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THE EFFECT OF DOE-LITTER SEPARATION ON PRODUCTION PERFORMANCE IN RABBIT DOES AND THEIR KITS

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ABSTRACT : Pannon White does were subjected to artificial insemination between 9 and 11 days subsequent to kindling (no. of AI = 931). Controlled suckling was systematically applied from 0 to 18 days post partum. In addition to the control group (group C, n = 236), the does of the three experimental groups were prevented from suckling on the day prior to insemination (48h doe-litter separation, or MLS). The following day some of the does were inseminated two hours before suckling (B-2, n = 229), some immediately after suckling (B-0, n = 234), and some two hours after suckling (B+2, n = 232). The effect of biostimulation led to only slight, non-significant improvement in receptivity (0-7 %) and fertility rate (1-5 %). Litter sizes increased by 0.74 and 0.70 kits in groups B-2 and B-0 respectively, with no increase being observed in group B+2. Nevertheless, compared with the control group, suckling applied at the time of insemination improved the productivity at birth (+ 0.9 kits born alive per insemination). The most considerable but non-significant effect of MLS was recorded at the first kindling subsequent to separation. On the day after the omission of suckling the quantity of milk produced by the does increased by 22 %; on the three subsequent days milk secretion in these does lagged behind that of the does of the control group by 33 %, 15 % and 6 % respectively. In addition, two days after the omission of suckling, the milk secreted was found to contain higher levels than previously of dry matter (by 4.2 %), fat (by 1.7 %), protein (by 2.6 %) and ash (by 0.53 %). These values later returned to levels approaching the original values. Due to the omission of one suckling the weight of suckling and growing rabbits declined by 26-34 g. No compensatory growth was observed either before or after weaning (live weight controlled at 70 days: 2102 g in the control and 2068 g for rabbits of the 3 experimental groups). As some of these results are in opposition with previous reports using hybrids, the authors conclude that the effect of such a biostimulation could vary according to the genotype and/or breeding systems (particularly with controlled or free suckling applied before and after the stimulation).

RESUME : Effet de la séparation mère-portée sur les performances de production des lapines et de leurs lapereaux. Entre 9 et 11 jours après la mise bas, des lapines Pannon White ont été soumises à l'insémination artificielle (nombre d'IA = 931). Entre 0 et 18 jours post partum la tétée a été systématiquement contrôlée. Dans les trois groupes expérimentaux, l'allaitement a été interdit le jour précédant l'insémination (séparation mère-portée de 48 heures : MLS) contrairement au groupe témoin (groupe C, n = 236). Le jour suivant cette séparation, 229 lapines ont été inséminées deux heures avant l'allaitement (groupe B-2), 234 ont été inséminées immédiatement après l'allaitement (groupe B-0) et 232 ont été inséminées 2 heures après l'allaitement (groupe B+2). La biostimulation n'a qu'un effet léger, non significatif, sur l'amélioration de la réceptivité (0 à 7 %) et sur le taux de fertilité (+1 à 5 %). Des tailles de portées supérieures de 0,71 et 0,70 nés vivants ont été enregistrées dans les groupes B-2 et B-0 respectivement, alors qu'aucune augmentation n'a été observée dans le groupe B+2. L'effet numériquement le plus important mais non significatif de la séparation mère-portée a été observé après la première mise bas suivant la séparation. Le jour suivant la restriction d'allaitement, la quantité de lait produite par lapine a augmenté de 22 % ; les trois jours suivant, la production laitière de ces lapines passent en dessous de celle des lapines du lot témoin de 33, 15 et 6 % respectivement. En même temps, deux jours après la privation d'allaitement, la matière sèche du lait excrété a augmenté de 4,2 %, la matière grasse de 1,7 %, les protéines de 2,6 % et les minéreaux de 0,53 %, Les teneurs redeviennent ensuite proches de celles de départ. La privation d'une tétée conduit à une perte de poids de 20-34g chez les lapereaux, sans compensation ultérieure avant ou après sevrage (poids contrôlé à 70 jours : 2102 g pour le témoin et 2068 g pour les 3 lots expérimentaux). Certains de ces résultats étant en opposition avec d'autres précédemment obtenus avec des lapins hybrides, les auteurs concluent que les effets d'une telle stimulation peuvent varier avec la race et/ou avec le système d'élevage, spécialement en fonction du moment où la tétée contrôlée ou libre est appliquée : avant ou après la stimulation.

INTRODUCTION

Most of the studies involving doe-litter separation (MLS), one of the various methods of biostimulation, were performed by the research team of the IRRG. The majority of these experiments involved hybrid does, suckling being free, with the exception that the does were prevented from suckling their young on the day preceding insemination on the 9th-11th day. On that day insemination took place immediately after suckling. The majority of the reports indicate that MLS exerts a beneficial effect on receptivity (+20-40 % : PAVOIS *et al.*, 1994; MAERTENS, 1998; BONANNO and ALABISO, 1999) and on fertility rate (+10-20 %: PAVOIS *et al.*, 1994; MAERTENS, 1998; ALVARIÑO *et al.*, 1998; BONANNO and ALABISO, 1999; THEAU-CLÉMENT, 1999; VIRÁG *et al.*, 1999). In some cases litter size increases (MAERTENS, 1998), while litter weight and individual weight decrease (MAERTENS, 1998; ALVARIÑO *et al.*, 1998 and 1999; BONANNO and ALABISO, 1999; THEAU-CLÉMENT, 1999).

This experiment used purebred does with a mature weight higher by 0.4-0.8 kg than the hybrids used in previous studies. Suckling was controlled from 0 to 18 days post partum, and, for the purpose of examining growth compensation capacity, the weight of the offspring at the end of the fattening period was recorded. It is a new initiative to examine the effect exerted on reproduction by the length of the interval between insemination and suckling. The influence of the omission of one suckling on

doe milk production and milk composition in the subsequent days was also recorded.

MATERIALS AND METHODS

Animals

The investigations were performed on the Pannon White does (SZENDRŐ *et al.*, 1998) kept on the experimental rabbit farm at the Kaposvár university faculty. Only primiparous and multiparous lactating does were considered in the experiment.

Accommodation

The rabbits were housed in flat-deck cages (800 x 500 mm) in a closed building. In winter, warm air was blown in via a pressure system (minimum temperature 14 °C). Large windows allowed free air exchange from spring until autumn, but on the hottest summer days the temperature in the building rose above 25 °C. Adequate lighting was ensured for at least 16 hours per day: on the shortest days supplementary fluorescent strip lighting was required, while on the longest days of summer the sunlight entering through the windows provided more than 16 hours of light in the building.

Diet and feeding

The does were fed *ad libitum* with a commercially with available feed mix (CP: 16.5 %; CF: 15.5 %; 10.3 MJ/kg DE). Water was available *ad libitum* from self-drinkers.

Insemination and care of offspring

The does were inseminated every 42 days on Fridays. The interval between kindling and AI varied from 9 to 11 days. To induce ovulation 1.5 μg GnRH analogue was administered (D-Phe 6-GnRH; Ovurelin inj. ad us. vet., Reanál), insemination with individual fresh sperm being performed simultaneously. After kindling, when litters contained more than ten kits, some were fostered to smaller litters in the same treatment group. The range of litter size after equalisation was between 7 and 10 kits. Suckling was controlled between parturition and the 18th day after kindling. The does were only able to enter the kindling box between 8:00 and 9:30 a.m. each day. The young rabbits were weaned at 35 days.

Weight recording

All litters (n = 483) were weighed at 21 days of age and all rabbits (n = 4253) were weighed individually at 42 and 70 days. A group of rabbits (n = 216) was weighed daily to monitor the effect of MLS.

The suckling rabbits were weighed in the morning before suckling. The daily milk production of the does (n = 30) was established on the basis of the difference in weight of the litter before and after suckling. Milk production was determined every 2 days between days 3 and 9, and 12 and 18 but daily between days 9 and 12. Milk samples (n = 14) were obtained by means of oxytocin (3 NE) injection followed by the extraction of milk from one of the first pair of teats through a vacuum pump till they were completely emptied on day −1, +1, +2 and +4 (day 0 was when the suckling was omitted). Prior to chemical analysis these samples were stored at -20 °C.

Chemical analysis

Immediately before chemical analysis was performed the frozen milk samples were thawed. The dry matter content of milk samples was determined by drying to constant weight at 105 °C (Hungarian Standard No. 3744-67) and fat by the Gerber method (Hungarian Standard No. 3703-78). Constant weight was generally reached at 6 h, but more time was required when the fat content was high. Total protein content was measured using the Kjel-Foss nitrogen analyser (protein equivalent = N%x6.38) (Hungarian Standard No. 6830/4-81). Ash content was determined by heating the samples to 550 °C for 8 h (Hungarian Standard No.3926/2-76).

Experimental groups

Prior to insemination for each kindling, the does were allocated at random into four groups, based on the age of each doe and the number of kits being suckled.

* The does of the control group (C) were allowed to suckle their young every day (n = 236, n = number of AI).
* The does of the experimental group (B) were prevented from suckling on the day before insemination (a Thursday in each case), and were inseminated on the following day, according to the following schedule:
  - the does of group B-2 were inseminated two hours before suckling (n = 229);
  - the does of group B-0 were inseminated at the time of suckling (n = 234);
  - the does of group B+2 were inseminated two hours after suckling (n = 232).

  (The nestboxes were opened on Friday between 8 and 10 a.m.)

Statistical analysis

Only data for the first inseminations performed after kindling (on the 9^{th}-11^{th} day) was considered. Receptivity, based on vulval turgidity and color (red or violet), and fertility rate of the groups were compared by means of the chi-square test. The comparison of mean individual weight was performed with single factor variance analysis. To analyse for the effects of litter size, the following model was applied:

$$Y_{ijk} = \mu + B_i + P_j + e_{ijk}$$

Where

Y_{ijk} = individual data
μ = overall mean
B_i = effect of treatment (C, B-2, B-0, B+2)
P_j = effect of parity (primiparous or multiparous)
e_{ijk} = residual random error

Version 7.5 of the SPSS for Windows program package was used in the evaluation.

RESULTS AND DISCUSSION

Receptivity and fertility rate

No significant difference could be ascertained between the groups with respect either to receptivity or to fertility

rate (Table 1). Receptivity changed from the control group (C) from -2.3 to 6.2%. The increase in fertility rate was only 1.1 to 5.3 %. This lack of effect of MLS is in contrast to most reports in the literature (PAVOIS *et al.*, 1994; MAERTENS, 1998; THEAU-CLÉMENT, 1999). Only CASTELLINI (1998) found no significant effect when biostimulation was applied three days before A.I.. However, all these authors systematically used free suckling. An hypothesis could be that in using controlled suckling as regular "doe-litter separation" longer separation would only slightly effect reproductive performance. Another hypothesis may be the fact that the mature weight of the Pannon White breed exceeds that of hybrids by 0.4 to 0.8 kg. The larger bodied types suffer fewer burdens due to pregnancy and the rearing of offspring, while at the same time their temperament is less lively than that of the smaller bodied breeds. Due to these factors Pannon White rabbits do not show such a degree of reaction as hybrids to the various effects and probably, to the omission of suckling.

Table 1 : The effect of doe-litter separation on doe receptivity, fertility rate and litter size

Parameter	Experimental groups				SD
	C	B-2	B-0	B+2	
No. of inseminations	236	229	234	232	
No. of kindlings	149	147	160	156	
Receptive does, %	55.9[a]	62.6[a]	53.6[a]	62.1[a]	
Fertility rate, %	63.1[a]	64.2[a]	68.4[a]	67.2[a]	
No. of young in litter					
Total	8.83[ad]	9.65[bc]	9.69[ac]	8.67[d]	3.21
born alive	8.33[ab]	9.04[a]	9.03[a]	8.10[b]	3.37

Table 2 : The effect of doe-litter separation on the reproduction parameters (according to number of kindlings)

Parameter	Experimental groups				SD
	C	B-2	B-0	B+2	
After 1st kindling					
No. of inseminations	51	56	52	53	
Number of kindlings	26	35	32	33	
Receptive does, %	32.6	41.7	43.2	35.6	
Fertility rate, %	51.0	62.5	61.5	62.3	
No. of young in litter					
Total	8.12	9.23	8.78	8.33	2.65
born alive	7.69	8.63	8.31	7.67	2.91
After 2nd kindling					
No. of inseminations	40	35	36	34	
Number of kindlings	21	20	21	20	
Receptive does, %	53.1[ab]	71.9[a]	44.1[b]	48.3[ab]	
Fertility rate, %	52.5	57.1	58.3	58.8	
No. of young in litter					
Total	9.14[ab]	9.95[ab]	10.90[a]	8.65[b]	3.00
born alive	8.52[ab]	9.10[ab]	9.90[a]	7.80[b]	3.34
After 3rd - 5th kindling					
No. of inseminations	77	70	80	76	
Number of kindlings	53	46	56	54	
Receptive does, %	62.3	64.6	54.1	61.2	
Fertility rate, %	68.8	65.7	70.0	71.1	
No. of young in litter					
Total	9.09[a]	10.57[b]	10.32[b]	8.85[a]	3.17
born alive	8.57[a]	9.93[b]	9.66[ab]	8.59[a]	3.41
After 6th kindling					
No. of inseminations	68	68	66	69	
Number of kindlings	49	46	51	49	
Receptive does, %	67.2	72.1	66.1[a]	87.7[b]	
Fertility rate, %	72.1	67.6	77.3	71.0	
No. of young in litter					
total	8.96	8.85	8.75	8.86	3.58
born alive	8.55	8.50	8.25	8.33	3.57

[a,b] Different letters in the same row indicate significant differences ($P<0.05$).

Although no effect of biostimulation could be detected in this study, after the first kindling the effect of MLS led to numerical increases in receptivity (3.0 to 10.6 % higher) and in fertility rate (10.5 to 11.5 % higher) in comparison with the does of group C (Table 2). MAERTENS (1998) and VIRÁG *et al.* (1999) reported similar results, the effect of biostimulation also having been highest after the first kindling in their investigation. Results summarised by XICCATO (1996) indicate that in primiparous does the burden of pregnancy and suckling leads to the development of a state of negative energy balance, i.e. energy deficit. This would explain the weak levels of reproductive performance observed subsequent to the first kindling. The results obtained in the present study indicate that the effect of doe-litter separation is substantial in this particular period. It has been observed that PMSG treatment is also more effective in less favourable conditions (THEAU-CLÉMENT, 1998).

Litter size

The most beneficial results on litter size were obtained in groups B-2 and B-0, while no difference was observed between groups B+2 and C (Table 1). Examination of the data taking into account how many times the does had kindled (Table 2) reveals that, up to the 6th kindling, the does of groups B-2 and B-0 in every case produced the largest litters. Only MAERTENS (1998) has shown the effect of MLS to lead to a significant improvement (+1.2) in the number of young born alive per litter. In the present study the largest difference observed between groups C and B-2 or B-0 occurred at the peak of production (between the 2nd and the 6th kindling). The number of young born alive per insemination was 5 .3, 5.8, 6.2 and 5.5 for groups C, B-2, B-0 and B+2 respectively. Compared with the control group, suckling applied at the time of insemination improved the productivity at birth (+ 0.9 born alive per insemination).

Table 3 : The effect of doe-litter separation (one-day prevention of suckling) on doe milk composition

Composition	Group mean values		Deviation from level recorded before omission of suckling
	C	B	
One day before omission of suckling			
Dry matter, %	36.1	31.9	-
Fat, %	17.1	15.5	-
Protein, %	14.4	13.1	-
Ash, %	2.09	1.91	-
One day after omission of suckling (the day of AI)			
Dry matter, %	34.6	35.7	+3.8
Fat, %	17.2	16.6	+1.1
Protein, %	13.2	15.1	+2.0
Ash, %	2.19	2.47	+0.56
2 days after omission of suckling			
Dry matter, %	35.3	36.1	+4.2
Fat, %	17.1	17.2	+1.7
Protein, %	15.1	15.7	+2.6
Ash, %	2.34	2.44	+0.53
4 days after omission of suckling			
Dry matter, %	35.4	33.9	+2.0
Fat, %	17.9	16.8	+1.3
Protein, %	13.4	13.2	+0.1
Ash, %	2.31	2.20	+0.29

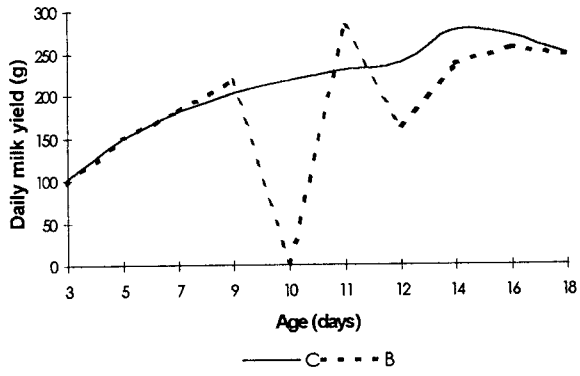

Fig. 1 : Effect of doe-litter separation on the milk production of does (The suckling was omitted on day 10 in the biostimulated group /B/)

Milk production

On the day following the MLS the does of group B produced 22 % (50 g) more milk than those of the control group (C) (Figure 1). It seems that rabbit does are not capable of secreting and/or accumulating milk in greater quantities than this. In the group of does which were not allowed to nurse for 48 hours, the mammary gland did not increase in a greater proportion than it did during the period of 24-28 hours after suckling (CALVERT *et al.* 1985). Those authors showed milk secretion to decline to slightly less than a half of its original value between 24 and 32 hours after suckling and to a tenth of this in the subsequent 8 hours.

On the three days following the slight increase observed, milk production in the does of the experimental group (B) lagged behind that of the does of group C by 33 %, 15 % and 6 % respectively (Figure 1). After that time, this gap practically closed up. These data verify that if one suckling is omitted the milk flow begins to dry up, while restarting suckling has the effect of restoring milk flow to its normal level.

Symptoms of drying up derived from the omission of one suckling are also apparent in changes in milk composition: the dry matter, fat, protein and ash content of the milk secreted showed an increase in the first two days, subsequently decreasing to approach the original levels (Table 3). Similar changes were observed on comparison of the composition of milk from does successfully inseminated immediately after kindling with that of the milk of non-pregnant does (KUSTOS *et al.*, 1996). Milk production in the does which became pregnant post partum decreased sharply after the lactation peak, and the milk produced by these does also showed more rapid changes in composition than did that of the non-pregnant does, which produced more milk. CALVERT *et al.* (1985) observed a rise in milk fat and protein content and a reduction in lactose content when 12 hours had elapsed after the omission of suckling.

Weight of suckling and growing rabbits

Due to MLS, the weight of the young rabbits was observed to decrease by an average of 23 g (12.5 %) in parallel with the milk production of the does (Table 4). In this study the weight difference between groups C and B increased slightly in absolute terms up to the point of weaning. The kits of the control group were, on average, 34 g heavier than those of group B at 28 days. The data recorded throughout the experiment verify that compensatory growth did not occur either while rabbits were still suckling or subsequent to weaning (up to the age of 70 days). No substantial change was observed in weight difference between groups C and B (Table 4). MLS results in a significant lag fluctuating between 26 and 34 g throughout the entire experimental period.

The results obtained in this study support observations that a one-day prevention of suckling leads to a decrease in weaning weight of between 2 and 10 % (PAVOIS *et al.*, 1994; MAERTENS, 1998; ALVARIÑO *et al.*, 1998 and 1999; BONANNO and ALABISO, 1999; THEAU-CLÉMENT, 1998). These data also verify that fattening rabbits will not have the capacity to make up for this shortfall after weaning. The experimental data indicate that because of MLS, rabbits take one more day to reach slaughter weight, although this is not considered a substantial delay.

Table 4 : Changes in the weight of the suckling and growing rabbits

Age	Group						Difference		Degree of significance
	C			B			g	%	
	n	mean	SD	n	mean	SD			
Experimental group (g)									
Day 1	96	60.7	8.3	120	63.6	8.5	+2.9	4.6	*
Day 7	93	120	21	114	120	21	0	0	NS
Day 9	93	166	29	114	169	29	+3	1.8	NS
Day 11	93	184	30	114	161	28	-23	12.5	***
Day 12	93	201	33	114	181	31	-20	10.0	***
Day 14	92	232	33	114	207	36	-25	10.8	***
Day 21	91	379	45	113	352	52	-26	6.9	***
Day 28	90	607	70	112	573	78	-34	5.6	**
All kits (g)									
Day 21	120^N	361	61	363^N	335	60	-26	7.2	***
Day 42	1099	1126	138	3154	1097	137	-29	2.6	***
Day 70	965	2102	259	2825	2068	267	-34	1.6	***

N = Number of litters, *P<0.05, **P<0.01, ***P<0.001

CONCLUSIONS

On the basis of the findings of this experiment and literature data relating to MLS, the hypothesis may be formed that the effect of biostimulation varies according to genotype and/or the breeding system (particularly with controlled or free suckling). Regular controlled suckling (corresponding to short doe-litter separation) could limit or suppress the positive effect of 48h doe-litter separation in increasing reproductive performance. It is considered important to examine these hypotheses in relation to the effect of genotype with different adult weight categories, or the effect of free and controlled suckling on the results of MLS.

MLS leads to changes similar to those observed when the drying up of milk flow commences. Despite a higher quantity of milk being produced the following day, milk production continues to be depressed for several days, while changes in milk composition also occur. MLS for one suckling also gives rise to a decrease of 20-34 g in the weight of suckling and growing rabbits. The rabbits do not have the capacity to compensate for this shortfall, either prior to, or subsequent to, weaning.

Knowledge of the hormonal changes some hours before and after AI would be useful to better understand the underlying physiological mechanism of the differences between the experimental (B-2, B-0, B+2) groups.

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