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GENETIC COMPARISON OF MILK PRODUCTION AND COMPOSITION IN THREE MATERNAL RABBIT LINES

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Abstract: The aim of this study was to compare 3 Spanish maternal rabbit lines (A, V and LP) in terms of milk production and composition. These lines were founded on different criteria but selected for litter size at weaning. A total of 194 mature does in their third or higher parity were used. The milk yield of does was recorded at 1, 2, 3, 4, 8, 9, 10, 11, 15, 16 and 17 d post-partum (dpp). The milk production traits studied were weekly milk yield (WMY; g/wk) and milk conversion ratio (MCR; grams of litter gain per grams of milk suckled during the first 21 dpp). The milk composition traits studied were fat (%), protein (%), ash (%), lactose (%) and total solids (%). The milk samples to be analysed were collected from each doe at 18 dpp. Data were analysed using single trait mixed and fixed models with and without covariates; the covariates were number born alive (NBA) and doe weight at kindling (DW). The overall mean of WMY, during the first 3 wk, was 1547±16 g/wk. Milk yields during the different lactation weeks were for line A 872±39, 1503±39 and 1865±39 g for first, second and third lactation weeks, respectively. In line V, the corresponding values were 919±35, 1633±35 and 2004±35 g, and in line LP, they were 1043±36, 1819±36 and 2254±36 g. Means of MCR were 0.41±0.01, 0.41±0.01 and 0.42±0.01 for A, V and LP lines, respectively. Overall means of fat, protein, ash, lactose and total solids (%) were 14.62±0.17, 11.10±0.07, 1.89±0.04, 2.67±0.12 and 30.27±0.24, respectively. The differences between lines for milk production traits were significant except for MCR, while the differences between lines for milk composition traits were not significant. NBA had significant effects on all milk yield traits but had no significant effects on milk composition traits. DW only had a significant effect on weekly milk yield. The parity order had no significant effect either for milk production traits or milk composition traits in multiparous does, except for ash %.

Key Words: rabbits, milk production, milk composition, maternal lines.

INTRODUCTION

Rabbit does nurse their kits till weaning (4-6 wk of age) and kits exclusively depend on milk until 18-19 d of age (Fortun-Lamothe and Gidenne, 2000). During this period, newborn rabbits have high energy requirements and a low thermal isolation. Thus, the survival of the kits and their weight gain depend exclusively on the doe's milk production. For these reasons, milk yield of the does is an important factor in rabbit production (Iraqi *et al.*, 2010). Lactation requires a great energy effort from the doe and is closely related to certain traits such as body condition, fecundity and foetal growth (Fortun-Lamothe and Bolet, 1995; Xiccato *et al.*, 2004).

Current maternal rabbit lines have an average litter size of over 10 kits and low individual weaning weight (Garreau *et al.,* 2004), and consequently high requirements for lactation. However, due to the fact that they are expensive to record, milk production and composition traits are not used as selection criteria, but there are many traits associated with them, such as litter size at weaning, litter weight at weaning and mortality during lactation, that certainly are

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used as selection criteria. Lukefahr *et al.* (1983) detected a strong positive association within breed between milk production of the doe and both litter size and weight at 21 d of age. In intensive rabbit meat production, it is common to use crossbred does from lines selected for litter size and thereby indirectly improve milk yield (Garreau *et al.*, 2004). To date, genetic studies concerning milk production and composition in purebred and crossbred rabbits are scarce. Milk production and milk composition of crossbred does depend on the value of these traits in the maternal lines used to produce those does, as well as on the heterosis effects for these traits. So, the aim of this study was to assess milk production ability and milk composition of three maternal rabbit lines, all of them selected for litter size at weaning but differing in the foundation criteria.

MATERIALS AND METHODS

Animals and management

Animals used in this study belong to 3 Spanish maternal rabbit lines (A, V and LP) reared at the selection nucleus of the Institute for Animal Science and Technology, Universitat Politècnica de València. Line A originated in 1980 from New Zealand White (NZW) rabbits reared by farmers near Valencia, Spain. The criteria used to form this line were that the founders were healthy and fulfilled the standard characteristics of the NZW breed. Since its foundation, line A has been selected for litter size at weaning by evaluation of candidates using a family index (Estany *et al.*, 1989). Line V was founded in 1982 from crosses between 4 specialised maternal lines; it has subsequently been selected for litter size at weaning (Estany *et al.*, 1989). Line LP was founded more recently, by selecting females from commercial farms that showed extremely long productive lives, measured as the number of parities, but keeping prolificacy, measured as the mean number of liveborn per parity, near or above the average for the Spanish commercial rabbit population (Sánchez *et al.*, 2008). This line has been selected since 2003 to increase litter size at weaning. In V and LP lines, animals are genetically evaluated using a repeatability animal model and selection decisions are based on Best Linear Unbiased Predictions (BLUP) of their additive genetic values. In the present study, all animals were kept in the same rabbitry and under the same environmental and management conditions during all the production stages. Bucks and does begin reproduction at 17 to 18 wk of age by natural mating. On day 12 post-mating, each doe is palpated to detect pregnancy, and non-pregnant does are returned, generally, to the same buck. Does are mated 11 d after kindling and litters are examined each morning during the suckling period to remove dead kits. Kits are reared by their own dams without fostering and weaned at 28 d post-partum (dpp). Then, animals are individually identified by a number tattooed on the left ear and transferred to standard progeny wire cages for fattening. Both breeding animals and progeny are fed the same pelleted commercial diets *ad libitum*. The selection processes are in their 42nd, 38th and 8th generations for lines A, V and LP, respectively. Animals used in our study were sampled from these generations. For a suitable comparison between lines, mature females both in body weight and mammary gland development were used, so does with 3 parities or more were sampled. Table 1 shows the number of females used for each type of trait studied, distributed by line and parity order.

Data collection

Number born alive (NBA) and doe weight at kindling (DW) have been controlled during the experiment. Milk yield of does was recorded at 1, 2, 3 and 4 dpp during the first week of lactation; 8, 9, 10 and 11 dpp during the second week and 15, 16 and 17 dpp during the third week on 194 does (Table 1). Fernández-Carmona *et al.* (2004) reported that

Table 1: Numbers of does by line, class of parity order and type of trait studied.

	Milk Yield		Milk composition	
Line	Class I	Class II	Class I	Class II
A	41	20	37	18
V	49	23	45	21
LP	26	35	22	29
Total	116	78	104	68

Class I= 3 to 5 parities; Class II= 6 to 9 parities.

3-d weekly sampling seems to be adequate to achieve acceptable precision when measuring weekly milk yield. The maximum milk yield is reached during the third week of lactation; milk yield declines gradually in the fourth week and kits start eating rations as of day 18-19 of lactation. No records were conducted in the fourth week of lactation, so differences between lines in the persistency of milk production could not be studied. Milk yield was measured using the weigh-suckle-weigh method described by Lukefahr *et al.* (1983). This method basically consisted of weighing the dam before and after suckling, so on control days it is only necessary to allow access to the dams once per day for about 5-10 min. Daily milk production was computed as the difference between pre- and post-suckling doe weight. Daily milk yield for each week was calculated as the average of daily milk production measurements for that week. One trait analysed was weekly milk production (WMY) during the first (WMY1), second (WMY2) and third (WMY3) week of lactation. These quantities were computed as seven times the corresponding average daily milk yield for each week. Weekly milk yields were assumed to be repeated measurements of the same trait. Milk conversion ratio (MCR) during the period from kindling up to 21 dpp was computed as litter weight gain divided by milk intake (WMY1 + WMY2 + WMY3) during that period.

Milk samples for milk composition records were collected manually and after allowing their kits to suckle for only 30 s by gently massaging the mammary gland after 2 min of injection with 0.5 mL of oxytocin hormone into the marginal ear vein to enhance maximum concentration of epithelial cells. Samples were taken from 172 does in the morning of the 18th dpp; volumes of 25 to 30 mL per doe were obtained. The samples were cooled and transferred immediately to the laboratory for the chemical analyses. Milk samples were analysed for total solids using a stove at 105°C and for ash according to the procedure outlined in AOAC (1980). Fat was determined immediately after sample collection by Gerber method, as described by Case *et al.* (1985). Nitrogen was assessed by the standard micro-Kjeldahl method (AOAC 1980), then a nitrogen conversion factor of 6.38 was used to calculate protein content. Lactose was determined by subtracting the sum of fat, protein and ash from the total solids.

Statistical analysis

Weekly milk production traits were analysed using the following mixed model:

$$Y_{iwjk}=LW_{iw}+PO_j+\beta_1\times NBA_k+\beta_2\times DW_k+p_k+e_{iwjk}$$

where Y_{iwjk} corresponds to the record of the k doe from line i, obtained in week w and in parity order class j. LW_{iw} with 9 levels is the fixed effect resulting from the combination of the line (3 levels; A, V and LP) and the week (3 levels) of lactation; PO_j is the fixed effect of parity order class (2 levels: where class 1 includes parity orders 3, 4 and 5 and class 2 includes parities 6, 7, 8 and 9); β_1 is the regression coefficient of the trait on number born alive in the litter (NBA_k); β_2 is the regression coefficient of the trait on doe weight at kindling (DW_k); p_k is a random permanent effect of doe, which includes permanent environmental effects plus all genetic effects of the doe k (for k = 1, …,194); and e_{iwjk} is the residual.

MCR and milk composition traits were analysed using the following fixed model:

$$Y_{ijk}=L_i+PO_j+\beta_1\times NBA_k+\beta_2\times DW_k+e_{ijk}$$

where L_i is the fixed effect of line i (3 levels; A, V and LP). The other terms are the same as defined in the previous model.

In a previous step, the permanent and residual variances were estimated using the remlf90 program (Misztal *et al.*, 2002). These variance components were used in the blupf90 program (Misztal *et al.*, 2002) to obtain the estimates of the fixed effects, as well as the (co)variance matrix of the errors and the differences between lines, weeks and different order of parities. For instance, the contrast between lines A and V will be computed as:

$$A–V=⅓(LW_{A1}+LW_{A2}+LW_{A3})–⅓\ (LW_{V1}+LW_{V2}+LW_{V3})$$

and the contrast between the first and the second week as:

$$1^{st}–2^{nd}\ wk=⅓(LW_{A1}+LW_{V1}+LW_{LP1})–⅓\ (LW_{A2}+LW_{V2}+LW_{LP2})$$

The contrasts of interest were tested using F statistic tests. The contrasts between lines and parity orders were computed excluding the covariates to evaluate the total differences between them, and with the complete model, including the covariates, to show the differences not depending on the differences in the covariates. The difference between 2 specific lines for milk yield at a given week was compared with the difference between the same lines in another week as a measure of the corresponding interaction.

The experiment was designed to evaluate the differences between lines in adult does, as the most direct and simple way to assess the variability between them. In this sense, the model of analyses included those factors to characterise this variability. Thus, both experimental design and statistical models would prevent the study of the effect of factors such as growth and ageing of the does, overlapping of lactation and gestation, and others; all these factors could be relevant, but their study remains beyond our objectives and capabilities, given our experimental design.

RESULTS AND DISCUSSION

WMY1, WMY2 and WMY3 were considered in our analysis as repeated measures of the weekly milk yield, including in the model the permanent effect of the doe that allowed the estimation of the repeatability of the trait. This estimate was 0.56 for the model with covariates and 0.62 for the model without covariate, showing that weekly milk yield is a relatively high repeatable trait.

Effect of NBA and DW on milk traits

NBA had significant effects on all milk yield traits. An increase in litter size by one kit born alive increased weekly milk yield by 49.21 g/wk; this confirmed the positive relationship between litter size and milk production in maternal rabbit lines already reported in the literature. Lukefahr *et al.* (1983) reported a correlation coefficient of 0.78 between total 21 d lactational yield and litter size at 21 d. Iraqi and Youssef (2006) estimated that an increase in litter size by one kit resulted in an increase by 42.9, 84.4, 102.4, 84.6 and 306.9 g of milk for the 1st, 2nd, 3rd, 4th week and total milk yield, respectively. Other studies confirmed this positive phenotypic relationship between litter size and milk production in rabbits. Pascual *et al.* (1996) reported that milk yield was 32% greater for does with ten kits (P<0.01) than for does with 7-8 kits. Also Theilgaard *et al.* (2009) found that milk yield was 49% greater for those does with nine kits (P<0.01) than for does with five kits.

Regarding the effect of prolificacy on MCR, an increase by one kit born alive decreased MCR by 0.07.

DW had a significant effect only on weekly milk yield, an increase of one gram of the female body weight led to an increase of weekly milk yield of 0.14 g. This result is in accordance with those reported by Singh (1996), who stated that doe weight at kindling was a significant source of milk yield variation. Neither NBA nor DW had significant effects on milk composition traits.

Table 2: Overall means (± standard errors) and contrasts of factors affecting weekly milk yield (g/week) using the model with and without covariates.

	With covariates	Without covariates	Δ_{NBA}	Δ_{DW}
Overall mean	1552±17	1547±16		
Contrast				
A-V	–79±40[a]	–105±36[a]	–0.86	128
A-LP	–204±45[a]	–292±41[a]	–1.17	–139
V-LP	–125±43[a]	–186±38[a]	–0.30	–267
1st-2nd wk	–706±18[a]			
1st-3rd wk	–1096±18[a]			
2nd-3rd wk	–389±18[a]			
Class I-Class II	2±37	31±34	0.30	60

[a]Effect significantly different from 0, α=0.05; Δ_{NBA}: Difference of number born alive between lines and parities; Δ_{DW}: Difference of doe weight at kindling between lines and parities; Class I: 3 to 5 parities; Class II: 6 to 9 parities.

Milk production traits

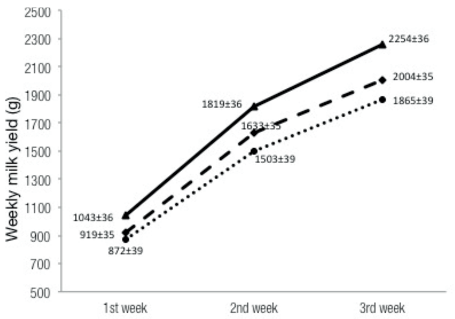

Figure 1: Least-square means±standard errors for weekly milk yield (g/wk) of the 3 lines using the model without covariates. ···●··· Line A; – ◆ – Line V; —▲— Line LP.

Overall means of weekly milk yield (WMY) and contrasts between the levels of main factors affecting milk yield are presented in Table 2. The values were calculated using models with and without covariates and the differences in the contrasts between both models are well explained by the differences between the covariates. Results show that LP does had higher weekly milk yield than A and V does (P<0.05), A having the lowest yield. It can be seen (Table 2) that the differences between lines are partially due to the differences in the covariates and, consequently, the differences decreased when the comparisons were made at the same value of NBA and DW. Different published studies have already reported significant variability between breeds or lines of rabbits. Lukefahr *et al.* (1983) reported that New Zealand White does were superior to Californian rabbits in terms of milk yield (P<0.01). Iraqi *et al.* (2010) stated that Moshtohor line and Gabali breed does were superior in total milk yield (during 28 d) relative to line V.

Regarding total milk yield during the first 21 dpp, LP does showed the highest production (5116±80 g) while V and A lines had the lowest averages, 4556±78 and 4241±89 g, respectively (Figure 1). These results are compatible with those reported by Theilgaard *et al.* (2009), who found that LP does had a higher milk yield (+10%; P<0.01) than V does during the first weeks of lactation.

The contrasts between weeks showed that WMY was increasing significantly throughout the first 3 wk of lactation (Table 2). These results are in agreement with the findings by El-Sayiad *et al.* (1994) and Zerrouki *et al.* (2005). In contrast, El-Maghawry *et al.* (1993) found that the peak of milk yield in NZW was attained at the second week; this discrepancy may be due to the hot climate environment in which that experiment was conducted.

The interactions between genetic lines and weeks on WMY are presented in Table 3, showing that the differences between lines are not constant throughout the weeks. The only significant interaction involving the 1st and 2nd wk was for A and LP lines. The difference between A and LP lines during the first week was 171 g, whereas in the second week it was 316 g (Figure 1), which means an interaction of 145 g more in favour of the LP line, as indicated in Table 3. There were no significant interactions concerning the 2nd and 3rd wk, but all interactions regarding the 1st and 3rd wk were significant. The differences between lines were increasing, thus reflecting the fact that the sign of the interactions did not change over the weeks.

Table 3: Estimates of genetic line×week interactions and their standard errors for weekly milk yield (g/wk) using the model without covariates.

	Contrast[1]	Interaction
Weeks 1 and 2	(A1st-V1st)-(A2nd-V2nd)	82±43
	(A1st-LP1st)-(A2nd-LP2nd)	145±44[a]
	(V1st-LP1st)-(V2nd-LP2nd)	63±43
Weeks 2 and 3	(A2nd-V2nd)-(A3rd-V3rd)	9±43
	(A2nd-LP2nd)-(A3rd-LP3rd)	73±44
	(V2nd-LP2nd)-(V3rd-LP3rd)	64±43
Weeks 1 and 3	(A1st-V1st)-(A3rd-V3rd)	92±43[a]
	(A1st-LP1st)-(A3rd-LP3rd)	218±44[a]
	(V1st-LP1st)-(V3rd-LP3rd)	126±43[a]

[1]Xyth: Least square mean of line X (A, V and LP) at week yth (1st, 2nd and 3rd); [a]Effect significantly different from 0, α=0.05.

Table 4: Overall means (±standard errors) and contrasts of factors affecting gross chemical composition of milk (%) using the model without covariates.

	Fat	Protein	Ash	Lactose	Total solids
Overall mean	14.62±0.17	11.10±0.07	1.89±0.04	2.67±0.12	30.27±0.24
Contrast					
A - V	–0.57±0.38	–0.08±0.16	0.15±0.09	–0.13±0.26	–0.63±0.53
A - LP	–0.13±0.44	0.32±0.19	–0.27±0.10[a]	0.03±0.31	0.04±0.62
V - LP	0.45±0.40	0.40±0.17[a]	–0.42±0.09[a]	0.16±0.28	0.59±0.56
Class I - Class II	0.06±0.37	0.08±0.16	–0.24±0.09[a]	0.36±0.26	0.26±0.53

[a]Effect significantly different from 0, α=0.05; Class I: 3 to 5 parities; Class II: 6 to 9 parities.

The parity order did not have any significant effect on WMY, whether the correction by the covariates had been performed or not (Table 2); this result could be expected, as the does in the present study had 3 or more parities, so no differences between does in mammary gland development might be expected. Increases by up to 20% in milk yield were observed from 1st to 2nd lactation (Maertens *et al.*, 2006), even after a correction for the difference in litter size. A number of experiments (McNitt and Lukefahr, 1990; Hassan *et al.*, 1992; Khalil, 1994 and Quevedo *et al.*, 2006) have already reported that the increment of milk production with the parity order could be correlated with development of the mammary glands.

Differences between lines in MCR were not significant; this could be a consequence of not observing significant differences between lines in milk composition traits, as will be seen below (Table 4). The overall mean of MCR was 0.42; this figure was higher than those reported by Ramadan (2005) (0.38) and Al-Saef *et al.* (2008) (0.36). In contrast, the MCR mean reported here was lower than that reported by Iraqi (2008), who found that MCR was 0.53 in NZW rabbits, and also lower than those reported by Khalil and Al-Saef (2012), who found that the means of MCR were 0.54 and 0.74 in V and Saudi lines, respectively.

Milk composition traits

Overall means of milk composition traits (fat %, protein %, ash %, lactose % and total solids %) and contrasts between lines and between parity orders are presented in Table 4. The overall means of milk composition traits in our study were similar to those reported in previous studies involving many different lines (Coates *et al.*, 1964, Castellini *et al.,* 2004, Khalil *et al.*, 2004, Al-Sobayil *et al.*, 2005, Abou Khadiga *et al.,* 2012, Khalil and Al-Saef, 2012). This uniformity in overall means across studies is an indication of the low variability across lines for milk composition traits. Thus, significant differences between lines were only observed between the LP line and both A and V lines for ash %; and for lines LP and V for protein %. The difference between V and LP line for protein % was significant (P<0.05), in favour of V line. Between these 2 lines, a lower WMY was observed for the V line, although a strong association such as higher yield and lower percentage of milk components cannot be postulated. El-Sayiad *et al.* (1994) found similar results to those observed in the present study; they stated that the differences between NZW and Californian rabbits were not significant for fat, protein, lactose and ash content of their milk. Moreover, Maertens *et al.* (2006) found no significant differences in milk composition traits between commercial hybrids, nor did Cowie (1968) between New Zealand White and Dutch rabbits.

Significant differences among the 3 lines in terms of milk yield traits without any important effect on milk composition enhance the idea of some independency between milk yield and milk composition in rabbits.

In the present study, parity order was not a significant source of variation for any milk composition trait except for the ash % (Table 4). Khalil (1994), El Sayiad *et al.* (1994), Pascual *et al.* (1999) and Iraqi *et al.* (2007) observed a clear difference in milk composition traits between first parity and older females, following a trend consistent with the development of the mammary glands and physiological capacity of the does. As commented above, a homogenous sample of adult females, with 3 or more parturitions, were considered and, once again, the effect associated with mammary gland development was not observable in our data.

No effect of NBA on milk composition traits was observed. In contrast, Mennicken *et al.* (1999) reported that kits from does with 7 kits tended to grow faster than those with 8 or 9 kits although individual milk intake was similar;

this indicates a possibly lower energy or nutrient content of the milk of does with a higher milk production as a consequence of having larger litters.

CONCLUSIONS

Although the LP line showed superiority on milk production over the A and V lines, its milk components were not affected. There were some important interactions between line and week of lactation for WMY, showing that the differences between lines increased over the weeks. No significant differences were observed between parity orders higher than the second, either for WMY or for milk composition traits. Differences between lines in MCR were not significant. Number born alive (NBA) had significant effects on all milk yield traits, but not on milk composition traits.

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