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EFFECTS OF REARING FEEDING PROGRAMME ON THE YOUNG RABBIT FEMALES' BEHAVIOUR AND WELFARE INDICATORS

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Abstract: Restriction of young rabbit females during rearing is a widespread management technique that could have negative consequences on their welfare and behaviour. In the present work, a total of 24 young rabbit females aged 9 wk were used to evaluate 3 rearing feeding programmes until first parturition: 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)]; CR, receiving the C diet restricted (140 g/d) from 12 wk of age; and F, fed *ad libitum* with a low energy/high fibre diet [F: 8.7 MJ DE and 88 g DP per kg DM]. F females presented lower body weight than CAL and CR females at week 18 (–0.4 kg and –0.2 kg; $P<0.05$), but differences in body weight disappeared at parturition. Feeding programme affected the daily feed intake of young females during rearing and gestation periods (on av. of 2 periods: 140, 127 and 179 g DM/d, for CAL, CR and F females, respectively; $P<0.001$). Blood levels of glucose and insulin decreased with the age of rabbits (from 97 to 73 mg/dL for glucose and from 11 to 6 µUI/mL for insulin at 13 and 20 wk, respectively; $P<0.001$). Concentration of non-esterified fatty acids was higher in the blood of CAL females (+0.13 mmol/L compared to F; $P<0.05$), while corticosterone was higher in F females (+0.7 µg/dL compared to CAL; $P<0.05$). The type of feeding schedule affected the lying still and eating behaviour ($P<0.01$) of CR females, especially before and after feeding supply, as well as their behavioural stressed indicators (stereotypies; $P<0.01$), which were more frequent in CR females before feeding supply at 20 weeks of age. Therefore, *ad libitum* use of a low energy/high fibre diet is an adequate feeding programme for young rabbit females, which does not alter their behavioural patterns.

Key Words: rabbit females, rearing, feeding, welfare, behaviour.

INTRODUCTION

Management of young rabbit females during the rearing period could help ensure better pubertal development and growth, avoiding problems related with over-conditioning during their future reproductive life. In the last decade, some works have assessed the possible impact of different feeding programmes during the rearing period on female development and reproduction. These programmes included either feed restriction of a diet for reproductive rabbit females with high energy content (Rommers *et al.*, 2004) or the use of fibrous, roughage rich diets but poor in energy content (Niza *et al.,* 1997; Xiccato *et al.*, 1999; Pascual *et al.*, 2002; Quevedo *et al.*, 2005). Rearing feeding programmes aim to develop adequate body growth by promoting voluntary feed intake in order to increase the long-term productivity and lifespan of rabbit females (Savietto *et al.*, 2012), and positively influence the behaviour and welfare of females (Krohn *et al.*, 1999).

Some authors have compared these 2 feeding programmes and evaluated their effects on performance, body condition and blood parameters (Rebollar *et al.*, 2011; Martínez-Paredes *et al.,* 2012). However, there is a lack of information about their possible effect on behaviour and welfare of animals. Under farm conditions, feed restriction

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programmes have 2 major differences in relation to the feeding behaviour of wild rabbits: i) the energy content and bulk of diets and ii) the period of the day with access to feed (Krohn *et al.*, 1999), which could modify rabbit behaviour and welfare (Gidenne *et al.*, 2012).

In this work, we evaluated the effects of a diet for reproductive rabbit females provided *ad libitum* or restricted during the rearing and gestation periods compared to a feeding programme based on the *ad libitum* use of a low energy/high fibre diet on development, normal and abnormal behaviours and welfare of young rabbit females.

MATERIAL AND METHODS

The experimental procedure was approved by the committee of ethics and animal welfare of the Universitat Politècnica de València (UPV) and followed the European Union recommendations on care and protection of animals used for experimental purposes (European Union, 2003).

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 females [11.0 MJ digestible energy (DE), 114 g digestible protein (DP) and 277 g acid detergent fibre (ADF) per kg dry matter (DM)] was formulated following the recommendations of De Blas and Mateos (2010). In addition, a low energy diet with a high fibre content (F) was also formulated [8.7 MJ DE, 88 g DP and 394 g ADF per kg DM].

Animals and experimental procedure

A total of 24 young rabbit females (line A from UPV, selected over 36 generations for litter size at weaning) were used from 9 wk of age to first parturition. The animals were housed in a traditional building under controlled environmental conditions, with a 16:8 light cycle.

Until 9 wk of age, young rabbit females were caged collectively (50×80×32 cm), receiving a commercial growing diet *ad libitum* and subsequently housed individually (50×70×32 cm) and allocated to one of the experimental groups. There were 3 experimental groups. The first group included females that received the C diet *ad libitum* until first parturition (CAL group). The second group included females that received the C diet *ad libitum* until 12 wk of age and then 140 g per day until first parturition, with a 7-d flushing period (C diet *ad libitum*) around artificial insemination (CR group). The third group included females that received the F diet *ad libitum* until first parturition (F group).

Females were artificially inseminated at 18 wk of age and at 28th day of pregnancy cages were provided with a nest for the litter.

The traits monitored in young rabbit females were body weight (BW) and feed intake at 9, 12, 16, 18 (artificial insemination), 20 and 23 (parturition) wk of age. Total and live litter size and weight at partum were also recorded.

All females from each group were recorded by a video camera placed 2 m above the cages and 3 ethograms of 1 h each were recorded at 13 and 20 wk of age. The observations were made before (8:00) and after feeding provision in the CR group (9:00) and at the moment of high activity in feeding behaviour of non-restricted rabbits (17:00).

Blood samples were collected at 13 and 20 wk of age from the central ear artery into ethylenediaminetetraacetic acid (EDTA)-containing tubes at 11:00, after a 4-h fasting period. Blood samples were centrifuged immediately after sampling (3000×g, 4°C and 10 min) and plasma was stored at –20°C until analyses of hormones and metabolites.

Blood metabolite and hormone analyses

Plasma concentrations for glucose, non-esterified fatty acids (NEFA), insulin, leptin, tri-iodothyroxine (T3) and corticosterone were determined.

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 by Brecchia *et al.* (2006).

Table 1: Ingredients and chemical composition of experimental diets.

Ingredient (g/kg)	C	F
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)[2]		
Dry Matter (DM, g/kg)	899	900
Ash	90	103
Ether Extract	52	29
Crude Protein	179	146
Starch	205	63
Neutral Detergent Fibre (NDF)	358	476
Acid Detergent Fibre (ADF)	277	394
Acid Detergent Lignin (ADL)	59	88
Gross Energy (MJ/kg DM)	18.24	18.67
Digestible Energy (DE; MJ/kg DM)[3]	11.03	8.72
Digestible Protein (DP)[3]	114	88
DP/DE (g/MJ)	10.3	10.1

[1] Per kg of feed: Vitamin A: 8375 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.

[2] Chemical analysis of diets were performed following the AOAC (2000) methods for DM, ash, ether extract and crude protein (934.01, 942.05, 920.39 and 976.06, respectively). Ether extract was determined after acid hydrolysis. Starch was determined according to Batey (1982), by a two-step enzymatic procedure and the resulting glucose being measured by the hexokinase/glucose-6-phosphate dehydrogenase/NADP system (R-Biopharm, Darmstadt, Germany). 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).

[3] Apparent digestibility coefficients of gross energy and crude protein were determined for each diet, using a total of 30 three-way crossbred rabbits, aged 42 d with an average body weight of 1.32 (s.d. 0.07) kg according to Perez *et al.* (1995).

Plasma insulin concentrations were analysed 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 by 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 corticosterone 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.

Behavioural measurements

Frequency and duration of four normal behaviours (lying still, sitting still, self-grooming and eating) and frequency of 2 abnormal behaviours or stereotypies (biting the bars and turning round) were measured in each ethogram.

Lying still, sitting still, self-grooming and biting the bars were stated according to Kronh *et al* (1999), eating was considered as when the rabbit was eating the pellet from the hopper or visiting the empty feeder and turning round was an abnormal behaviour defined as when the rabbit turned around herself twice or more without any external stimuli. When the animal was performing a behaviour not mentioned above, including movement, it was considered other behaviour.

Statistical Analysis

The model used to analyse BW, feed intake, hormonal and metabolic data of young rabbit females was a mixed model (Proc MIXED; SAS, 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 and F), the week of age (9, 12, 16, 18, 20 and 23 wk for BW and feed intake, or 13 and 20 wk for hormones and metabolites), and their interactions as fixed effects. Random terms in the model included a permanent effect of each animal and the error term. To analyse litter data at first parturition, a lineal model (Proc GLM of SAS, 2002) including the feeding programme (CAL, CR and F) as fixed effect was used.

The frequency of the different behaviours monitored were analysed using a generalised linear mixed model (Proc GLIMMIX; SAS, 2002). A log link was used and an underlying Poisson distribution was assumed. The model included the feeding programme (CAL, CR and F), the week of age (13 and 20 wk), the time of day (8:00, 9:00 and 17:00) and their interaction as categorical fixed variables. Overdispersion was accounted for by adding a random residual component. Least square mean comparison was performed using a Bonferroni correction.

RESULTS

Animal performance

The evolution of BW and feed intake of young rabbit females during rearing and pregnancy are presented in Figures 1 and 2. BW at 9 wk of age was 2.1±0.04 kg (least square mean±standard error). BW was lower for CR and F compared to CAL females from 16 to 20 wk (on av. –250 g and –340 g, respectively; $P<0.05$). Differences in BW among feeding programmes disappeared at parturition. Females fed *ad libitum* with F diet showed significantly higher feed intake throughout the entire experimental period than CAL and CR females (on av. +37 and +45 g DM/d, respectively; $P<0.05$) and in CR females daily intake was lower that CAL from 12 to 18 wk (–38 and –13 g DM/d from 12 to 16 wk and 16 to18 wk, respectively; $P<0.05$). During the last 3 wk of pregnancy, F females presented higher DM, DE and DP intake (171 g, 1493 kJ and 15 g/d, respectively) than CR and CAL (on av. 92 g, 1016 kJ and 11 g/d; $P<0.05$).

The feeding programme adopted during rearing and gestation periods had no effect on litter traits at the first parturition, averaging 7.5 kits (6.7 live/litter) with 53 g/kit.

Blood metabolic and hormonal parameters

The plasma profiles of females for glucose, NEFA, insulin, leptin, T3 and corticosterone at rearing (13 wk) and early pregnancy (20 wk) in the different feeding programmes are shown in Table 2. Circulating blood concentrations of glucose, insulin and T3 were lower while leptin was higher at early pregnancy than at 13th wk of age (–25, –46, –14 and +36%, respectively; $P<0.05$), but the feeding programme had no effect. NEFA plasma concentrations were

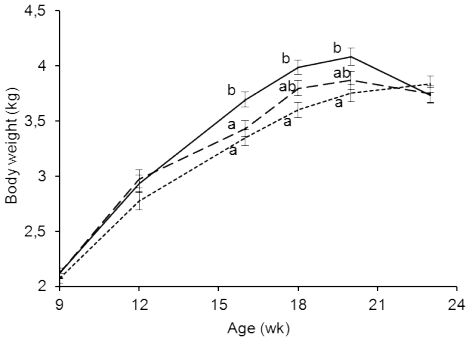

Figure 1: Evolution of body weight (kg) of young rabbit females from 9 wk of age to first parturition fed with a diet for reproductive rabbit does *ad libitum* (CAL), or restricted at 140 g DM/d (CR), or with a low energy/high fibre diet *ad libitum* (F). Values at a same age with differing letter (a,b) were different at $P<0.05$. —— CAL, – – – CR, ------ F.

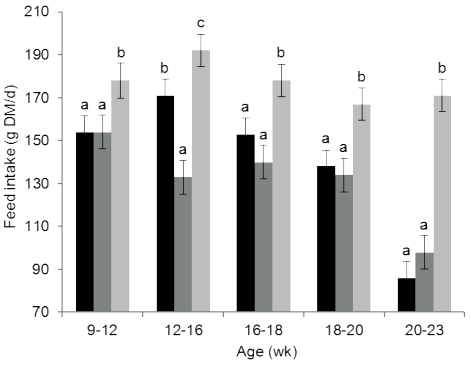

Figure 2: Feed intake (g DM/d) of young rabbit females from 9 wk of age to first parturition fed with a diet for reproductive rabbit does *ad libitum* (CAL), or restricted at 140 g DM/d (CR), or with a low energy/high fibre diet *ad libitum* (F). Values at a same age differing letter (a,b) were different at $P<0.05$. ■ CAL, ■ CR, ■ F.

lower in F than in CAL and CR females (on av. –23%; $P<0.05$). Plasma corticosterone was higher for F related to CAL females (+15%; $P<0.05$) and CR females showing intermediate values.

Behavioural measurements

Figure 3 shows the percentage of total time of different natural behaviours of young rabbit females at 13th and 20th wk of age (panel a), as well as with the different feeding programmes (panel b).

The inactive behaviour (lying still+sitting still) represented 60% of the time, lying still increasing and sitting still decreasing at 20th wk ($P<0.01$). Females in CR group showed less inactive behaviour than CAL and F groups (–9.5 points of percentage; $P<0.001$), because they spent less time lying still in the morning (–25.6 points of percentage; $P<0.001$).

Table 2: Blood plasma glucose, non-esterified fatty acids (NEFA), insulin, leptin, tri-iodothyroxine (T3) and corticosterone concentrations in young rabbit does during rearing (13 week of age) and early first pregnancy (20 week of age) with the different feeding programmes.

	Feeding programme[1]				Week			*P*-value	
	CAL	CR	F	SE	13	20	SE	Feed	Week
Glucose (mg/dL)	86.7	78.8	90.1	5.3	97.2	73.2	4.2	0.3170	0.0005
NEFA (mmol/L)	0.588[b]	0.544[b]	0.433[a]	0.03	0.504	0.539	0.02	0.0022	0.3867
Insulin (µUI/mL)	7.13	5.65	9.65	1.26	11.11	4.79	1.17	0.2768	0.0002
Leptin (ng/mL)	4.32	4.86	4.69	0.35	3.92	5.34	0.23	0.5459	0.0001
T3 (nmol/L)	3.11	3.41	3.40	0.17	3.50	3.11	0.13	0.3767	0.0239
Corticosterone (µg/dL)	4.61[a]	4.89[ab]	5.30[b]	0.17	4.84	5.03	0.14	0.0385	0.3459

[1] Feeding programme: CAL group received a diet for reproductive rabbit does (C) *ad libitum* until 1st parturition; CR group received the C diet *ad libitum* until 12 wk and then, 140 g/day until 1st parturition; F group received a low energy/high fibre diet *ad libitum* from 9 wk until 1st parturition.

[a,b] Means of feeding programme within a row not sharing any superscript are significantly different at $P<0.05$.

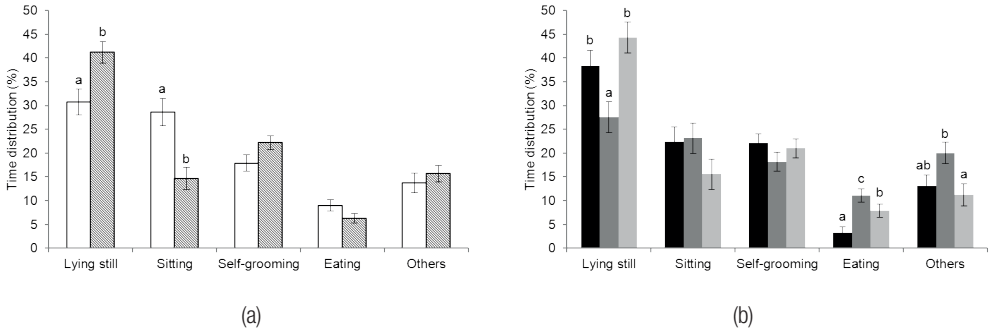

Figure 3: Time distribution (%) for the different behaviours of young rabbit females, a) at 13 (☐) and 20 (▨) wk of life, and b) when fed with a diet for reproductive rabbit does *ad libitum* (■ CAL), restricted at 140 g DM/d (■ CR), or with a moderate energy diet *ad libitum* (■ F). Values for a same behaviour differing letter (a,b,c) were different at $P<0.05$.

Self-grooming represented 20.4% of time and no differences were found between groups, weeks of age or time of day monitored.

Eating was significantly modified by the feeding programme ($P<0.001$), and eating pattern in CR group varied depending on day, time and age ($P<0.05$; Figure 4). For CAL and F females, no great differences were observed in the time spent eating throughout the day and at the different weeks of age monitored. However, F females seemed to spend more time eating than CAL ones (7.9 and 3.4%, respectively; $P<0.05$). On the other hand, CR females visited the feeder frequently before feeding provision (8:00) at 13th wk, but not at 20th wk (8.7 *vs.* 1.3%, respectively; $P<0.05$), and spent longer periods of time (16 to 19%) eating after feeding provision (9:00) compared to the other groups (on av. 4.4%).

Percentage of rabbit does showing stereotypic behaviours are shown in Figure 5. Turning round was observed sporadically (on av. 0.3 and 2.6 times/h at 13th and 20th wk, respectively) and associated with the same rabbit females over time. Frequency higher than 3 times/h of turning round behaviour was only shown in one rabbit doe from the CAL group at 17:00 at 13th wk of age and did not appear in F group, but in CR group at 8:00 at 20th wk of age this behaviour affected 50% of rabbit females ($P<0.05$).

Bar-biting behaviour was observed in all rabbit does in CR group at 8:00 at 20th wk of age, with a medium to high frequency (on av. 14 times/h; $P<0.01$). Moreover, frequency of this behaviour in CAL and F groups was higher at

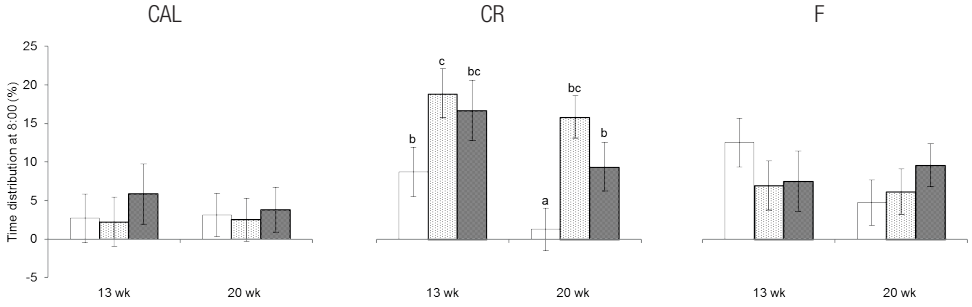

Figure 4: Time distribution (%) of eating behaviour of rabbit at 13 and 20 wk of life, when fed with a diet for reproductive rabbit does *ad libitum* (CAL), restricted at 140 g DM/d (CR), or with a moderate energy diet *ad libitum* (F) before (8:00 h ☐) and after (9:00 h ▨) feeding provision to CR does and in the afternoon (17:00 h ■). Values for a same dietary treatment differing letter (a,b,c) were different at $P<0.05$.

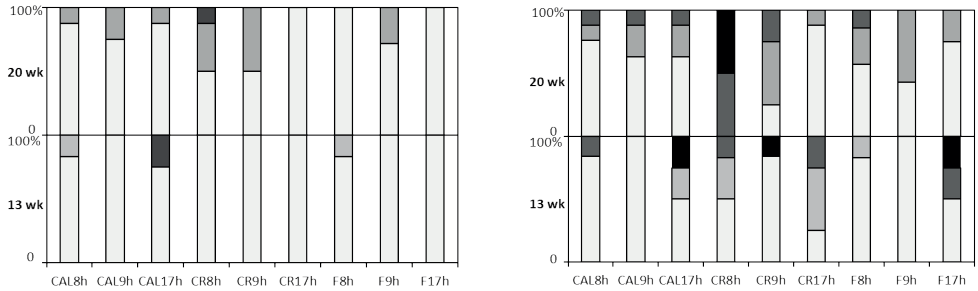

Figure 5: Frequency of young rabbit females showing stereotypic behaviours *a)* turning round (■ >3, ■ 1-3, □ 0), and b) biting bars at 8:00, 9:00 and 17:00 h (■ >10, ■ 6-10, ■ 2-5, □ 0-1), monitored at 13 and 20 week of life when fed with a diet for reproductive rabbit does *ad libitum* (CAL), restricted at 140 g DM/d (CR), or with a moderate energy diet *ad libitum* (F).

17:00 (5 times/h), while during the rest of the periods observed the frequency was lower than 10 times/h and always associated with the same rabbit females.

DISCUSSION

The data on body weight, feed intake, blood metabolites and hormones were similar to those obtained by other authors (Rommers *et al.*, 2004; Brecchia *et al.*, 2006; García-García *et al.*, 2011; Rebollar *et al.*, 2011; Menchetti *et al.,* 2015a; Martínez-Paredes *et al.*, 2012), except for blood glucose and insulin concentrations at 20th wk of age, which were very low in all groups. Compared with values at 13th wk of age, blood glucose fell in mid gestation, as a result of its increased metabolic demand by foetal and maternal tissues (Fortum-Lamothe, 2006) and, accordingly, circulating insulin concentration also decreased. This fall in blood insulin level should be even greater if compared with that recorded by Martínez-Paredes *et al.* (2012) at insemination (18 wk of age). However, insulin blood concentration at mid gestation was very low compared to that provided by Menchetti *et al.* (2015a), but this discrepancy could be due to differences in the sampling protocol, in our case in the morning and after four hours of fasting. A lower glucose and insulin blood level, together with a higher NEFA blood level, has been related to pregnancy toxaemia risk, but in our case NEFA level did not increase and the performance at partum was not affected.

Leptin level increased at mid gestation, probably as a result of fat deposition during rearing and early gestation (Pascual *et al.*, 2013). However, T3 concentration was higher at 13th wk, as required in a growing animal due to the function of T3 in animal growth. Martínez-Paredes *et al.* (2012) found an increase in T3 values from 9 to 18 wk of age.

The changes in endocrine and metabolic status linked to feed restriction are directly related to the actual nutrient intake during the restricted period. This may vary depending on severity and duration (Rommers *et al*, 2004), moment of application (Menchetti *et al.*, 2015b) and feed intake adaptation to the nutritive values of feed (Arias-Alvarez *et al*, 2009; Rebollar *et al*, 2011). In our experiment, the feeding programme had no effect on nutrient intake from 9 to 13 wk of age (1658 kJ DE/d and 17 g DP/d) and from 18 to 20 wk of age (1487 DE/d and 15 g DP/d). Consequently, blood values for glucose, insulin, leptin and T3 were not affected. However, F and CR programmes provided fewer nutrients before insemination at 18 wk than CAL programme (on av. –195 kJ DE/d and –2 g DP/d; P<0.01), while F group had higher nutrient supply during the last 3 wk of gestation than CR and CAL groups (on av. +477 kJ DE/d and +4.6 g DP/d; P<0.01). Despite these figures, there were no differences in litter traits at the first parturition. It can be hypothesised that the 3 feeding programmes allowed us to achieve the leptin threshold and body maturity level necessary for reproductive activity of young rabbit does (Arias-Alvarez *et al*, 2009; Martínez-Paredes *et al.*, 2012). On the other hand, Martínez-Paredes *et al.* (2012) also observed that a higher energy intake achieved during late gestation by using a low energy/high fibre diet did not affect litter performance at partum.

Blood concentration of NEFA varied with the feeding programme, being higher for those females receiving diet C (both *ad libitum* and restricted) compared to F females. A possible explanation could be related to the greater BW (and probably body condition) of these females. Previous studies have found that fasting NEFA concentration was greater in animals with high body condition score (Frank *et al.*, 2006; Suagee *et al.*, 2013).

Undernutrition during rearing and/or gestation is a stressor for animals that should reflect on hormones, especially corticosterone, as well as on behavioural patterns, such as inactivity, eating and stereotypic behaviours. According to Brecchia *et al.* (2006), undernutrition increased plasma concentration of corticosterone, as in metabolic adaptation to complete food deprivation, but interactions with other hormones could exist. In our case, feed restriction in CR group did not significantly increase corticosterone levels, but the difference between CAL and F groups was significant, which could indicate a higher stress when a low energy/high fibre diet was used as compared to animals fed *ad libitum* with a commercial diet.

However, the behavioural patterns were affected by the feeding programme. In the CR group, the females were less inactive in the morning because they were more anxious before feeding provision (at 8.00), spending 10% of the time visiting the empty feeder at 13th wk of age or showing more stereotypic behaviours at 20th wk of age, and increasing the eating events at 9.00 (19% of this time) after feed provision, as was related by Gidenne *et al.* (2012) with restricted growing rabbits. On the contrary, low eating activity was recorded in F (7%) and CAL (3%) groups in the morning, in accordance with the normal behaviour of rabbits (Gunn and Morton, 1995; Krohn *et al.*, 1999; Fernández-Carmona *et al.*, 2005; Gidenne *et al.*, 2012).

From the results of the present work, it may be concluded that restricted access to a diet formulated to cover the needs of lactating rabbit does for the whole rearing period and gestation could alter eating behaviour of rabbit females as a consequence of increasing feed intake in the morning immediately after feed provision. This feeding programme seems more stressful, as the rabbits appear more anxious, showing stereotypic behaviours before feed provision. As an alternative, the use of a low energy/high fibre diet *ad libitum* seems more appropriate, as it does not alter the animal's behavioural patterns, but could increase the blood corticosterone level.

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