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Comparison of different reproduction protocols for rabbit does: effect of litter size and mating interval

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Abstract

The aim of the study was to compare different reproductive protocols based on fixed (11 days after kindling) or alternating remating intervals (1 day post-partum and post-weaning alternatively) and two litter sizes (six vs. eight kits). Weaning of kits occurred at 26 days of age. Four groups of 35 New Zealand White females were inseminated for eight consecutive cycles. Does with six kits had a higher sexual receptivity, fertility rate and a lower energy deficit. The milk production was lower but the milk suckled per kit was higher. This greater milk availability determined heavier kits till 70 days of age and a lower mortality rate. Does submitted to the alternating rhythm showed the same kindling interval as the respective fixed groups although the mating interval was 3 days longer. This rhythm in comparison with the fixed one seemed more adapted to doe physiology and improved the receptivity and the fertility, even under more critical conditions (high milk output).

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1. Introduction

The economic efficiency of a rabbitry depends mainly on the reproductive performance of the doe, which in turn is affected by their fertility and prolificacy and by the weight gain and mortality of the young rabbits. All of these traits are affected by many factors such as genetic strain, feeding, environment and management and a stable balance between them is difficult to achieve.

In Europe the introduction of cycled production

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and artificial insemination (AI) has improved the management and productivity of the doe. At the same time, the mortality rate of young rabbits (25–35%) and the health state of the doe (replacement/year more than 120%) have worsened (Facchin et al., 1993; Filleul, 1996). To avoid these problems, greater attention to better matching rabbit behaviour is needed.

The current management protocol is based on the AI of does 11 days after kindling and on the weaning of young rabbits at 28–30 days of age. This protocol is adapted to the cycled production, which required the insemination of groups of animals on fixed days of the week. However, at the time of AI the sexual receptivity is not particularly high and the use of

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synchronising hormones is often required to reach a suitable fertility rate (Castellini and Boiti, 1999). Sexual receptivity in rabbit does is aphasic and shows a peculiar trend: it is very high immediately after kindling, then decreases with a non-predictable trend; another peak appears only after weaning (Theau-Clément et al., 1990).

During the reproductive season, wild rabbits mate immediately after kindling and the nursing of kits lasts 25 days (Hudson et al., 1996). Domestic rabbits maintain this natural pattern (Bell, 1984). Hence more attention to this behaviour could improve productive performance.

Previous studies have shown that a continuous post partum (pp) rhythm increases the number of kindlings/year/doe (Cervera et al., 1993), but also the litter size, fertility rate and length of reproductive activity decrease (Parigi-Bini et al., 1989). Large litter size (Garcia and Perez, 1989) and hyperprolific strains exacerbate these negative aspects (Maertens and Okerman, 1988), and only few does can sustain a fixed pp rhythm.

The aim of this study was to investigate a reproductive protocol for rabbit does based on alternating mating interval and on reducing litter size, with early weaning.

2. Materials and methods

Four groups of 35 young New Zealand White (NZW) rabbit does each were bought by the National Association of Italian Rabbit Breeders (ANCI) that selected them from NZW populations reared in Italy. At 120 days of age the does were transferred to the experimental rabbitry of the Animal Production Department (University of Perugia). The environmental temperature ranged from +15 to +20 °C and relative humidity from 65 to 70%. The treatments were the following: fixed (F) reproductive rhythm (AI 11 days after kindling) or alternating (A) rhythm (AI 1 and 27 days after kindling, alternatively) (Fig. 1). In each treatment, at 24 h from the birth, the number of suckling kits was adjusted to six/litter (L6) or eight/litter (L8).

Young does were first inseminated at 4.5 months of age for eight consecutive AI cycles (n = 1071). Does non-pregnant for three consecutive AI were eliminated. Since the simultaneous occurrence of

lactation—gestation during the first lactation is particularly severe (Xiccato, 1996), all the primiparous does were inseminated 1 day after weaning. Palpation was done at the 12th day from AI. The kits were always weaned at 26 days. Does of the four groups were submitted to the same treatments during all of the trial.

At 16 days of lactation a feeder was put inside the nest box to favour early feed ingestion by the kits.

Diet formulation and analytical data are presented in Table 1. Diet L was administered ad libitum to rabbit does during lactation whereas diet W, specific for early weaning (Xiccato et al., 2000), was administered to young rabbits from 16 to 42 days of age. Chemical analysis was done according to AOAC (1995) procedures and digestible energy (DE) was estimated according to Maertens et al. (1988).

DE requirement was estimated according to the equations developed by Parigi-Bini and Xiccato (1998) and DE deficit was estimated as the difference between requirement and ingestion.

No additives (coccidiostatic, antibiotic or sulfamides) were administered during the trial.

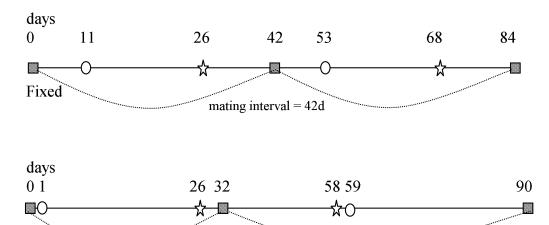
Kits were nursed once a day and milk output was determined by weighing the doe immediately before and after the milking. During lactation, feed consumption of the does was recorded daily.

Sexual receptivity was evaluated by analysing the vulva colour and its turgescency and two classes were established: receptive (red or red-violet and turgescent) and non-receptive (white and no-turgescent vulva).

AI was performed in the morning immediately after milking by inseminating 0.5 ml of diluted fresh semen, containing about 10 million sperms (Castellini and Lattaioli, 1999). No hormonal synchronisation was done. Ovulation was induced by inoculating 10 µg of GnRH (Lutal-Hoecst).

The fertility rate was estimated as the percentage of kindlings/AI. Litter size was recorded at kindling, at 45 and 70 days of age.

The following indexes of efficiency were calculated: the global productivity, as the number and the weight of rabbits sold/year/doe, and the efficiency of the system as the percentage of the actual production with respect to the theoretical one (theoretical production considering fertility rate = 100 and mortality of the young rabbits = 0).



mean mating interval = 45d



Alternating

Fig. 1. Scheme of reproductive protocol.

Statistical analysis was done with a linear model (SAS/GLM, 1990) considering the interactive effect of litter size and rhythm of insemination. Since the aim of the study was to compare the global efficiency of the experimental protocols, the effect of the physiological phase of the does (lactating or not) and the day of insemination (1, 11, 27 or multiple) were not analysed; the kindling order was also absorbed.

Non-parametric variables (sexual receptivity, fertility rate and percentage of doe replacement) were analysed with a chi-square test (procedure CAT-MOD/SAS).

3. Results

The average body weight of does during lactation was lower (P < 0.05) when females suckled a higher number of kits (Table 2).

This was particularly notable when females were

submitted to the fixed reproductive rhythm, although this group gained more body weight (+ 8.3%) from kindling until weaning.

DE intake, energy requirement, energy deficit and milk production were higher for the L8 group regardless of the reproductive rhythm.

The daily weight of does is shown in Fig. 2. The L8 fixed group was lighter than both L6 groups. This trend was highly significant at kindling, successively the differences decreased and leaner does gained more weight. At 12 days (the day after AI) the fixed groups showed a significant reduction in body weight and energy intake (Figs. 2 and 3).

During lactation the digestible energy intake was higher in L8 but the higher milk production (Fig. 4) increased the energy requirement and the energy deficit.

Reproductive performance of the does (Table 3) was affected by the treatments: the fixed reproductive rhythm with eight kits/litter decreased the sexual receptivity (P < 0.05), the fertility rate (P < 0.05)

Table 1 Formulation (%), chemical composition (%) and nutritional value of diets

Ingredients		Diet L	Diet W
Barley meal		21.0	8.0
Wheat bran		14.5	25.0
Dehydrated alfalfa meal		37.0	30.0
Soybean meal 44% crude protein		14.0	6.0
Soybean extruded		7.0	_
Sunflower meal 30% crude protein		-	8.0
Fat		1.0	2.0
Skimmed milk powder		-	2.0
Beet pulp		-	15.0
Beet molasses		3.0	2.0
Calcium diphosphate		0.5	0.52
Vitamin-mineral premix ^a		0.85	0.30
Limestone		0.50	0.50
Salt		0.50	0.50
DL-Methionine		0.05	0.08
HCl Lysine		0.10	0.10
Crude protein		18.75	16.00
Ether extract		4.80	3.97
Crude fiber		14.70	16.44
Ash		9.17	8.20
NDF		29.22	31.59
ADF		18.53	18.36
ADL		3.30	3.99
Cellulose		14.53	13.77
Hemicelluloses		10.69	13.23
Estimated digestible energy ^b	MJ/kg	10.92	10.45

 $^{^{\}rm a}$ Added per kg: vit. A 11 000 U.I.; vit. D $_{\rm 3}$ 2000 U.I.; vit. B $_{\rm 1}$ 2.5 mg; vit. B $_{\rm 2}$ 4 mg; vit. B $_{\rm 6}$ 1.25 mg; vit. B $_{\rm 12}$ 0.01 mg; vit. E 25 mg; biotine 0.06 mg; vit. K 2.5 mg; niacine 15 mg; folic acid 0.30 mg; p-pantotenic acid 10 mg; coline 600 mg; Mn 60 mg; Cu 3 mg; Fe 50 mg; Zn 15 mg; I 0.5 mg; Co 0.5 mg.

0.05) and consequently increased the kindling interval (P < 0.01). Kindling interval was also negatively affected by litter size under the alternate rhythm.

Does submitted to the alternate rhythm showed the same kindling interval as the respective fixed groups although the re-mating interval was 3 days longer.

The mortality rate and the number of weaned rabbits were higher in L8 groups (Table 3).

The performance of young rabbits is summarised in Table 4. The smaller litters had a higher live weight at birth; later, the higher individual milk intake resulted in heavier and more viable kits.

The indexes of global productivity (Table 5) showed that the L8 group under the alternate reproductive rhythm produced the highest number and weight of rabbits sold/year/doe.

The total mortality (Table 4), the doe replacement and the non-pregnant does for three consecutive AI were higher in the L8 groups, which also showed the worst production efficiency (Table 5).

4. Discussion

The weight of does during lactation was higher when only six kits/litter were suckled. Although the does of the FL8 group gained more body weight (Fig. 2), this gain probably does not correspond to a net body gain (Xiccato, 1996), but was due to the higher feed intake (about +4%) and therefore to the higher weight of the digestive tract. Consequently, the energy deficit was lower in L6 does and this

Table 2 Effect of mating interval and litter size on the weight and some productive traits of rabbit does (n = 770)

Mating interval Litter size		Fixed		Alternating		MSE
		L6	L8	L6	L8	
Weight of doe during lactation	alg	4122 ^{bc}	4049 ^a	4167°	4102 ^b	375
Weight of doe at kindling	g	3900 ^b	3803°	3942 ^b	3904 ^b	435
Weight of doe at weaning	g	4204 ^b	4119 ^a	4200 ^b	4160^{ab}	408
Weight variation (weaning-kindling weight)	%	7.7 ^{ab}	8.3 ^b	6.5 ^a	6.7 ^a	3.5
DE intake during lactation	kJ/day/kg ^{0.75}	1220ª	1273 ^{bc}	1245 ^{ab}	1290°	182
Estimated DE requirement ^a	kJ/day/kg ^{0.75}	1341 ^a	1423 ^b	1362°	1430 ^b	189
Energy deficit	kJ/day/kg ^{0.75}	121 ^A	150 ^B	117 ^A	140 ^B	45
Milk production	g/day	195°	210^{b}	196°	211 ^b	58

Values in the same row with differing superscripts differ (P < 0.05 lower-case and P < 0.01 upper-case letters).

^b According to Maertens et al. (1988).

^a According to Parigi-Bini and Xiccato (1998).

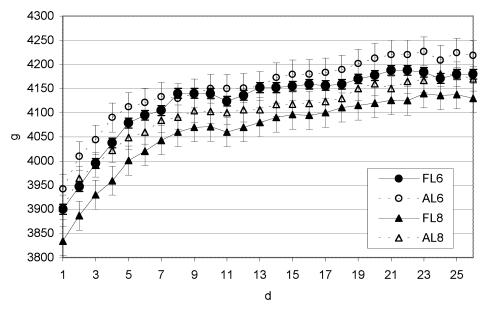


Fig. 2. Body weight of does during lactation (95% upper and lower confidence interval).

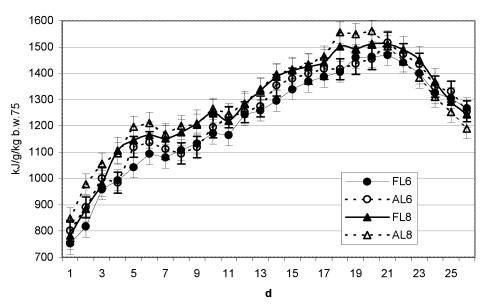


Fig. 3. Digestible energy intake of the different groups during lactation (95% upper and lower confidence interval).

better body condition improved the sexual receptivity and the fertility rate, confirming the positive relationship between sexual receptivity and fertility (Theau-Clément and Roustan, 1992).

The results obtained in the FL8 group confirm that the reproductive performance of does is influenced by the number of milking kits, and by the milk production, which affects the degree of energy deficit (Fortun-Lamothe, 1998).

It has been widely proven that lactation determines a very high energetic output due to the high number of suckling kits and to the caloric value of milk; and,

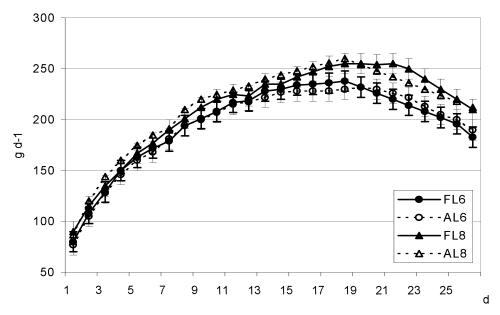


Fig. 4. Milk production of experimental groups (95% upper and lower confidence interval).

Table 3 Reproductive performance of doe and pre-weaning mortality

Mating interval		Fixed L6 L8		Alternating		MSE
Litter size				L6	L8	
Sexual receptivity	%	67.9 ^b	61.8ª	78.7 ^d	73.9 ^{cd}	_
Fertility	%	73.6 ^b	63.4°	78.6 ^b	72.3 ^b	_
Kindling interval	days	53.1 ^A	57.4 ^B	55.2 ^A	58.1 ^B	15.5
Live born	n	8.5	8.2	8.4	8.5	3.1
Litter size at weaning	n	5.6 ^A	6.8 ^B	5.7 ^A	$7.0^{^{\mathrm{B}}}$	1.4
Pre-weaning mortality ^a	%	6.7 ^A	15.0 ^B	5.0 ^A	12.5 ^B	10.8

Values in the same row with differing superscripts differ (P < 0.05 lower-case and P < 0.01 upper-case letters).

Table 4 Performance of young rabbits

Mating interval Litter size		Fixed		Alternating		MSE
		L6	L8	L6	L8	
Weight of live kits at kindling	g	50.4 ^b	48.6ª	49.2 ^b	47.9ª	6.0
Milk/kit	g/day	33.5 ^B	28.6 ^A	33.1 ^B	28.2 ^A	7.6
Kit weight at 19 days	g	353 ^B	292 ^A	340 ^B	292 ^A	175
Kit weight at weaning	g	629 ^B	503 ^A	610 ^B	508 ^A	188
Litter at 45 days	n	5.4 ^a	5.8 ^{ab}	5.3°	6.2 ^b	3.1
Litter at 70 days	n	5.2°	5.6 ^{ab}	5.1 a	$6.0^{^{\mathrm{b}}}$	2.8
Individual weight at 45 days	g	1210 ^b	1141 ^a	1250 ^b	1154 ^a	295
Individual weight at 70 days	g	2340 ^b	2299°	2367 ^b	2302°	190
Mortality from weaning to 70 days	%	7.1 a	17.6 ^b	10.5°	14.3 ^{ab}	38.5

Values in the same row with differing superscripts differ (P < 0.05 lower-case and P < 0.01 upper-case letters).

^a Calculating starting from the adjusted litter size.

Table 5 Indexes of global productivity

Mating interval		Fixed		Alternating		MSE
Litter size		L6	L8	L6	L8	
Rabbits sold/year/doe	n	35.7ª	35.6ª	33.8ª	37.7 ^b	3.4
Live weight sold/year/doe	kg	83.5 ^b	81.9 ^{ab}	79.8°	86.7°	6.0
Real/theoretical production	%	67.9°	49.9°	70.0°	57.2 ^b	9.8
Total mortality (kits milked-sold)	%	13.3°	$30.0^{\rm b}$	15.0°	25.0 ^b	42.5
Annual replacement of doe	%	60°	90 ^b	60°	80 ^b	_
Doe with three consecutive negative AI	%	30°	50 ^b	30°	50 ^b	_

Values in the same row with differing superscripts differ (P < 0.05 lower-case and P < 0.01 upper-case letters).

at the end of lactation there is a negative energy balance (Xiccato, 1996).

Fortun-Lamothe et al. (2000), comparing does with four or seven milking kits, showed that the digestive tract of females suckling larger litters was significantly greater (about 10%), whereas adipose tissue was less (-41%). Even 35 days after parturition, females with the larger litters (10 vs. 4) had a great depletion of body stores (-35.8%; Fortun-Lamothe and Gidenne, 2000) which can be detrimental for future reproductive activity.

Regarding the reproductive rhythm it should be noted that the alternating pp and post-weaning (pw), in comparison with the fixed insemination at 11 days, seemed more adapted to doe physiology. This improved the receptivity and the fertility, even under more critical conditions (high milk output) without any oestrus synchronisation. Thus, the longer remating interval did not reduce the productivity per year.

It seems that the antagonism between a continuous intensive rhythm and the reproductive performance (Parigi-Bini et al., 1989; Cervera et al., 1993) can be made less stressful by providing the doe with time to recover a suitable fat supply.

The combination pp-pw rhythm should be further investigated. Although under alternating rhythm insemination, time did not cause any difference in receptivity and fertility (always above 70%—data not shown), some effects could take place. For example, pp reduced the prolificacy at the following kindling by about 1.1 live kits (7.9 vs. 9.0—data not shown) probably due to the lower ovulation rate (Theau-Clément et al., 1990; Fortun-Lamothe, 1998) or higher foetal mortality.

The effect of the alternate rhythm should be

analysed by studying the net body variation of the doe and the hormonal profile.

Twenty-six days of milking seemed sufficient for a correct weight gain of the litters. The weight of kits at kindling was higher when does milked only six kits, which is in agreement with the findings of Fortun and Lebas (1994), due to the higher individual milk intake (+19%) that contributed to lower the mortality rate.

Fortun-Lamothe and Gidenne (2000) reported a similar relation between milk availability (-15%: 10 vs. 4) and litter weight at weaning; even if the mortality rate from 16 to 42 days was 12% higher in the more numerous litters.

These results, in agreement with those of Scapinello et al. (1999), suggest that the growth and survival of young rabbits are lower if the rabbits are reared in large litters; earlier and greater ingestion of solid food, due to the lower milk availability, only partially compensates for this lack.

The differences in the fertility rate, weight of young rabbits and their survival permitted to obtain a global productivity with a smaller litter size similar to that obtained with a larger litter size. This reduction is advisable under the fixed rhythm.

The results obtained showed that cycled production amplifies the importance of the fertility rate with respect to litter size (Castellini, 1996). In fact cycled production implies that non-pregnant does follow the same insemination schedule as pregnant ones and when the fertility rate is low many does must wait until the next insemination cycle and the mean kindling interval increases.

The alternating rhythm with eight kits/litter seemed to be the protocol that best matches the

compromise between the productivity of the doe, the litter requirement and the mortality rate, resulting in the highest number and weight of rabbits sold/year/doe.

From the point of view of animal welfare, smallersize litters reduced the energetic stress of the doe, increased the length of its reproductive activity and reduced the total mortality of rabbits. The mean efficiency of the rearing protocol was about 69% in L6 groups and about 53% in L8. Although the latter efficiency is economically conceivable it should be considered too low to consider the system sustainable. Increasing the efficiency will require related genetic, feeding and management. For example, selection toward does with larger litters and higher milk production is inefficient because of the negative aspects of such increases including the high energy deficit, fertility reduction, high doe replacement rate and mortality rate of the kits. Probably selection for higher feed intake should give animals more balanced and adapted to the actual rearing conditions. In fact some authors (Parigi-Bini and Xiccato, 1998; Xiccato et al., 1999) have shown that with does of the current hybrid the reduction of the energy deficit by nutritional strategies is not yet possible, since a higher energy intake causes a simultaneous increase in milk output.

The alternative rhythm seems more suitable than the fixed mating interval, since it is more respectful of physiological behaviour (sexual receptivity, energy requirement, and length of reproductive activity). Naturally this approach must be further studied, perhaps simplified and tested with other strains and litter size.

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References

AOAC, 1995. In: 15th Edition. Official Methods of Analysis.Association of Official Analytical Chemist, Washington, DC.Bell, D.J., 1984. The behaviour of rabbits: implications for their

- laboratory management. In: Standard in Laboratory Animal Management. UFAW, Potters Bar, UK.
- Castellini, C., 1996. Recent advances in rabbit artificial insemination. Proceedings 6th World Rabbit Congress, Toulouse Vol. 2, 13–28
- Castellini, C., Boiti, C., 1999. Le linee guida per la riproduzione. Riv. di Coniglicoltura 3, 20–23.
- Castellini, C., Lattaioli, P., 1999. Effect of number of motile sperms inseminated on reproductive performance of rabbit does. Anim. Reprod. Sci. 57, 111–120.
- Cervera, C.J., Fernandez-Carmona, J., Viudes, P., Blas, E., 1993.Effect of remating interval and diet on the performance of female rabbits and their litters. Anim. Prod. 56, 399–405.
- Facchin, E., Castellini, C., Cappiotti, P., 1993. Dispensa di coniglicoltura. Lapival—EnAIP Veneto ed Istituto Zooprofilattico Sperimentale Venezie, Verona.
- Filleul, J.P., 1996. Enteropatie del coniglio: situazione attuale in Francia. In: Atti Convegno scientifico ASIC sulle Enteropatie del Coniglio, Vol. 43. Fondazione Iniziative Zooprofilattiche e Zootecniche, Brescia, pp. 89–98.
- Fortun-Lamothe, L., 1998. Effets de la lactation, du bilan énergétique et du rythme de reproduction sur les performances de reproduction chez la lapine primipare. Mem. 7èmes Journées de la Recherche Cunicole, Lyon, 257–260.
- Fortun, L., Lebas, F., 1994. Influence of the number of suckling young and the feed level on fetal survival and growth in rabbit does. Ann. Zoot. 43, 163–171.
- Fortun-Lamothe, L., de Rochambeau, H., Lebas, F., Tudela, F., 2000. Influence of number suckling young on reproductive performance in intensively reared rabbits does. In: Proceedings 7th World Rabbit Congress, Valencia, pp. 125–132.
- Fortun-Lamothe, L., Gidenne, T., 2000. The effects of size of suckled litter on intake behaviour, performance and health status of young and reproducing rabbits. Ann. Zoot. 49, 517– 529.
- Garcia, F., Perez, A., 1989. Effectos de la lactation y numero de lactantes sobre la monta, ovulacion y suervivencia fetal hasta el parto, evaluados por laparoscopia, en conejas multiparas. ITEA 80, 3–10.
- Hudson, R., Schaal, B., Bilko, A., Altbacker, V., 1996. Just three minutes a day: the behaviour of young rabbits viewed in the context of limited maternal care. In: Proceedings 6th World Rabbit Congress, Toulouse, Vol. 2, pp. 395–403.
- Maertens, L., Okerman, F., 1988. Le rythme de reproduction intensif en cuniculture. Cuniculture 82, 171–177.
- Maertens, L., Moermans, R., De Groote, G., 1988. Prediction of the apparent digestible energy content of commercial pelleted feeds for rabbits. J. Appl. Rabbit Res. 11, 60–67.
- Parigi-Bini, R., Xiccato, G., Cinetto, M., 1989. Influenza dell'intervallo parto-accoppiamento sulle prestazioni riproduttive delle coniglie fattrici. Riv. di Coniglicoltura 26, 51–57.
- Parigi-Bini, R., Xiccato, G., 1998. In: De Blas, C., Wiseman, J. (Eds.), The Nutrition of Rabbit. CABI.
- SAS, 1990. SAS/Stat[®], User's Guide, Version 6. SAS, Cary, NC. Scapinello, C., Gidenne, T., Fortun-Lamothe, L., 1999. Digestive capacity of the rabbit during the post-weaning period, according to the milk/solid feed intake pattern before weaning. Reprod. Nutr. Dev. 39, 423–432.

- Theau-Clément, M., Bolet, G., Roustan, A., Mercier, P., 1990.
 Comparaison de differents modes d'induction de l'ovulation chez les lapines multipares en relation avec leur stade physiologique et la réceptivité au moment de la mise a la reproduction. Mem. 5èmes Jour. Rech. Cunicole, Paris, Comm, 6.
- Theau-Clément, M., Roustan, A., 1992. A study on relationships between receptivity and lactation in the doe, and their influence on reproductive performances. In: Proceedings 5th World Rabbit Congress, Corvallis, A, pp. 412–421.
- Xiccato, G., 1996. Nutrition of lactating does. In: Proceedings 6th World Rabbit Congress, Toulouse, Vol. 1, pp. 29–47.
- Xiccato, G., Bernardini, M., Castellini, C., Dalle Zotte, A., Queaque, P.I., Trocino, A., 1999. Effect of post-weaning feeding on performance and energy balance of female rabbit at different physiological states. J. Anim. Sci. 77, 416–426.
- Xiccato, G., Trocino, A., Sartori, A., Queaque, P.I., 2000. Early weaning of rabbits: effect of age and diet on weaning and post-weaning performance. In: Proceedings 7th World Rabbit Congress, Valencia, C, pp. 483–490.