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ASSOCIATION BETWEEN LITTER SIZE AND THE K-CASEIN GENOTYPE IN THE INRA RABBIT LINES

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Abstract: The reproductive traits of 276 rabbit does belonging to an intercross generation of two synthetic INRA lines, which differed by their genotypes at the κ-casein locus being either *AA* or *AB*, were recorded at birth and at weaning for a total of 743 litters. They were the daughters of 77 dams whose κ-casein genotype was *AB* and 18 sires whose genotype was *AA*. A significant association between the κ-casein genotype and reproductive traits at birth was found in favour of the *AB* females, with an increase in litter size (+0.56; $P<0.009$) as well as in litter weight (+27 g; $P<0.023$). After standardisation of the litter size at birth, the genotype of the dam was found to have no effect on weight gain and the viability of the young between birth and weaning or on the litter weight at weaning. Work is now in progress to explain this association.

Key words: κ-casein, reproduction, litter size, litter weight, rabbits

INTRODUCTION

The polymorphism of the κ-casein gene has been characterised (Hiripi *et al*., 1998) and it was shown that the mRNA transcribed from the two alleles, named *A* and *B*, accumulate at similar levels and are translated into identical κ-casein. In an earlier study, the frequency of the A allele (71.6%) was found to be higher in two populations of New Zealand White rabbits (Hiripi *et al*., 1998). This result was extended recently in 12 European rabbit breeds (Bolet *et al*., 2002). A preliminary analysis was performed to address the question whether the k-casein genotype of does correlates with economical performances, in particular with the growth of young rabbits before weaning (Bösze *et al*., 2000) because of its possible effect on milk composition. A significant relationship was shown, but on a reduced number of data from different breeds. The aim of the present study was to verify these first results about the relationship between the κ-casein genotype and the reproductive traits.

MATERIAL AND METHODS

Experimental design

The reproductive performances were recorded on an intercross generation of two synthetic INRA lines in 2000 and 2001: the INRA 1077 line originating from New Zealand White rabbits and the INRA 2066, originating from Californian and Himalayan rabbits. Both were selected for 30 generations for litter size. INRA 1077 does from generations 29 and 30 were inseminated with semen from INRA 2066 males in four groups in March, April, September 2000, and March 2001. Their genotypes for the κ-casein gene were determined. All INRA 2066 males were *AA*, whereas INRA 1077 females were *AA*, *AB* and *BB* with a frequency of allele *A* of around 50%. Only daughters from the *AB* dams were selected

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in order to compare the reproduction of *AA* and *AB* does originating from the same sires and dams (unfortunately, given the genotype of the males, no *BB* does were available).

Each of these 4 groups of about 120 daughters was housed in a building, in individual wired cages. The females were first inseminated when they were 18 weeks old, they were then inseminated every 6 weeks, whatever the result of the previous insemination. We pooled the semen of 8 males, all bearing the AB κ-casein genotype. During four parities, litter size at birth and at weaning (at 35 days) was recorded, whereas litter weight was recorded only during the first three parities. In the first two litters, litter size was standardised for all females to 9 (first litter) or 10 (second litter) young, by removing or adding young, which had not been previously genotyped and therefore were randomly distributed among the does. This crossfostering allowed analysing the effect of the genotype of the dam whatever the genotype of the young.

Determination of the κ-casein genotype

Genomic DNA was isolated from blood samples by a standard protocol. PCR was performed on genomic DNA and κ-casein genotypes were determined by the size difference in the first intron, as previously described (Bösze *et al.*, 2000).

Statistical analysis

The effect of the κ-casein genotype on the traits was analysed by analysis of variance (the GLM procedure of SAS software). The model included the fixed effects of the κ-casein genotype (AA or AB), parity (4 levels: 1, 2, 3 or 4), group (4 levels) and the random effect of the female nested within the κ-casein genotype and group. Preliminary analysis showed that there were no significant interactions between the fixed effects. The effect of the κ-casein genotype was tested using either the residual or the animal mean square as an error term. For the litter size at weaning, litter size at birth was included as a covariate.

Table 1: Influence of the κ-casein genotype of females on their reproductive performances: nº of data, means and probability of the F-test, least square means for the two kappa genotypes.

	Litter size			Weight at birth (g)		Weight at weaning (g)	
	Total born	Born alive*	Weaned	Litter	Average	Litter	Average
Nº litters	743	734	723	622	622	511	511
Mean	11.86	11.29	9.60	612	56.1	5329	574
RSD	2.61	2.91	1.22	140	8.8	803	66
R^2	0.52	0.46	0.66	0.60	0.56	0.75	0.65
Kappa / RMS (*P*)	0.0091	0.0227	0.2705	0.0884	0.1940	0.2306	0.6346
Kappa/dam MS (*P*)	0.0271	0.0353	0.2574	0.1177	0.2387	0.2243	0.6697
Dam within kappa (*P*)	0.0010	0.0753	0.6938	0.0688	0.0449	0.5849	0.0441
Parity (*P*)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Batch (*P*)	0.0005	0.0019	0.0143	<0.0001	0.2705	<0.0001	<0.0001
Covariate: *P*			<0.0001				
Regression coefficient			0.65±0.05				
Kappa							
AA	11.66±0.17	11.15±0.19	9.64±0.08	596±173	56.6±9.8	5613±65	578± 65
AB	12.22±0.14	11.67±0.16	9.75±0.07	623±158	55.8±9.9	5680±56	579±4.6

*Excluding litters with only stillborn young.RSD: Residual standard deviation. *P*: *P*-value. Kappa/RMS: F test using the residual mean square as the residual error. Kappa/dam MS: F test using the dam mean square as the residual error.

RESULTS

Data from 743 litters were recorded on 276 females, daughters of 77 *AB* dams and 18 *AA* sires. One-hundred-and-eleven females were *AA* and 165 *AB*, which was significantly different from the expected genotypic ratio 1:1 (χ^2=10.6).

There was a significant effect of group and parity on all the traits (except for the average weight of litter at birth). Reproductive performances fluctuated between the groups and most traits increased significantly from parity 1 to parity 3, as expected.

Effect of the κ-casein genotype on litter size

There was a significant effect of the κ-casein genotype of does on litter size at birth, in favour of the *AB* females. The total number of young was higher by 0.56 (P<0.009) and the number of young born alive was higher by 0.52 (P<0.023). Using the dam mean square as a residual error, these effects were also significant, but at a lower level.

Effect of the κ-casein genotype on litter weight

There was a slight effect of the genotype in favour of the AB dams on litter weight at birth (+27 g; P<0.088), as a consequence of their larger litter size, but there was no effect on the average individual weight of young rabbits at birth. After standardisation of the litters at birth, there was no effect on litter size at weaning (i.e. on the viability of the young) nor on the litter weight at weaning (i.e. on weight gain from birth to weaning).

DISCUSSION

Reproductive traits are of major economical interest in farm animals. Litter size is a trait whose heritability is low in all polytoccous species, and consequently difficult to improve by selection. Marker assisted selection could potentially be employed in conjunction with traditional selection methods to accelerate the rate of change in those traits.

Our results clearly showed that there is no effect of the κ-casein genotype of females on the weight of young at birth or at weaning but, unexpectedly, there is a significant relationship between the κ-casein genotype of females and their litter size at birth. Since *AA* and *AB* females come from the same *AB* dams, their genetic background is the same, and their difference in prolificacy is clearly related to their genotype at the κ-casein locus or nearby loci. It is important to point out that the higher litter size of *AB* dams was not accompanied by a lower individual weight at birth. Such an increased litter size in combination with no change in average birth weight has already been described in association with certain alleles of the prolactin receptor gene in the pig (Vincent *et al*., 1998). The fact that the two κ-casein alleles do not affect milk quality (Hiripi *et al*., 1998) is coherent with the fact that litter size or litter weight gain at weaning were not affected by the κ-casein genotype of the does. Further investigations are needed, on the one hand to compare the reproductive performances of the *BB* females (by comparing the progeny of *AB*×*AB* parents) and on the other hand to understand this relationship. However, if we consider the fact that the ratio of AB and AA animals in the progeny of AB dams and AA sires is unbalanced and in favour of the AB genotype, we can suggest an interpretation: if this imbalance is due to a higher mortality of the AA embryos, it is coherent that the litter size of the AA females is lower than that of the AB females, since the expected proportion of AA embryos is higher. This is consistent with the observation that the embryonic mortality is higher in the INRA2066 strain, where the A allele is fixed, than in the INRA1077 strain, where both alleles are present (Bolet and Theau-Clement, 1994). This point has to be thoroughly invesytigated.

Candidate gene markers for litter size have been identified in sheep (Davis *et al*., 1991; Montgomery *et al*., 1994) and in the pig, including the oestrogen receptor locus (Rothschild *et al*, 1996), prolactin

receptor gene (Vincent *et al*., 1998), follicle stimulating hormone beta (Li *et al*., 2001) and retinol-binding protein 4 gene (Rothschild *et al*., 2000), although Linville *et al*. (2001) were not able to confirm the role of these candidate genes. The casein locus has been mapped to position OCU 15q2.3 (Pauloin *et al*., 2002). Rabbit chromosome 15 is homologous to human chromosome 4, pig chromosome 8 and sheep chromosome 6. The Booroola fecundity gene (*FecB*) maps to chromosome 6 in sheep and the distance to the casein locus was estimated to be 12 cM (Montgomery *et al*., 1994; Mulsant *et al*., 2001). Three QTL influencing the ovulation rate or litter size as well as candidate genes for prolificacy have been assigned to pig chromosome 8 (Jiang *et al*., 2002; Chen and Wu, 1998). Since the intronic polymorphism of the κ-casein gene is not likely to be the causative mutation that influences prolificacy, comparative genome mapping experiments are in progress to identify a gene(s), which is located in a nearby locus, using a microsatellite-based map (Chantry-Darmon *et al*., 2005, 2006).

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