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DIET DENSITY IN REARING AND REPRODUCTIVE PHASES INFLUENCES CARCASS COMPOSITION, PREGNANCY RATE AND LITTER PERFORMANCE OF PRIMIPAROUS RABBIT DOES

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Abstract: The objective was to evaluate the effect of the interaction of diet density in the rearing phase×diet density in the reproductive phase on carcass composition, pregnancy rate, and litter performance of primiparous rabbit does. The experiment followed a 2×2×2 factorial (2 seasons, 2 diet densities in the rearing phase and 2 diet densities in reproductive phase, that is, from mating to weaning of the first litter). The reference diet (RD) contained 184 g/kg of crude protein (CP), 165 g/kg of acid detergent fibre (ADF) and 10.5 MJ/kg of digestible energy (DE). The low-density diet (LD) had 147 g/kg of CP, 24 g/kg of ADF and 8.4 MJ/kg of DE. The treatments were applied from 70 d of age until weaning of the first litter at 35 d of age. Ninety-six females from the Botucatu Genetic Group (24 females/experimental group) were mated at 142 d of age. On day 12 of gestation, 23 does were slaughtered to evaluate weights of carcass, organs and dissectible fat, and embryo implantation rate. No effects of diet density in the rearing or in the reproductive phases were detected on feed intake of does during the reproductive phase. Does fed LD during the rearing phase showed lower body weight at mating (3574±47 *vs.* 3866±43 g, P=0.0001) and during most of the reproductive phase, but they lost less weight in the peripartum. Perirenal fat was lighter in these does (72.8±10.0 *vs.* 102.1±9.6 g, P=0.048) and they showed a lower pregnancy rate (76.1 *vs.* 91.7%, P=0.045). The does fed RD in the reproductive phase were heavier during this phase (4055±40 g *vs.* 3887±41 g, P=0.0044). The does fed LD in rearing phase and RD in the reproductive phase showed larger litters at weaning, due to decreased kit mortality, than those fed RD in both phases (6.16±0.47 *vs.* 3.93±0.71, P=0.0361). Litters were lighter at weaning when LD was fed in the reproductive phase (3582±201 *vs.* 4733±187, P<0.0001). Feeding a low-density diet during the rearing phase and a reference diet during the reproductive phase is the best alternative to improve reproductive performance at the first parity.

Key Words: embryo implantation rate, litter size, perirenal fat, pregnancy rate, rabbit.

INTRODUCTION

Primiparous rabbit does are prone to an intense energy deficit during lactation because their feed intake capacity is not completely developed and they are still growing during the first reproductive cycle (Fortun-Lamothe, 2006). This energy deficit has been demonstrated during the lactation of does kindling for the first or second time and may lead to decreased reproductive performance and high replacement rate (Rommers *et al.,* 1999). A higher intake was observed in multiparous does, which suggests that the energy deficit could decrease as parity increases (Rommers *et al.,* 2004, Xiccato *et al.,* 2004).

Rabbit does increase feed intake by 25 to 50% during pregnancy. Until the 3rd wk of gestation, nutrient requirements for foetal growth are small and energy balance remains positive; as a result, body fat reserves increase. However,

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during the last week of gestation, the energy requirement for foetal development shows a sharp rise, whereas feed intake decreases during the last few days of pregnancy. Nevertheless, the overall energy balance at the end of the first pregnancy remains positive (Fortun-Lamothe, 2006).

Lactating does have high protein and energy requirements (De Blas and Mateos, 2010), especially when the reproductive rhythm is intense. Despite the great increase in feed intake during this period, it may not be enough to cover maintenance, growth and lactation requirements, resulting in a negative energy balance during the first lactation (Fortun-Lamothe, 2006). Variations in perirenal fat weight or thickness were considered suitable indicators of mobilisation of body reserves and change in body condition of young female rabbits (Pascual *et al.* 2000, 2004).

The adoption of management measures and feeding practices only during the reproductive period does not seem to be effective to mitigate the problem of negative energy balance during the first lactation (Rommers *et al.,* 1999). Management programmes involving feed restriction (Rommers *et al.,* 2001; 2004) or the use of fibrous, low density diets in the rearing period (Nizza *et al.,* 1997; Xiccato *et al.,* 1999; Pascual *et al.,* 2002) have been proposed, but the results are still controversial. Among the factors that influence the outcome of these programmes are the environmental conditions, the young doe's age at the beginning of the programme and the source of fibre used (Rebollar *et al.,* 2011). Moreover, feed restriction in the rearing phase may affect animal welfare by increasing anxiety and stereotyped behaviour before feed provision (Martínez-Paredes *et al.,* 2015).

Thus, the aim of this study was to evaluate the effect of the interaction of diet density in the rearing phase×diet density in the reproduction phase on carcass composition, pregnancy rate and litter performance of rabbit does during their first reproductive cycle and under natural mating. It was expected that the does fed low diet density in the rearing phase would eat more in the reproductive phase, due to an increased intake capacity, and that it could have a positive impact on their performance in the first reproductive cycle.

MATERIAL AND METHODS

Animals and facilities

The experiment was conducted from December 14 to July 6 (season 1) and replicated from May 10 to October 15 (season 2) under the tropical conditions of Botucatu, Brazil (22°51'S, 48°26'W). The first season corresponded to the end of summer and the fall, whereas the second season comprised winter and the beginning of spring. The mean high and low ambient temperatures were 29.0 and 17.3°C for the first season and 26.3 and 15.8°C for the second season. Facility space limitation was the reason for repeating the experiment in the subsequent season, and not the interest in the seasonal effect by itself. Ninety-six Botucatu does were used, 48 in each season. Teixeira *et al.* (2013) discussed the seasonal effect on the performance of young females during the rearing phase.

The Botucatu genetic group is a synthetic strain, originated from Norfolk 2000 rabbits (Moura *et al.,* 2000). According to that breeding company, Norfolk hybrids originated from a two-generation crossbreeding programme involving the New Zealand White, Californian and Bouscat Giant breeds. This genetic group has now undergone more than 40 years of local adaptation and 2 decades of selection for growth rate and litter size (Moura *et al.,* 2001; Khalil and Al-Saef, 2008).

During the rearing phase, does were first housed in collective cages, in groups of four, from 70 up to 119 d of age. Then, at 119 d they were moved to individual cages. A detailed description of the performance of young does in the rearing phase was provided elsewhere (Teixeira *et al.,* 2013). The reproductive phase began with mating at 142 d of age. An east-west oriented opened building equipped with plastic adjustable curtains and commercial cages (0.80×0.60×0.45 m) was used for rearing and reproductive phases. All cages had a circular feeder and a nipple drinker. Animals were provided with 13 h of light (natural plus artificial) every 24 h.

Experimental diets

Two pelleted diets were manufactured (Table 1). The reference diet (RD) was formulated to attain the following contents: 184 g/kg crude protein (CP); 165 g/kg acid detergent fibre (ADF); and 10.5 MJ/kg digestible energy (DE), on an as-fed basis (De Blas and Mateos, 1998). The low-density diet (LD) was formulated to attain 147 g/kg CP; 240 g/kg ADF and 8.4 MJ/kg of DE. Both diets were formulated to contain approximately 18 g CP/MJ DE.

Two diet batches were manufactured in each season. Two samples were taken from each batch, one just as it had come out of the pelleting machine, and another one as the batch was about to be used up. The samples were analysed according to the recommendations from EGRAN (2001) and A.O.A.C. (2000) methods for dry matter (DM) (930.15), CP (984.13), ether extract (EE) (920.39), ashes (942.05) and ADF (973.18). For neutral detergent fibre, cellulose and lignin, the sequential method of Van Soest *et al.* (1991) with the notation indicated by Mertens (2002) was used (Table 2). The regression equation proposed by Fernández-Carmona *et al.* (2004) was applied to estimate the DE content, based on the ADF content. The estimated DE contents were, on a 900g/kg DM basis: 10.7 and 9.5 MJ/kg, for RD and LD, respectively. Thus, RD ended up having an estimated DE content 4% below the recommendations of De Blas and Mateos (1998) for breeding does (11.1 MJ/kg on a 900 g/kg DM basis) and 2% above those for fattening rabbits (10.5 MJ/kg). The estimated DE content of LD was 14% and 10% below the above requirements. The final estimated CP/DE ratios were 16.3 and 14.9 g/MJ, respectively.

Table 1: Ingredients and planned chemical composition of the experimental diets, on an as-fed basis.

Ingredient (g/kg)	Reference diet	Low-density diet
Soybean meal	286.21	166.15
Corn grain	150.00	116.18
Wheat bran	100.00	100.00
Citrus pulp	126.84	21.06
Black oat hay	252.95	516.19
Limestone	1.65	11.39
Dibasic calcium phosphate	24.55	25.23
L-threonine	0.36	1.39
Vitamin and mineral premix[1]	5.00	5.00
Kaolin	20.00	10.00
Soybean oil	27.44	22.41
Salt	5.00	5.00
Planned chemical composition		
Crude protein (g/kg)	184	147
Digestible energy (MJ/kg)	10.5	8.4
Acid detergent fibre (g/kg)	165	240
Starch (g/kg)	126	100
Calcium (g/kg)	12.0	12.0
Phosphorus (g/kg)	6.0	6.0
Methionine (g/kg)	2.67	2.18
Sulphur-containing amino acids (g/kg)	5.60	4.49
Lysine (g/kg)	10.05	7.52
Threonine (g/kg)	7.00	7.00
Tryptophan (g/kg)	1.78	1.12

[1]"Supervit Coelho" 5:1. In order to reach the following additional levels (per kg of diet): vit. A, 7000 UI; vit D_3, 1250 UI; vit. E, 35 mg; vit K_3, 2 mg; vit. B_1, 3 mg; vit. B_2, 5 mg; vit B_6, 2 mg; Vit. B_{12}, 12.5 µg; calcium pantothenate, 10 mg; niacin, 30 mg; folic acid, 1 mg; antioxidant, 200 mg; anticoccidial agent, 33 mg; choline,125 mg; selenium, 0.2 mg; manganese, 60 mg; iron, 80 mg; copper, 12 mg; iodine, 1 mg; zinc, 50 mg .

Table 2: Actual chemical composition of the diets (g/kg on a 900 g/kg dry matter basis).

	Chemical components						
Diet	CP	EE	Ash	NDF	ADF	Lignin	Cellulose
Season 1							
Reference	193.0	51.1	71.3	287.0	160.1	27.0	125.6
Low-density	157.0	33.0	83.0	381.5	223.1	38.4	174.2
Season 2							
Reference	201.0	52.4	82.0	270.0	168.0	29.7	125.2
Low-density	156.3	42.2	78.0	398.1	230.2	43.0	175.0

CP: crude protein; EE: ether extract; NDF: neutral detergent fibre; ADF: acid detergent fibre.

Experimental treatments

Four treatments were used: 1) RD offered during the rearing phase, from 70 to 142 d of age and also during the reproductive phase, from mating (on day 142) to weaning of first litter; 2) RD offered during rearing phase and LD during reproductive phase; 3) LD offered during rearing phase and RD during reproductive phase; and 4) LD offered during both phases. In each season, 12 does were used per experimental group, therefore 24 does were included per group, considering the 2 seasons (Table 3).

Analyses of the evolution of body weight and feed intake during the rearing phase (from 70 d up to mating at 142 d of age) were previously reported (Teixeira *et al.,* 2013). Therefore, the present study focused exclusively on the performance of does during the reproductive phase, that is, from mating at 142 d up to weaning of the first litter. Feed intake and body weight were assessed weekly during the reproductive phase, beginning at the first mating attempt (142 d of age). Feed intake was estimated by weighing the total amount of feed offered and left in the feeder weekly. From mating up to 16 d post-partum, feed intake reflected only the doe's intake. From then on, the kits started eating solid feed; thus, the intake depended on litter size.

No synchronisation treatments were used. Each female was taken to a male's cage for mating when she reached 142 d of age. If she refused to mate (no mating within approximately 3 min), another male was randomly assigned. If the female still refused to mate, she was taken back to her cage and presented again to a male 1 wk later. Change from rearing to reproduction diet in treatments 2 and 3 occurred only after actual mating took place. On day 10 after mating, pregnancy was diagnosed through abdominal palpation; in the event of a negative diagnosis, the doe was mated again. To obtain a sufficient number of pregnancies, up to four mating attempts per doe were allowed during the first season and up to 2 attempts in the second season.

On day 12 of gestation, 3 does/treatment/ season were randomly chosen, weighed and slaughtered (Close *et al.,* 1997). Reference carcass weight was obtained according to Blasco and Ohayoun (1996). The empty digestive tract

Table 3: Evolution of number of does and litters according to diet density in rearing and reproductive phases.

	Diet density			
	RD rear.		LD rear.	
	RD reprod.	LD reprod.	RD reprod.	LD reprod.
Initial	24	24	24	24
Receptive[1]	24	24	23	23
Pregnant	21	23	19	16
Slaughtered[2]	6	6	6	5
Litters born	15	17	13	10[3]
Litters weaned	12	17	13	8

RD: reference diet; LD: low density diet; Rear.: rearing phase, Reprod.: reproductive phase.

[1]Over all mating attempts.

[2]On day 12 of pregnancy.

[3]One doe from this group was diagnosed pregnant, but did not give birth.

was weighed after previous cleaning and washing. Scapular, inguinal and perirenal fat depots were dissected and weighed. The entire reproductive tract was dissected and weighed. The numbers of corpora lutea (CL) in both ovaries and of implanted embryos in both uterine horns were computed. The number of live embryos was estimated by normal uterine swellings, whereas small uterine swellings were considered as degenerated embryos (Santacreu *et al.* 1992). Embryo implantation rate (IE) was computed as follows:

IE (%) =100×(number of live implanted embryos /number of corpora lutea in both ovaries)

Dams were not remated after parturition. Pregnancy rates at first mating and overall mating attempts were computed as 100*number of pregnant does/ number of mated does. Wooden nests (0.45×0.25×0.30 m) containing approximately 3 cm wood shavings were provided for each pregnant doe on the 28th d of pregnancy. Nests were inspected on the day of parturition to record total number born and number born alive per litter, and litter weight at birth. Stillborn kits were removed and nest bedding was replaced. No adoptions of kits took place. All nests were inspected daily during lactation to record kit mortality. Free nursing of litters was allowed, that is, no lactation control technique was adopted. Litter size and weight were recorded again on day 21 and at weaning (day 35).

Statistical procedures

A 2×2×2 factorial (2 seasons×2 diet densities in the rearing phase×2 diet densities in the reproductive phase) was adopted, in a completely randomised design. For the analysis of doe weight and feed intake, a repeated measures statistical model was adopted. It included the fixed effects of season, diet density during rearing phase, diet density during reproductive phase, week, the two and three-way interactions of the last three effects, and the random effect of the residuals. The weekly weighings and measurements of feed intake were considered as repeated measures. Litter size at weaning was included in the model as a covariate in the analysis of maternal feed intake. The model used for the analysis of carcass composition and embryo implantation rate included the fixed effects of season, diet density during rearing phase, diet density during reproductive phase, the two-way interaction between the last two effects and the random effect of the residuals The MIXED procedure of SAS (2003) was used in these analyses.

Pregnancy rate data were analysed by means of the LOGISTIC procedure of SAS (2003). The model included the effects of season, diet density in the rearing phase, diet density in the reproductive phase and the two-way interaction between the latter two effects and the random effect of the residuals.

For the analyses of litter size at birth, at 21 and 35 d of age the statistical model included the fixed effects of season, diet density in the rearing phase, diet density in the reproductive phase, and the two-way interaction between the latter 2 effects and the random effect of the residuals. For the analyses of litter weight, the fixed effect of age (birth, 21 d and weaning) and its two-way and three-way interactions with diet density in the rearing phase and diet density in the reproductive were included in the previous model. The weighings were considered as repeated measures. These analyses were implemented in the MIXED procedure of SAS (2003). Appropriate contrasts were computed when interaction effects were significant.

RESULTS

Physical space limitation in facilities was the reason for splitting the experiment between 2 seasons, not an interest in the seasonal effects; they were therefore not shown.

Feed Intake and body weight of does from mating to weaning of the first litter

No effects of diet density in the rearing phase (P>0.05) or in the reproductive phase (P>0.05) were detected on feed intake of does from mating to weaning of the first litter. However, a week effect (P<0.0001) was found on feed intake: it was lower in the prepartum period than in the postpartum period. In prepartum, intake remained constant (on av., 180.3±13.2 g/d), but increased during postpartum (on av., 435.5.4±13.8 g/d), and this increase became more intense from the eighth week onwards, i.e., from the third week of lactation onwards, when the kits began to eat solid feed.

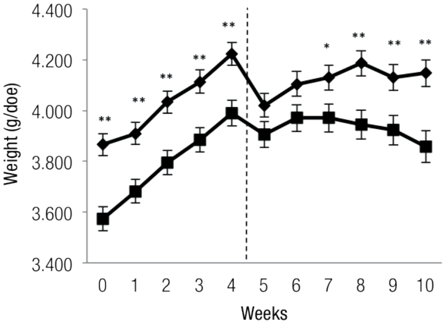

Figure 1: Effect of the nutritional density in the rearing phase×week interaction on the evolution of body weight of does in the reproductive phase (from 142 d of age up to weaning of the first litter), including gestation (before vertical dotted line) and lactation (after vertical dotted line) periods. —◆— reference diet in rearing phase; —■— low density diet in rearing phase. (*P<0.05; **P<0.01).

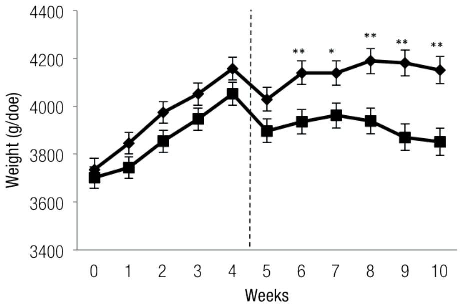

Figure 2: Effect of the nutritional density×week interaction on the evolution of body weight of does in the reproductive phase (from 142 d of age up to weaning of the first litter), including gestation (before vertical dotted line) and lactation (after vertical dotted line) periods. —◆— reference diet in reproductive phase; —■— low density diet in reproductive phase. (*P<0.05; **P<0.01).

No effects (P>0.05) of the interaction of diet density in the rearing phase×diet density in the reproductive phase were found on feed intake and body weight of does from mating to weaning of the first litter.

Diet density in the rearing phase influenced (P=0.0003) does' body weights in the reproductive phase. Does fed RD during the rearing phase showed higher body weight at mating (3866±43 *vs.* 3574±47 g, P =0.0001) and during most of the reproductive phase (4079±39 *vs.* 3863±42 g, P=0.0003) than those fed LD during the rearing phase. However, a diet density in the rearing phase×week interaction effect (P=0.0018) was detected on body weight of does in the reproductive phase. Whereas no difference in body weight between the 2 groups was detected at partum and during the first 2 wk of lactation, does fed LD in the rearing phase were lighter during gestation and became lighter than those fed RD as lactation progressed (Figure 1). Weight loss in the peripartum was lower in does fed LD in the rearing phase.

Does fed RD in the reproductive phase were heavier during the reproductive phase than those fed LD in the reproductive phase (4055±40 g *vs.* 3887±41 g, P=0.0044). A diet density in the reproductive phase×week interaction effect (P=0.0172) showed that body weights of these 2 groups were similar during gestation, but during the lactation period does fed RD were heavier than those fed LD in the reproductive phase (Figure 2).

Pregnancy rate

Feeding LD in the rearing phase decreased pregnancy rate both at first mating and over all mating attempts (Table 4), whereas diet density in the reproductive phase did not affect pregnancy rates. Additionally, no effects (P>0.05) of the interaction of diet density in the rearing phase×diet density in the reproductive phase were detected on pregnancy rate at first mating or overall mating attempts (Table 4).

Embryo implantation rate and carcass composition of does

Twenty-three does slaughtered on day 12 of pregnancy were used for the determination of embryo implantation rates and carcass composition (Table 3). Due to the lower number of pregnant does in the group fed LD in rearing and reproductive phases, only five, instead of six does from this group were slaughtered on day 12 of gestation (Table 3).

The does fed LD in the rearing phase were lighter at slaughter and produced lighter carcasses (Table 5). No effects (P>0.05) of diet density were observed on the weights of the reproductive tract, empty gastrointestinal tract, scapular or inguinal fat depots, despite the difference in body weight. Perirenal fat, however, was lighter in does fed LD in the

Table 4: Pregnancy rate according to diet density in rearing and reproductive phases.

	Diet density				*P*-value		
	RD rear.		LD rear.				
Trait	RD reprod.	LD reprod.	RD reprod.	LD reprod.	Rear.	Reprod.	Rear.×Reprod.
Pregnancy rate (%)							
At first mating	62.5 (15/24)	75.0 (18/24)	39.1 (9/23)	47.8 (11/23)	0.0097	0.2392	0.9696
Overall attempts	87.5 (21/24)	95.8 (23/24)	82.6 (19/23)	69.6 (16/23)	0.0448	0.7115	0.2217

RD: reference diet; LD: low density diet; Rear.: rearing phase, Reprod.: reproductive phase.
In brackets, number of pregnant does/number of mated does.

rearing phase (Table 5). No effects (*P*>0.05) of the interaction of diet density in the rearing phase x diet density in the reproductive phase were found on carcass composition of does (Table 5).

No effects (*P*>0.05) of the interaction of diet density in the rearing phase×diet density in the reproductive phase, or of any of these factors alone, were detected on embryo implantation rate (Table 5). Four out of 6 does fed RD in rearing phase and LD in reproductive phase showed at least one degenerated embryo at slaughter on day 12 of pregnancy, whereas only one doe from each of the other 3 groups presented at least one degenerated embryo.

Litter performance

The number of litters born and weaned according to diet density in the rearing and reproductive phases is presented in Table 3. At 21 d of age, the number of live litters was the same as at weaning. Five whole litters were lost between birth and weaning, from which 3 belonged to the does fed RD in both rearing and reproductive phases and 2 were from does fed LD in both growth and reproductive phases.

No effect (*P*>0.05) of diet density fed during either rearing or reproductive phases or of the interaction between these 2 effects was found on total number born or number born alive (Table 6). However, effects of the interaction of diet density fed in the rearing phase×diet density fed in the reproductive phase were found on litter size at 21 and at 35 d of age (Table 6).

Decomposing the effect of density fed during rearing phase, within each density fed in reproductive phase, revealed that when RD was fed in the reproductive phase, the diet fed in rearing phase affected litter size at 21 and at 35 d of age. Thus, when LD was fed in the rearing phase litter size increased at 21 and 35 d, compared to when RD was fed in the rearing phase (Table 6). In contrast, when LD was fed in the reproductive phase, no effect of the diet density in the rearing phase was detected on litter size (Table 6).

Table 5: Least-squares means (standard errors) for carcass composition traits and embryo implantation rate on day 12 of gestation, according to diet density in rearing and reproductive phases.

	Diet density				*P*-value		
	RD rear.		LD rear.				
Trait	RD reprod.	LD reprod.	RD reprod.	LD reprod.	Rear.	Reprod.	Rear.×Reprod
Live weight (g)	4142 (96)	4008 (96)	3752 (96)	3907 (105)	0.0220	0.9177	0.1578
Carcass (g)	2146 (65)	2128 (65)	1916 (65)	2313 (71)	0.0187	0.5594	0.3971
ERT (g)	45.8 (4.6)	37.5 (4.6)	38.6 (4.6)	45.1 (5.0)	0.9588	0.8470	0.1338
EGT (g)	302.0 (12.8)	295.3 (12.8)	274.4 (12.8)	270.8 (14.1)	0.0636	0.7018	0.9083
Scapular fat (g)	25.4 (3.9)	22.9 (3.9)	24.4 (3.9)	21.2 (4.3)	0.7394	0.4884	0.9412
Inguinal fat (g)	21.7 (4.0)	27.0 (4.0)	22.7 (4.0)	19.3 (4.4)	0.4296	0.8248	0.2992
Perirenal fat (g)	113.8 (13.4)	90.3 (13.4)	77.9 (13.4)	67.8 (14.8)	0.0481	0.2393	0.6337
EIR (%)	86.2 (10.1)	56.9 (9.2)	78.0 (9.2)	84.3 (10.1)	0.3330	0.2520	0.0816

RD: reference diet; LD: low density diet; Rear.: rearing phase, Reprod.: reproductive phase; ERT: entire reproductive tract; EGT: Empty gastrointestinal tract; EIR: Embryo implantation rate.

Table 6: Least-squares means (standard-errors) of total number born, number born alive and litter size at 21 and 35 d of age, according to diet density in the rearing and reproductive phases.

	Diet density				*P*-value		
	RD rear.		LD rear.				
Trait	RD reprod.	LD reprod.	RD reprod.	LD reprod.	Rear.	Reprod.	Rear.×Reprod
Total number born	7.53 (0.71)	6.82 (0.67)	7.62 (0.76)	6.48 (0.87)	0.8741	0.2283	0.7766
Number born alive	6.86 (0.80)	6.35 (0.75)	7.31 (0.86)	5.89 (0.99)	0.9945	0.2634	0.5973
Litter size at 21 d	3.99 (0.72)[a]	5.58 (0.68)	6.32 (0.77)[b]	4.37 (0.88)	0.4681	0.8137	0.0252
Litter size at 35 d	3.93 (0.71)[c]	5.41 (0.66)	6.16 (0.76)[d]	4.18 (0.87)	0.5053	0.7403	0.0259

RD: reference diet; LD: low density diet; Rear.: rearing phase, Reprod.: reproductive phase.
[a,b] P=0.0320; [c,d] P=0.0361.

Diet density in the rearing phase had no effect (P=0.6266) on litter weight, although diet density in the reproductive phase influenced total litter (P=0.0023) and litter mean weights (P<0.0001), as expected. Nevertheless, a diet density in the reproductive phase×age interaction effect was also found on total litter (P=0.0100) and mean litter (P<0.0001) weights. Whereas no difference between LD and RD was detected on litter and kit mean weights at birth or at 21 d of age, litters were lighter and kit mean weight was also lower at weaning (on day 35) when LD was fed in the reproductive phase (Table 7).

DISCUSSION

The experiment was designed to evaluate the effects of diet density for young rabbit does on their performance in the first reproductive cycle. To this end, a reference or a low density diet were fed in the rearing (from 70 to 142 d of age) and reproductive phases (from mating at 142 d of age up to weaning of the first litter) to 96 Botucatu does under natural mating in a factorial design.

A previous study on the performance of young does in the rearing phase showed that those fed LD had higher intake and lower weight gain and body weight than those fed RD (Teixeira *et al.,* 2013). Therefore, it was expected that the does fed LD in the rearing phase would eat more in the reproductive phase, due to increased intake capacity (Xiccato *et al.,* 1999; Pascual *et al.,* 2002). The results, however, contradicted this expectation. We also anticipated that the does fed LD in the reproductive phase would eat more during this phase, in an attempt to meet their energy requirements. The results, once again, contradicted this expectation. Apparently, the higher ADF content of LD limited intake physically during the reproductive phase. In fact, Cheeke (1987) observed that the ability to increase intake to meet energy requirements is present when diets contain from 9.2 to 13.4 MJ/kg of DE, but the LD diet in this study was formulated to contain only 8.4 MJ/kg ED.

Table 7: Least-squares means (standard errors) for litter and kit mean weights according to diet density in the reproductive phase×litter age interaction.

	Diet density in reproductive phase		
Litter age	RD	LD	*P*-value
At birth			
Total litter weight (g)	387 (177)	364 (193)	0.9313
Litter mean weight (g)	56.7 (21.0)	62.2 (22.8)	0.8616
Day 21			
Total litter weight (g)	1790 (187)	1507 (201)	0.3039
Litter mean weight (g)	353.2 (22.1)	296.3 (23.7)	0.0820
Day 35			
Total litter weight (g)	4733 (187)	3582 (201)	<0.0001
Litter mean weight (g)	912.5 (12.1)	703.4 (23.7)	<0.0001

RD: Reference diet; LD: Low density diet.

Litter size has an impact on maternal feed intake during lactation; consequently, several authors have used litter standardisation at birth to evaluate maternal feed intake during lactation (Nizza *et al.* 1997, Xiccato *et al.* 1999). To account for this effect, we used litter size at weaning as a covariate in the model of analyses for maternal feed intake. The covariate effect was significant, as expected, but neither diet density in rearing and reproductive phases nor the interaction of these two effects influenced feed intake of does during the reproductive phase.

During lactation, rabbit does have a higher energy requirement, which explains higher intake after parturition, compared to during gestation (Fortun-Lamothe, 2006). Furthermore, the kits began to eat solid feed when they were approximately 16 to 18 d old (Gidenne *et al.,* 2010), thus increasing total intake at the end of lactation.

The does fed RD in the rearing phase were heavier at mating. This resulted from the higher growth rate observed in the RD-fed animals prior to mating and from the fact that all does were first presented to a male for mating at a constant age (142 d) (Teixeira *et al.,* 2013). The mature body weight of Botucatu does is approximately 4500 g. Considering that they should reach at least 80% of mature body weight for mating (Lebas *et al.,* 1997), even the does fed LD during the rearing phase were, on average, very close to this minimum body weight (3574±47 g, corresponding to 79.4%). However, as does fed LD in the rearing phase ate more during the rearing phase, the content of their gastrointestinal tract may have contributed more to body weight at mating.

During the pre-partum, rabbit does undergo a positive energy balance and gain weight (Fortun-Lamothe, 2006), which accounts for their increased body weight observed in this period. However, the does fed LD in the rearing phase remained lighter during the first gestation than those fed RD in that phase. At parturition, the weight difference between the 2 groups disappeared, mainly because the does fed RD in the rearing phase, despite being heavier, had a sharper decrease in body weight close to and right after kindling. It is hypothesised that the does fed the high fibre diet (LD) during rearing may have presented a lower mobilisation of lipids around parturition, compared to the does fed a standard diet (RD) during the same period (Rebollar *et al.,* 2011).

However, as lactation progressed, the does that received LD during the rearing phase became lighter, and lost weight from mid-lactation and on, presumably due to the mobilisation of body reserves. This finding contrasts with those from previous reports (Xiccato *et al.,* 1999; Pascual *et al.,* 2002), probably due to the lower diet density supplied in the present study.

The diet fed in the reproductive phase did not affect the weight of does during gestation. Thus, the groups that received LD and RD presented similar body weights from mating to kindling. During lactation, however, LD-fed does became lighter and the difference between the 2 groups increased as lactation progressed. The does fed LD during the reproductive phase began losing weight after mid-lactation and continued up to weaning, presumably due to mobilisation of body reserves for lactation caused by a persistent energy deficit (Fortun-Lamothe, 2006).

Diet density fed in the reproductive phase did not exert an effect on embryo implantation rate and on doe's carcass composition on day 12 of gestation. This can be attributed to the short time periods –7 to 8 d to implantation and 12 days to slaughter– as rearing diets were changed to reproduction diets only on the day of mating. However, the density of diet fed in the rearing phase affected body weight and carcass composition on day 12 of pregnancy to some extent.

Rabbit does fed LD in the rearing phase had lower body, carcass, and perirenal fat weights on day 12 of gestation, but the weight of the reproductive tract was unchanged, compared to does fed RD in the rearing phase. Perirenal fat weight or thickness were indicated as suitable measurements of body fat reserves in rabbit does (Pascual *et al.,* 2000, 2004). The above results, therefore, suggest that feeding a higher diet density in the rearing phase increased body weight mainly by increasing the amount of muscle and body fat reserves, but not the relative weight of the reproductive organs. Analogously, young does that were fed *ad libitum* a standard diet were heavier and showed more fat content 5 d after AI than those fed a restricted amount of the same diet (Rommers *et al.,* 2001). Nevertheless, in contrast with the present study, quantitative feed restriction in the rearing phase decreased the weight of full uterine horns.

Feeding LD in the rearing phase had a negative impact on pregnancy rate, both at first mating and over all mating attempts, but did not affect embryo implantation rate. Therefore, being heavier and fatter at mating increased the chance of becoming pregnant and giving birth to a litter, likely due to improved ovarian follicular development and higher ovulation rate (Rommers *et al.,* 2001). Likewise, quantitative feed restriction, imposed on rearing does from 11 wk of age until one week before the first AI, retarded puberty and depressed fertility, despite the fact that mating of restricted females was delayed by a week relative to controls (Rebollar *et al.,* 2011). Furthermore, increasing energy intake a few days before mating has been recommended to improve male acceptance and conception rate (Fortun-Lamothe, 1998).

Changing diet density of young does from LD in the rearing phase to RD in the reproductive phase, or vice-versa, seemed to have favoured litter survival. In these 2 groups, no deaths of entire litters were recorded. In the other 2 groups, at least 2 entire litters were lost. Diet density in rearing and reproductive phases did not influence number born alive or mortality at birth, but an interaction of these 2 factors affected litter sizes at 21 and 35 d of age, as well as cumulative mortality rates up to 21 d and up to weaning (data not shown).

For the does that were fed LD in the reproductive phase, the diet fed in the rearing phase had no effect on litter size or on litter mortality. This can be credited to the limiting effect of the LD diet, whose DE and protein content were too low to support lactation and litter growth. In contrast, the does that received RD in the reproductive phase produced larger litters at 21 and 35 d, only if they had been fed LD in the rearing phase. Otherwise, they produced smaller litters at 21 and 35 d due to an increased mortality rate from birth to weaning. A possible explanation for this deleterious effect is that the does that were fed RD in the rearing and reproductive phases were fatter and, due to fat infiltrated in the mammary glands, they did not produce enough milk for their offspring. Another possibility was that the does that received RD in the reproductive phase had a sharper decrease in body weight in the peri-partum and this mobilisation of body reserves had a negative impact on lactation later on.

This finding supports that of Nizza *et al.* (1997), who found larger litters at weaning in does that received a fibrous diet during rearing. However, they reported that those does ate more during reproduction than the does fed a standard diet during rearing. This was not observed here. What we observed was that the weight loss at parturition was more pronounced in does fed a higher density diet in the rearing phase. This may have reflected a higher mobilisation of body reserves (Rebollar *et al.,* 2011), which impacted milk production and, consequently, kits' survival and welfare later on. Low mortality rate is the most important welfare indicator in rabbit production (Hoy and Verga, 2006). A feed restriction programme applied from 5 to 10 wk of age, followed by a recovery period, showed an analogous effect, increasing litter size at weaning in the first reproductive cycle of New Zealand White does, compared to *ad libitum* fed does (Rommers *et al.,* 2004). In that case too, higher feed intake and lower weight loss during the first gestation was reported for does submitted to feed restriction in the rearing phase.

Feeding RD in the reproductive phase increased total and mean litter weights at 35 d of age, probably due to the combined effect of a richer diet on milk production and on the growth rate of kits as they begin to eat solid feed. This result agrees with the data conveyed in previous reports (Nizza *et al.* 1997, Santos *et al.* 2004).

CONCLUSION

Feeding young does a low-density diet during the rearing phase and a standard diet during the reproductive phase is the best alternative to improve reproductive performance in the first parity. These does were lighter at mating and showed lower pregnancy rate, but they weaned larger litters. Although they lost less weight around parturition, feed intake in the reproductive phase was unaffected by rearing diet. In order to improve male receptivity and pregnancy rate, when using a low density rearing diet, anticipating the change to a standard diet, at least a week before mating, is advisable. If AI were used, the need to anticipate the change to a standard diet would be even stronger, as all does are inseminated on the same day, and those that are not fertilised will stay open for at least 42 d, until the next insemination cycle occurs. A low density diet limits reproduction and litter performance and cannot be recommended for the reproductive phase.

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