YOUNG RABBIT DOES FED WITH FIBROUS DIET DURING REARING: SERICAL AND PRODUCTIVE PARAMETERS

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ABSTRACT

The aim of this work was to determine the effect of different feeding systems during rearing on serum leptin, non esterified fatty acids (NEFA) and total protein levels and some productive parameters of rabbit does. Thirty six animals 11-weeks old were randomly distributed in three experimental groups: group AL-C and group R-C were fed *ad libitum* and restricted (150 g/day) respectively a commercial diet (NDF 38% DM). One week before first artificial insemination (AI), R-C group was fed *ad libitum*. AL-F group fed an experimental diet with a higher level of fibre provided *ad libitum* (NDF 50% DM). First AI was made in AL-C does at 16 weeks of age and in AL-F and R-C does one week later. After first parturition, all animals fed *ad libitum* the commercial diet. During the rearing period, animals of group R-C had the lowest daily feed intake ($P<0.001$), digestible energy intake ($P<0.03$) and digestible protein intake ($P<0.001$), delaying the onset of puberty ($P<0.001$), decreasing their fertility at first AI ($P<0.02$) and having the lowest pre-puberal leptin concentration ($P<0.001$) in comparison with the other two groups. During pregnancy, AL-F does had a tendency to a higher feed intake with respect to other groups, and their fertility on day 11 *post partum* (pp) tended to be higher ($P<0.09$). All animals showed physiological high levels of total serum protein at 16 weeks of age. Serum NEFA level was high around parturition in all groups indicating mobilization of energy reserves, even though in AL-F group this mobilization was lower. *Ad libitum* feeding system with fibrous diets during rearing allowed to obtain similar reproductive performances in nulliparous rabbit does than feeding system with lower fibre inclusion.

Key words: Rearing, Feeding management, Fibre, Leptin, NEFA, Serum total protein.

INTRODUCTION

During rearing, feed restriction is usually applied in rabbit females to obtain uniformity in their body weight, to avoid fattening and high mortality around parturition (Rommers *et al*., 2001), to increase voluntary intake at beginning of the lactation period and to allow a long productive life (Partridge, 1986). Nevertheless, restricted rabbit females start their productive life (first pregnancy and lactation) with a deficient energy level. To avoid it, it is possible to provide *ad libitum* a diet with a high percentage of fibre. Although this type of diet has a low digestible energy, it could improve feed intake during first lactation due to the enlargement of the digestive tract (Nizza 1997; Pascual *et al*., 2002). The negative effect of caloric restriction on reproductive parameters exists, and it was demonstrated when Brecchia *et al*. (2006) observed a low pulse LH levels in fasted rabbit females. Nutrition and reproduction relation is established by means certain endocrine signals that communicate nutritional status of female to hypothalamus-pituitary axis (Boiti 2004). In this sense, leptin, a hormone released in the adipose tissue, communicates to hypothalamus the adequacy of peripheral energy store to sustain reproduction. During periods of high demand as lactation or pregnancy, total serum protein can be affected (Melillo, 2007) and the ability to mobilization of body reserves by means lipolysis of fat depot contributes to energy supply that is released into the blood and it can be measured determining the increase of serum concentration of non-esterified fatty acids (NEFA). The aim of this work was to determine the effect of different feeding treatments provided to rabbit females during rearing on serum parameters (leptin, NEFA, and total protein levels) and some productive parameters at the beginning of the reproductive life.

MATERIALS AND METHODS

Animals and Experimental Design

Animals were held on an experimental farm in Animal Production Department of UPM into individual flat-deck cages, under a constant photoperiod of 16h light per day, a temperature of 18-22ºC, and a relative humidity of 60-75% maintained by a forced ventilation system. Thirty six rabbit females 11-weeks old were randomly distributed until their first parturition in three experimental groups: Group AL-C: animals were fed *ad libitum* a commercial diet (Cunimax A, Cargill España) with 38% NDF on a DM basis, according to De Blas and Mateos (1998); group R-C: fed with the same previous diet restricted (150 g/d) and one week before first insemination *ad libitum;* group AL-F: fed *ad libitum* an experimental diet (Cunimax F Cargill España), with 50% NDF on a DM basis. Differences in fibrous ingredients between F and A diet were due to different proportion of alfalfa hay (57 vs. 14%) and defatted grape seed meal inclusion (20 vs 0%). After the first parturition and during the lactation period all animals fed *ad libitum* the commercial diet Cunimax A. Chemical composition of diets is shown in Table 1. First artificial insemination (AI) in AL-C group was made at 16 weeks of age and in AL-F and R-C groups at 17 weeks. After parturition, a second AI was made on day 11 *post-partum* (pp). Seminal doses, with more than 20 million spermatozoa in 0.5 ml of a commercial diluent (Magapor S.L.), were made using a pool of fresh heterospermic semen from bucks selected for growth performance. To induce ovulation, does were given an intramuscular injection of 10 µg buserelin (Suprafact, Hoechst Marion Roussel, S.A., Madrid). After parturition, litter size was manipulated to provide eight to ten kits per doe, which were then weaned at 35 days of age. Total number of kits born and stillborn (prolificacy) at birth and mortality during the lactation period were recorded. Body weight (BW) and feed intake (FI) at first fertile AI, at parturition and at weaning were recorded. Blood samples were taken from the ear artery (at 9:00-10:00 a.m., to avoid circadian variations) to determine leptin, NEFA and total protein levels of rabbit does at 11, 16 and 17 weeks of age, 24 hours before parturition, on day 11 (second AI) and 21 pp. Fecal digestibility was determined according to Perez et al. (1995), using 12 animals 15 weeks old per diet with a mean body weight of 2.8 ± 0.2 kg. All experimental procedures used in this research were approved by the Animal Ethics Committee of the Polytechnic University of Madrid and were in compliance with the Spanish guidelines for the care and use of animals in research (BOE, 2005).

Chemical Analyses and Serum determinations

Procedures of the AOAC (2000) were used to determine the concentrations of dry matter (DM) (934.01), ash (967.05), crude protein (CP) (968.06). Dietary NDF, ADF and ADL were determined sequentially by using the filter bag system (Ankom Technology, New York) according to Mertens (2002), AOAC (2000; procedure 973.187) and Van Soest *et al.* (1991), respectively. Energy was determined with a calorimetric adiabatic bomb. Quantitative determination of leptin was made by RIA using a commercial kit (Multi-species Leptin RIA, Linco Research). Intra and inter-assay coefficient of variation was 3.1% and 7.3%, respectively. The limit of detection after adjust the standard curve to rabbit values was 0.1 ng/mL HE (Human Equivalent). NEFA determination was performed using the acyl-CoA synthetase-acyl CoA oxidase (ACS-ACOD) method as prepared in the Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Serum total protein was determined by means Biuret method according to Tietz (1995).

Table 1: Chemical composition (% of DM) of diets

	Cunimax A	Cunimax F
Dry matter	92.34	94.11
Ash	7.89	9.49
Energy MJ/Kg	18.50	18.50
Crude protein	18.82	18.50
NDF	37.92	50.50
ADF	13.85	19.30
ADL	5.24	9.34

Statistical Analysis

Statistical analysis was carried out using the SAS statistical package (Statistical Analysis System 8.2; 2001). Data of productive parameters and apparent fecal digestibility were analysed as a completely randomized design with feeding system as main source of variation by using the General Linear Model (GLM). With respect to serum levels, a MIXED procedure was used according to an auto-regressive model to analyse repeated measures including the effect of feeding system, the time and their interaction. Doe was considered a random effect nested in the treatment. All means were compared using the protected LSD test.

RESULTS AND DISCUSSION

Digestibility results are shown in Table 2. DM, CP and EB digestibility coefficients of F diet decreased with respect to the A diet by around 10%, 6% and 12% ($P<0.0001$), respectively. It can be explained by the higher level of lignified fibre of F diet compared to A diet (De Blas *et al*., 1999). Consequently, it was observed a lower content of digestible energy. The negative effect of fibre on digestible CP was lower than on digestible E, resulting in a higher DP/DE ratio for F diet. It might be accounted for the relative high DP values of alfalfa hay and defatted grape seed meal (García *et al*., 1995; García *et al*., 2002). Productive parameters are shown in Table 3. At the beginning of experiment, mean BW of rabbit does was 2.4 ± 0.17 kg. Diets did not affect BW at first fertile AI, that is in accordance with the BW commendation to first time inseminated nulliparous does to achieve good results. There were not differences ($P>0.3$) in rabbit BW at parturition and at weaning between groups, being 3.6 ± 0.1 and 3.8 ± 0.1 kg, respectively. As it was expected, animals of group R-C had a lower daily FI than group AL-C and AL-F until the first fertile AI ($P<0.0001$) and DE and DP intake were lower, too ($P<0.03$). Therefore, fertility at first AI was lower in restricted rabbit does, indeed they delayed by a mean of 2 weeks the age of first fertile AI. During pregnancy, there were not differences in DP and DE intake, due to a tendency to a higher FI in group AL-F with respect to other groups. Maybe, for this reason, primiparous fertility (AI on day 11 pp) tended to be higher in rabbit does fed the fibrous diet. There were not differences in prolificacy at first parturition, and a mean of 7.2 born alive, 1.25 born dead and 7.1 weaned kits were obtained. Litter weight at 21 days of lactation and at weaning was similar between groups.

Table 2: Feed intake and apparent digestibility of nutrients

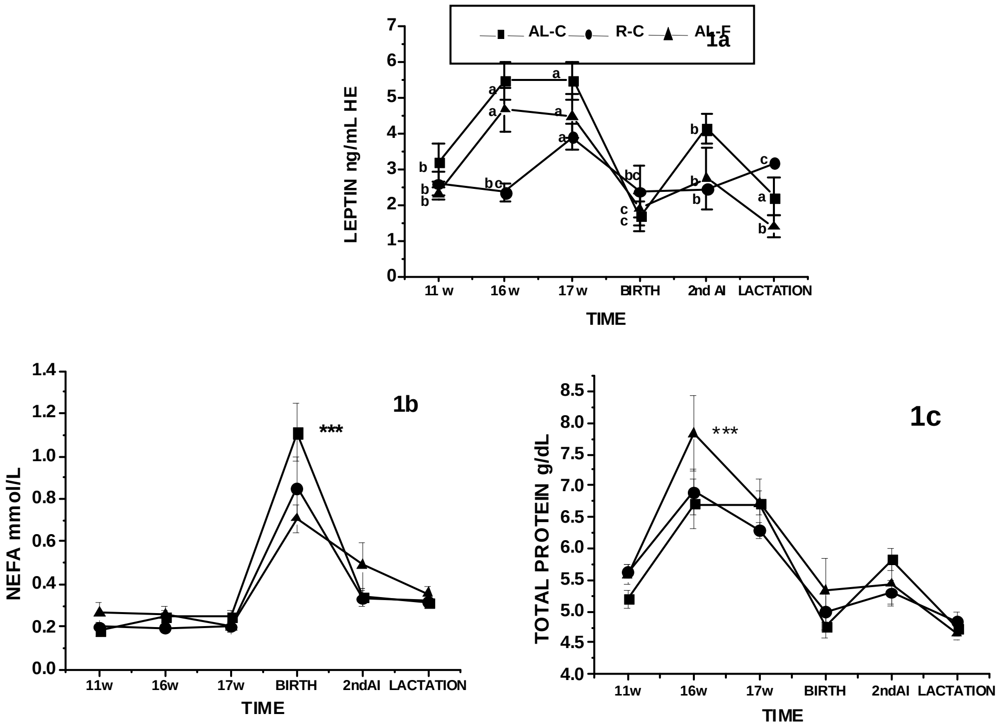
	Diets			
	Cunimax A	Cunimax F	SEM[1]	P
Doe weight (g)	2881	2771	61.9	0.22
Feed intake (g DM)	192	229	8.58	0.006
Digestibility coefficients (%):				
Dry matter	61.5	50.9	0.98	0.0001
Crude protein	72.1	66.3	0.75	0.0001
NDF	26.8	29.3	1.79	0.34
Energy	62.4	50.4	1.02	0.0001
Digestible energy (ED) (MJ/kg DM)	11.6	9.4	0.19	0.0001
Digestible protein (DP) (%)	13.6	12.3	0.14	0.0001
DP/DE (g/MJ)	11.6	13.1	0.21	0.0001

[1]SEM: Standard Error of means (n=12)

Table 3: Effect of feeding systems on productive performance

	Feeding system[1]			SEM[2]	P
	AL-C	AL-F	R-C		
Age at first fertile AI (w)	16 b	17.3 b	19.2 a	0.55	0.001
Doe BW at first fertile AI (g)	3534	3534	3659	100	0.59
Feed intake start-1st fertile AI (g/d)	195 b	223 a	144 c	10.7	0.0001
DE intake start-1st fertile AI (MJ/d)	2.09 a	1.97 a	1.54 b	0.11	0.003
DP intake start-1st fertile AI (g/d)	24.3 a	25.9 a	17.9 b	1.29	0.0003
Feed intake during pregnancy (g/d)	174 ab	206 a	169 b	14.2	0.15
DE intake during pregnancy (MJ/d)	1.87	1.82	1.81	0.15	0.96
DP intake during pregnancy (g/d)	21.7	23.9	21.05	1.75	0.48
Fertility at 1st AI (%)	100 a	95 a	72.6 b	6.80	0.02
Fertility at 2nd AI (%)	50 b	70 a	59.1 ab	6.11	0.09
Feed intake during lactation (g/d)	318	317	369	34.7	0.51

[1]AL-C: *Ad libitum* commercial diet; AL-F: *Ad libitum* fibrous diet; R-C: commercial diet restricted. [2]SEM: Standard Error of means (n=12). AI: Artificial insemination. BW: Body weight. FI: Feed Intake. DE: Digestible Energy. DP: Digestible Protein.



Figures 1a: Serum leptin levels; **1b**: NEFA levels; **1c**: Serum total protein levels in rabbit does at 11, 16 (1st AI in AL-C group) and 17 weeks of age (1st AI in AL-F and R-C groups), parturition day, 11 days pp (2nd AI) and 21th day of lactation. Different letters show differences between periods inside of each treatment (P<0.05) and ***: P<0.001

In fact, during the first lactation period FI was similar in all groups (around 335 g/d) and there were not differences in mortality at birth and during lactation (12.4 and 16.6%, respectively). At 11 weeks of age, leptin level (Figure 1a) was low in all groups, because rabbit does are in a pre-puberal status. From 11 to 16 weeks of age, does arrived to puberal age and total serum proteins (Figure 1c; P<0.0001) raised in all groups but remaining at physiological levels (Melillo, 2007). In accordance with Boiti (2004), at puberty, leptin controls the satiety sensations and feeding behaviour with the aim to regulate gonadotrophin release at the puberty onset. At this moment, both AL-F and AL-C groups had higher leptin level, and higher fertility at first AI, than rabbit of group R-C. In spite of restricted rabbit does have similar serum leptin level to AL-C and AL-F group at 17 weeks of age, their fertility was the lowest and their first fertile AI was delayed until reached similar weight than other groups. These results confirm that restriction results in retardation of puberty according to Rommers *et al*.

(2001) and the important connection role of leptin between nutrition and reproduction at this period. At the parturition, all groups showed a significant decrease of leptinemie, demonstrating an important energy loss occurred at the end of pregnancy. Does fed with the fibrous diet had a lower lipid mobilization around parturition (Figure 1b, P<0.001) whereas the highest lipid mobilization was observed in AL-C group. These results coincide with low values of total serum protein around parturition in all groups (Figure 1c). After parturition, all animals fed C diet *ad libitum* but it was not enough to increase leptin levels on day 11 pp. At this moment and on day 21 of lactation, serum leptin levels were lower than at 16 weeks and fertility was also low in all groups.

CONCLUSIONS

Restricted feeding in the rearing period dilated the onset of puberty and decreased fertility at first AI. Rabbit does fed with the fibrous diet had lower mobilization of lipids around first birth and the fertility obtained on day 11 pp tended to be higher. Our results could indicate that a fibrous diet provided *ad libitum* during rearing allows a regulation of body reserves mobilization, helping doe recuperation in their second reproductive cycle.

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