EFFECT OF REPRODUCTIVE RHYTHM AND LITTER WEANING AGE ON THE PERFORMANCE AND BODY ENERGY BALANCE OF RABBIT DOES

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ABSTRACT

One hundred twenty multiparous does were used. The does were synchronized to have parturition the same day (initial kindling). The trial lasted until the successive (final) kindling. At initial kindling, 22 does were selected for initial comparative slaughter. The remaining does were assigned to three reproductive rhythms, being mated 2 days *post-partum* (R2), 11 d *pp* (R11) or 26 d *pp* (R26). Within each rhythm, the does were further divided into two groups, whose litters were weaned at 21 (S21) or 25 (S25) d of age. A total of fifty-four does were pregnant and were slaughtered soon after the final kindling. *Effect of reproductive rhythm*. When increasing the kindling to mating interval, total milk production increased (5590 to 6065 g for R2 and R26 rhythms; P=0.05). Voluntary feed intake was not affected during lactation (364 g/d on average), but decreased during the dry period (182 to 169 g/d; P=0.05) and throughout the entire experiment (299 to 249 g/d; P<0.01) according to reproductive rhythm. At the final kindling, the number of kits born per litter was lower in does submitted to R11 rhythm (P<0.01). When increasing the kindling to mating interval, doe body water concentration decreased, while fat and energy increased (P<0.001) and a higher EB gain was recorded (from -123 to -4 to 97 g, P<0.001). As a consequence, body protein, fat and energy balances moved from negative values to equilibrium as reproductive rhythm became extensive (energy balance: -14.4%, -1.8% and +0.5% of the initial body content in R2, R11 and R26 does, respectively; P<0.001). Blood leptin concentration at 28 d after kindling was higher in R26 does (P<0.01), indicating higher body fat recovery due to lack of pregnancy. *Effect of weaning age*. Daily feed intake during the entire experiment was significantly lower in W21 does due to the longer dry period. At the final kindling, increasing weaning age from 21 to 25 d, both the number of kits born alive per litter (from 7.3 to 9.8; P=0.02) and doe body water concentration increased, while body fat and energy decreased (P<0.05). Therefore, W21 does showed an energy balance near equilibrium (-2.6%) while W25 does had negative fat (-14.1%) and energy (-7.9%) balances (P=0.08). Lower blood leptin concentration was recorded in W21 than W25 does (1.87 *vs*. 2.76 ng/ml, P=0.03).

Key words: reproductive rhythm, weaning age, milk production, energy balance, leptin

INTRODUCTION

Rabbit does are susceptible to intense body energy deficit during lactation, especially highly-productive commercial hybrids whose voluntary feed intake is often insufficient to fulfil requirements for lactation and concurrent pregnancy (PARTRIDGE *et al*., 1986a; PARIGI BINI and XICCATO, 1998; PASCUAL *et al*., 2000; XICCATO *et al*., 2004). As a result, young does frequently experience poor fertility rates (THEU-CLÉMENT and ROUSTAN, 1992). Although the causal link between nutrition and fertility is well known, precisely how nutritive resources act on reproduction remains unclear. Previous studies on nutritional strategies to stimulate rabbit energy intake and improve body condition have not yielded appreciable results (XICCATO, 1996; FORTUN LAMOTHE, 2003; PASCUAL *et al*., 2003). In contrast, management strategies intended to either prolong the energy recovery period by using less intensive reproductive rhythms or reduce the loss of energy during lactation by decreasing litter weaning age have been shown to be more effective (PARIGI BINI *et al*., 1996; XICCATO *et al*., 2004). Leptin is probably an important component in the long-term regulation of body weight and acts as a circulating hormone by adjusting mobilisation of energy stores through modulation of hypothalamic centres (MORASH *et al*. 1999). Leptin activity is probably modulated by other metabolic hormones, such as insulin-like growth factor (IGF-I). This study was carried out to establish how different reproductive rhythms and litter weaning ages affect multiparous doe voluntary feed intake, reproductive and lactation performance, body composition and energy reserves, and the blood concentration of two key metabolic hormones, leptin and IGF-I.

MATERIAL AND METHODS

One hundred twenty pregnant multiparous does from a Grimaud hybrid maternal line previously submitted to a 59-d kindling to kindling interval were used. The does were synchronized to kit on the same day (initial kindling) and the trial lasted until the successive (final) kindling. At the initial kindling, two does aborted and 22 does were slaughtered to estimate the initial empty body (EB) composition of the remaining does according to comparative slaughter technique (PARIGI BINI and XICCATO, 1998). The remaining 96 does were divided in three homogeneous groups and mated 2 d *post partum* (R2), 11 d *pp* (R11) and 26 d *pp* (R26), respectively. Within each reproductive rhythm, the does were further divided into two groups whose litters were weaned at 21 (W21) or 25 d of age (W25). During the trial, six does died or were excluded due to severe health problems. Only 54 does got pregnant and were slaughtered soon after final kindling: 17 does of R2 rhythm (11 of W21 and 6 of W25), 12 does of R11 rhythm (6 and 6), and 25 does of R26 rhythm (13 and 12). After initial kindling, the does were allowed to suckle their litters, standardized to ten kits, once daily for 10 min. Milk yield was measured daily by weighing the doe before and after suckling. Doe feed intake and live weight were measured daily throughout the trial. The does were fed *ad libitum* a lactation diet (CP: 19.1% DM; DE: 12.6 MJ/kg DM). Forty-eight does (8 per treatment) were randomly selected at the beginning of the experiment to collect post adsorptive blood samples by puncture of the marginal ear vein. Blood sampling was repeated on the same rabbits at 2, 15 and 28 d after initial kindling and soon after the final kindling.

Immediately upon collection, blood samples were centrifuged at 3000 *g* and plasma was stored at –20°C until assayed for leptin and IGF-I. Plasma leptin concentrations were determined by double antibody RIA (Linco Research Inc., St. Charles, MO, USA). Plasma levels of total IGF-I were assayed by an IRMA procedure (Active Non-Extraction IGF-I IRMA kit). The doe empty bodies were freeze-dried and analysed by AOAC (1990) methods. The energy and chemical balances of the 54 pregnant does were calculated on the basis of the difference between their EB composition at final kindling and their initial EB composition estimated on the initial slaughter group. The GLM procedure (SAS, 1991) was used for two-way analysis of variance (3 reproductive rhythms by 2 weaning ages with interaction). The Bonferroni "t" test was used to compare means by reproductive rhythms. No significant interaction (reproductive rhythm x weaning age) was recorded on doe performance and body balance.

RESULTS AND DISCUSSION

Effect of reproductive rhythm Initial and final live weight (LW) were not influenced by reproductive rhythm (Table 1), while total milk production increased (P=0.05) as the rhythm became extensive, due to the shorter overlapping of pregnancy and lactation. A sharp reduction of milk production in does under intensive reproductive rhythms has been associated to the competition between nutritional requirements for lactation and pregnancy caused by the exponential development of fetuses (PARTRIDGE *et al*., 1986b).

Table 1. Lactation and reproductive performance.

	Reproductive rhythm				Weaning age			
	R2	R11	R26	Prob.	W21	W25	Prob.	RSD
Rabbits	17	12	25		31	23		
LW at initial kindling (g)	4079	4118	4032	n.s.	4062	4090	n.s.	281
LW at final kindling (g)	3980	4096	4118	n.s.	4028	4101	n.s.	295
Total milk production (g)	5590^{a}	5913^{ab}	6065^{b}	0.05	5417	6296	<0.001	581
Food intake (g/d):								
- during lactation	353	352	363	n.s.	358	354	n.s.	33
- during dry period	182^{b}	181^{b}	169^{a}	0.05	179	176	n.s.	18
- from initial to final kindling	299^{C}	273^{B}	249^{A}	<0.001	267	280	0.04	21
No. kits born per litter	9.4^{AB}	7.9^{A}	11.4^{B}	<0.01	9.2	10.0	n.s.	3.0
No. kits born alive per litter	9.1	7.1	9.2	n.s.	7.4	9.6	0.03	3.4
Fertility rate (%)[1]	56.7^{a}	41.4^{a}	80.6^{b}	0.02	67.4	52.3	0.09	

[1]Calculated on the initial number of does excluding died or discarded does. Probability of Chi-square test.

Daily feed intake during lactation was similar among rhythms, while lower values were recorded both in the dry period (P=0.05) and in the entire experiment (P<0.001) in R26 does due to the different length of the dry period in the three groups (10, 19 and 34 d for R2, R11 and R26 rhythms). As reported by XICCATO *et al*. (2004), does maintain a high ingestion level for 3-4 d soon after early weaning and reach lower and stable ingestion within one week after weaning. Therefore, the shorter the dry period is (as with intensive rhythm), the higher the daily post-weaning feed intake is. Live and EB weight gain

increased linearly (P<0.01) from negative value in R2 does to positive values in R26 does (Table 2). Protein, fat and energy balances between initial and final kindling were close to equilibrium in R11 and R26 does but negative in R2 does. The main reason for increasing the kindling to kindling interval is to prolong the dry period, thereby increasing the recovery of body energy, as suggested by PARIGI BINI *et al*. (1996) who observed lower body energy deficit between first and second kindling in does mated 28 d *pp* (-15%) compared to does mated 12 d *pp* (-26%). In our study, body energy balance was negative in multiparous does submitted to intensive reproductive rhythm but positive in does submitted to semi-intensive and extensive rhythms. The variation of body energy balance might also explain the poorer fertility and prolificacy of R2 and R11 does in comparison with R26 does, thereby confirming the link between doe body condition and reproductive efficiency.

Table 2. Doe weight gain and balances of body chemical constituents and energy between initial and final kindling.

	Reproductive rhythm				Weaning age			
	R2	R11	R26	Prob.	W21	W25	Prob.	RSD
Live weight gain (g)	-99^{A}	-22^{AB}	86^{B}	<0.01	-34	11	n.s.	177
Gut content gain (g)	24	-18	-11	n.s.	-27	24	0.05	87
Empty body gain (g)	-123^{A}	-4^{AB}	97^{B}	<0.001	-7	-13	n.s.	142
Water balance (%)[a]	-0.1	-0.1	3.3	0.07	0.5	1.7	n.s.	4.9
Protein balance (%)[a]	-4.1^{A}	-0.4^{B}	0.9^{B}	<0.01	-1.2	-1.3	n.s.	4.4
Fat balance (%)[a]	-26.3^{A}	-1.6^{B}	1.4^{B}	<0.01	-3.6	-14.1	n.s.	23.7
Energy balance (%)[a]	-14.4^{A}	-1.8^{B}	0.5^{B}	<0.001	-2.6	-7.9	0.08	10.1

[a] Percentage variation of the empty body composition at initial kindling

Effect of weaning age Doe live weight at final kindling was not affected by weaning age, while the different lactation length obviously influenced total milk production (Table 1). Daily feed intake was similar in the two weaning groups both during lactation and dry periods but higher in W25 does if the entire experiment is considered (P=0.04) due to the shorter length of the dry period. When weaning age increased from 21 to 25 d, neither live weight nor empty body gain were affected but gut content gain increased (P=0.05) (Table 2). Body energy balance was always fairly negative but tended to be more pronounced (P=0.08) in W25 does and ascribed to higher fat losses. Increasing dry period length by early weaning therefore did not permit a complete recovery of body energy reserves due to the substantial decrease of feed intake after weaning. The persistence of a body energy deficit was observed also when weaning age was reduced from 32 to 21 d as a result of the marked reduction in daily DE intake (around -50%) during the dry period compared to the lactation period (XICCATO *et al*., 2004).

Blood metabolic hormones On day 2 *pp*, the mean blood concentration of leptin was 1.6 ng/ml with no difference between groups because the does were in the same physiological condition. At 15 d, leptin rose to 2.2 ng/ml on average regardless of the reproductive rhythm. In contrast, at 28 d, R26 does showed higher leptin levels (3.2 ng/ml; P<0.01) than R2 and R11 does (1.7 and 2.0 ng/ml, respectively), suggesting that the former were recovering greater fat stores than the latter probably due to the absence

of pregnancy. Interestingly enough however, in the R26 group, W25 does showed leptin levels almost double those of W21 does, thereby suggesting a weaning age effect with no clear explanation. After final kindling, leptin concentrations dropped to 1.3 ng/ml on average with no difference among groups. These low levels suggest that body energy stores were depleted by pregnancy. IGF-I concentrations in blood were also affected by reproductive rhythm, with the highest values in R11 does at 28 d (P=0.03) and in R26 does at final kindling (P=0.09). Although IGF-I concentrations were found to be positively linked to adequate nutrient intake in young growing rabbits (ROMMERS *et al.*, 2002), our study suggests that the level of this hormone might also depend on other factors such as the reproductive phase.

Table 3. Blood leptin and IGF-I concentrations at different reproductive phases.

	Reproductive rhythm				Weaning age			
	R2	R11	R26	Prob.	W21	W25	Prob.	RSD
Rabbits, no.	11	7	12		16	14		
Leptin (ng/ml)								
- 28 d after kindling[a]	1.7^{A}	2.0^{A}	3.2^{B}	<0.01	1.9	2.8	0.03	1.0
- final kindling	1.6	1.3	1.2	n.s.	1.3	1.4	n.s.	0.5
IGF-I (ng/ml)								
- 28 d after kindling	549^{ab}	620^{b}	456^{a}	0.03	518	565	n.s.	121
- final kindling	494	514	647	0.09	571	532	n.s.	163

[a] Interaction R x W (P=0.09): see text.

CONCLUSIONS

Increasing the kindling to mating interval to 11 or 26 d enabled multiparous does submitted to early weaning to recover certain body energy reserves and reach body energy equilibrium unlike the does mated 2 d pp which had a negative balance. The early weaning of litters at 21 d of age permitted does to reduce body energy utilization for milk production and approach body equilibrium more than weaning at 25 d. Certain potentially negative effects observed on reproductive performance caused by intensive rhythms and/or very early weaning deserve further investigation. Although leptin and IGF-I blood levels did not closely reflect the changes in energy balance caused by reproductive rhythm and weaning management, these hormones may prove useful as biological markers for the *in vivo* evaluation of body energy modifications throughout the rabbit reproductive career.

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REFERENCES

AOAC 1990. *Official Methods of Analysis of the Association of Official Analytical Chemist*, 15th Edition. Assoc. Off. Analyt. Chemists, Arlington, VA, USA.

FORTUN LAMOTHE L. 2003. Bilan énergétique et gestion des réserves corporelles de la lapine: mécanisme d'action et stratégies pour améliorer la fertilité et la longévité en élevage cunicole. *Proc. 10èmes J. Rech. Cunicole, Paris, France*, 89-103.

MORASH B., LI, A., MURPHY P.R., WILKINSON M., UR, E. 1999. Leptin gene expression in the brain and pituitary gland. *Endocrinology* **140**:5995-5998.

PARIGI BINI R., XICCATO G. 1998. Energy metabolism and requirements. In: *The Nutrition of the Rabbit*. (Edit. De Blas, C., Wiseman, J.) CABI Publishing, Wallingford, UK, 103-131.

PARIGI BINI R., XICCATO G., DALLE ZOTTE A., CARAZZOLO A., CASTELLINI C., STRADAIOLI G. 1996. Effect of remating interval and diet on the performance and energy balance of rabbit does. *Proc. 6th World Rabbit Congress, Toulouse, France*, Vol. 1, 253-258.

PARTRIDGE G. G., DANIELS Y., FORDYCE R. A. 1986a. The effect of energy intake during pregnancy in doe rabbits on pup birth weight, milk output and maternal body composition change in the ensuing lactation. *J. Agric. Sci.* **107**:697-708.

PARTRIDGE G. G., LOBLEY G. E., FORDYCE R. A. 1986b. Energy and nitrogen metabolism of rabbits during pregnancy, lactation, and concurrent pregnancy and lactation. *Br. J. Nutr.* **56**:199-207.

PASCUAL J. J., CERVERA C., FERNÁNDEZ-CARMONA J. 2000. The effect of dietary fat on the performance and body composition of rabbits in their second lactation. *Anim. Feed Sci. Technol.* **86**:191-203.

PASCUAL J.J., CERVERA C., BLAS E., FERNÁNDEZ-CARMONA J. 2003. High-energy diets for reproductive rabbit does: effect of energy source. *Nutr. Abstracts Rev.* **73**: 27R-39R.

ROMMERS J., BOITI C., BRECCHIA G., ZAMPINI D., MEJERHOF R., NOORDHUIZEN J.P.T.M., KEMP, B. 2002. Long-term caloric restriction to ameliorate the fertility by redirection of metabolism in female rabbits. *Reprod. Domestic. Anim*. **37**(4): 249 (P1.113).

SAS 1991. *User's guide, Statistics, ver. 6.03*. Edit. SAS Institute Inc., Cary, NC, USA.

THEAU-CLÉMENT M., ROUSTAN A. 1992 A study on relationship between receptivity and lactation in the doe, and their influence on reproductive performance. *Proc 5th World Rabbit Congress, Corvallis, USA*, Vol. A, 412-421.

XICCATO G. 1996. Nutrition of lactating does. *Proc. 6th World Rabbit Congress, Toulouse, France*, Vol. 1, 29-47.

XICCATO G., TROCINO A., SARTORI A., QUEAQUE P.I. 2004. Effect of doe parity order and litter weaning age on the performance and body energy deficit of rabbit does. *Livest. Prod. Sci.* **85**:239-251.