) insulin, (b) glucose, (c) non esterified fatty acids (NEFA), (d) leptin, (e) cortisol and (f) tri-iodothyroxine (T3) concentrations in young rabbit does during rearing and first pregnancy (9 to 23 wk of age) with the different feeding programmes (*CAL CR F FC FCF*; abbreviations as in Table 2). Data at 9 week of age are presented as a whole. Bars not sharing any superscript are significantly different at P<0.05*

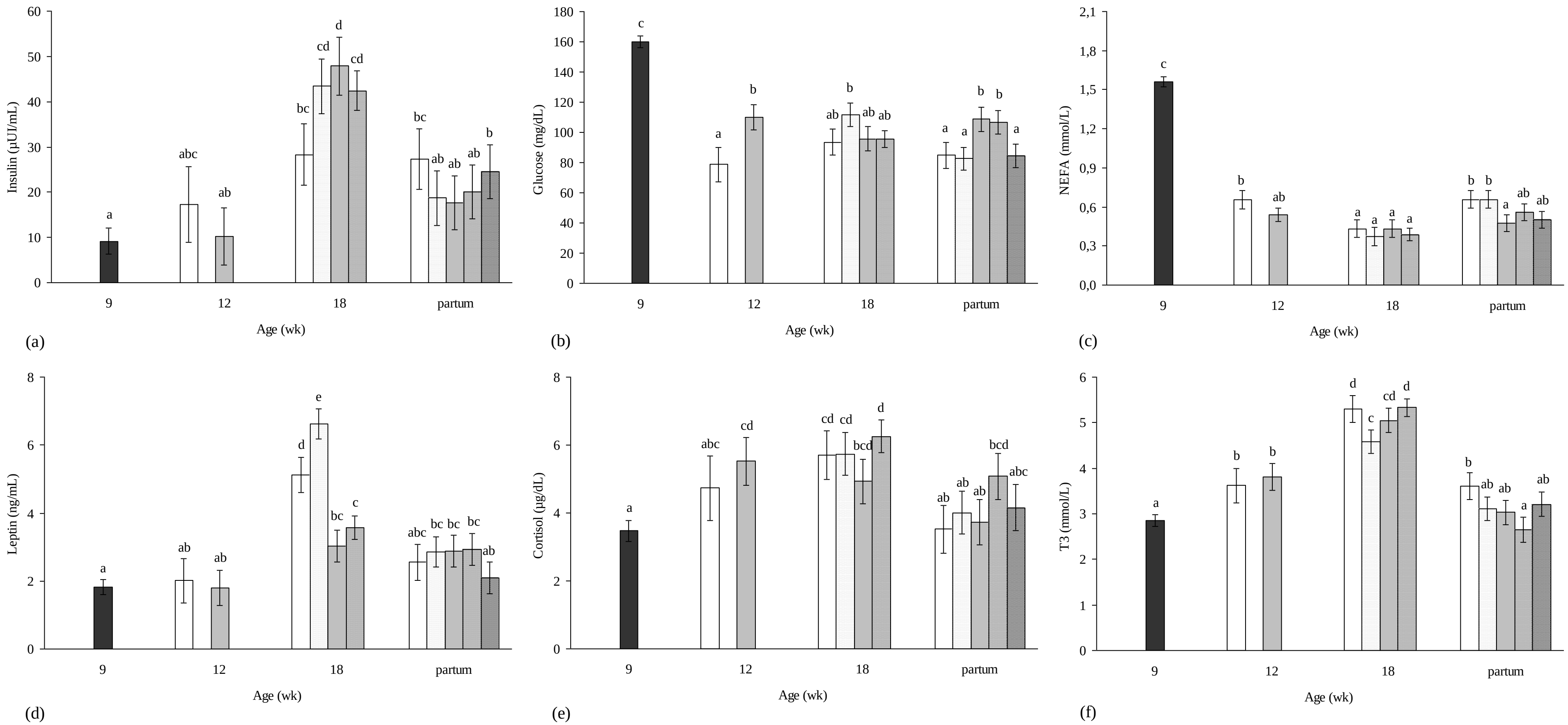


Figure 6 *Estimated energy content of young rabbit does at effective artificial insemination (AI) and parturition days with the different feeding programmes (abbreviations as in Table 2). Bars not sharing any superscript are significantly different at P<0.05*

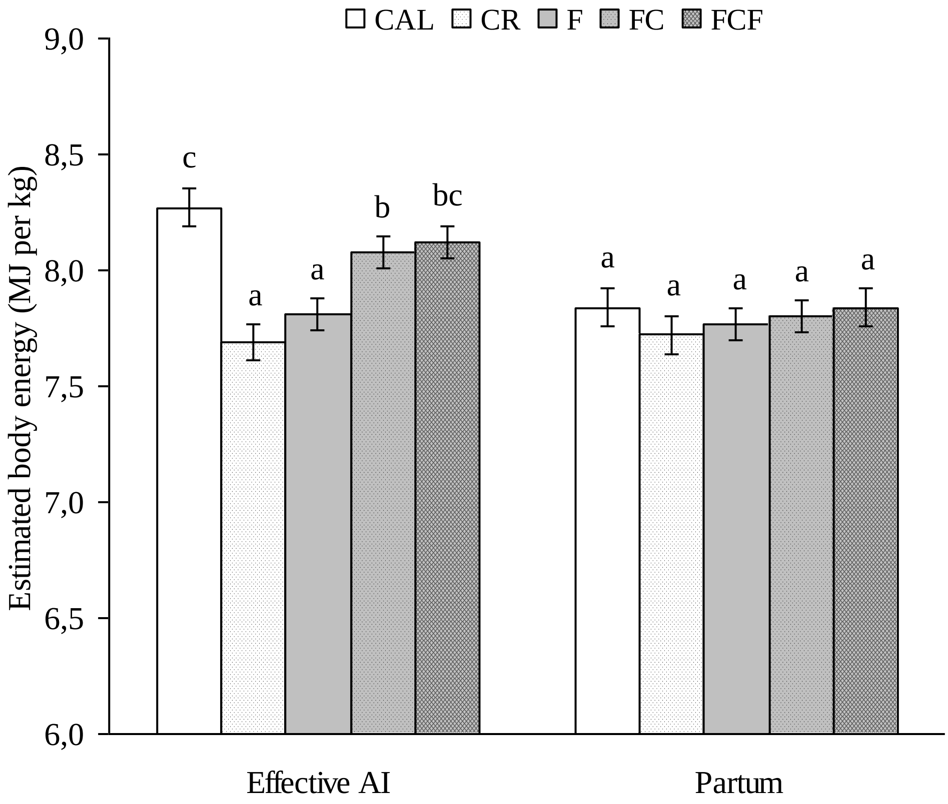


Figure 7 *Relationship between leptin levels in the blood of young rabbit does at first mating (16-18 wks of age) and the fertility observed during the first reproductive cycle. Data obtained from the present results and three previous works of the literature*