NOTA BREVE

EFFECT OF β-LACTAGLOBULIN GENOTYPES ON OVINE MILK COMPOSITION IN ALTAMURANA BREED

INFLUENCIA DEL GENOTIPO DE LA β-LACTOGLOBULINA SOBRE CARACTERES CUALITATIVOS DE LA LECHE EN LA RAZA OVINA ALTAMURANA

Dario, C., D. Carnicella and G. Bufano

Dipartimento di Sanità e Benessere degli Animali. Università degli Studi di Bari. Str. Prov.le per Casamassima, km 3. 70010 Valenzano (Ba). Italia. E-mail: c.dario@veterinaria.uniba.it

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PALABRAS CLAVE ADICIONALES

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SUMMARY

The distribution of the two main genetic variants of the B-lactoglobulin gene, LGB*A and LGB*B, was analyzed by PCR-RFLP and the effects of the substitution of Tyr^{20} with his encoding variants on ovine milk composition were studied. In the sampled population of Altamurana sheep the two genetic variants, the frequency of *LGB*A* and *B*, was 0.625 and 0.375, respectively. The chemical composition of milk did not differ considerably among the *LGB* genotypes; the most significant differences regarded fat for the AA and whey protein content for the BB genotype.

RESUMEN

El objetivo del presente trabajo, realizado sobre 48 ovejas de raza Altamurana, es la determinación de la influencia de los diferentes genotipos del *locus* LGB (AA, AB y BB) sobre la composición de la leche. La determinación del genotipo se ha realizado mediante PCR-RFLPs; el ADN se amplificó mediante PCR y, una vez amplificado, fue digerido con el enzima de restricción *Rsa*I. Las frecuencias alélicas obtenidas para el alelo A y B son 0,625 y 0,375 respectivamente. Se observó una influencia sólo por el porcentaje de grasa y la proteína de lactosuero.

INTRODUCTION

The attention surrounding sheep milk and its cheese production characteristics, especially in Mediterranean countries, has spurred considerable research into the genetic structure of native sheep populations and the possible relationships between the genetic variants of milk protein genes and milk related traits. This, in turn, has generated substantial interest in the practical applications of genetic markers in breed development programmes and preservation strategies, as well as awareness of the cultural value connected to safeguarding the biodiversity of autochthonous dairy sheep breeds. One of the most extensively studied milk protein polymorphisms is the substitution of the aminoacid Tyr[20] with His in the betalactoglobulin polypeptide (Kolde and Braunitzer 1983) resulting from a SNP substitution in the LGB gene, located on ovine chromosome 3. The T/C transversion also produces a RsaI restriction fragment length polymorphism which permits genotyping of animals with a PCR-RFLP method. (Feligini *et al.*, 1998).

MATERIAL AND METHODS

A total of 48 blood samples were randomly collected from a single flock of Altamurana sheep, an autochthonous dairy breed reared in the inland areas of the Apulian hills in southern Italy. For each animal genomic DNA was extracted from whole blood by a rapid method using the GFX genomic kit (Amersham). The PCR reaction, restriction endonuclease digestions, and gel electrophoresis were performed for *LGB* as described by Feligini *et al.*, 1998. The amplified 120 bp long PCR product was digested with *Rsa*I restriction endonuclease and separated on ethidium bromide- stained 2 p.100 agarose gel.

All the ewes were in the same lactation stage and were milked twice a day. Milk composition was assessed on individual milk samples, taken every two weeks from morning and evening milkings. Milk parameters were determined according to the ASPA standardized methods (ASPA, 1995). Data were analysed with a linear model which included fixed effects of the genotype at LGB *locus* (SAS, 1995).

RESULTS AND DISCUSSION

The three different genotypes detected for the Altamurana ewes were AA (66, 37 and 17), AB (103, 66, 37 and 17bp) and BB (103 and 17bp). The ovine *LGB* variants observed in the survey had been detected by electrophoretic patterns of proteins (Dario *et al.*, 2002). Only a few investigators had previously resorted to the PCR-RFLP method to study the ovine LGB gene (Feligini *et al.*, 1998; Vlatka *et al.*, 2002). The results of the present study provided more information on the genetic polymorphism and variability of the Altamurana breed associated with milk composition. As shown in **table I** the occurrence of genetic variants in the Altamurana breed was 0.625 for *LGB**A and 0.375 for *LGB** B. These figures are similar to the values found in the other two Italian breeds, the Barbaresca-siciliana

Table I. *Distribution of β-lactoglobulin genotype and allele frequencies.* (Distribución de las frecuencias genotípicas y génicas para *locus* LGB).

Breed		Genotypic frequencies			Allele Frequencies		X^2
	N=48	AA	AB	BB	A	B	
ALTAMURANA	obs	18	24	6	0.625	0.375	0.213
	exp	18.75	22.5	6.75			Df=1

and the Massese (Chiofalo and Micari, 1987; Rampilli *et al.*, 1997), where the A variant is the most frequent.

$\chi 2$ test showed no significant difference between expected and observed genotype frequencies has been detected by, so the population sample was in H-W *equilibrium*.

The least-squares means of the milk compositional parameters for the three LGB genotypes are shown in **table II**. The chemical composition of milk did not differ substantially among *LGB* genotypes in agreement with previous reports (Recio *et al.*, 1997; Taibi *et al.*, 1999). The LGB *locus* was found to have a significant effect on fat for the AA genotype and on whey protein content for the BB genotype.

In particular, a dominance effect of the LGB*A allele might be responsible for the differences (p>0.01) in fat content (AA= 7.84 *vs* BB= 7.48), while a dominance effect of the LGB*B allele seems to be involved in the whey protein content (BB, AB= 1.11 *vs* AA= 1.02). Some results are in disagreement with those of previous researches who reported a favourable effect of the BB genotype on milk quality (Giaccone *et al.*, 2000), while others point to a positive effect of the AA genotype on the fat and protein contents (Garzon and Martinez, 1992; Kukovics *et al.*, 1998), thus confirming the conflicting relationships regarding *LGB* polymorphism and milk composition. This inconsistency, which is also found in dairy cattle studies, may be ascribed to breed differences, population size, frequency distribution of genetic variants, the structure of the data analysed and the model used for statistical analysis and a failure to consider the relationships among animals.

Table II. *LS-means ± s.e. of the milk compositional parameters for the LGB genotypes.* (Medias de mínimos cuadrados ± e.s. de la calidad de la leche en función de los genotipos).

		LGB	
Parameter	AA	AB	BB
N_o	18	24	6
Fat (g/100 ml)	7.84 ± 0.06^{A}	7.80 ± 0.06^{A}	7.48 ± 0.11^{B}
Total Nitrogen (g/100 ml)	5.40 ± 0.10	5.52 ± 0.09	5.30 ± 0.18
Total Protein (g/100 ml)	5.20 ± 0.10	5.32 ± 0.09	5.25 ± 0.17
Whey protein (g/100 ml)	1.02 ± 0.02^{Aa}	1.11 ± 0.02^{B}	1.11 ± 0.04^{b}
Casein (g/100 ml)	4.18 ± 0.10	4.20 ± 0.09	4.14 ± 0.18
Lactose (g/100 ml)	4.62 ± 0.06	4.64 ± 0.05	4.58 ± 0.10
Total solids (g/100 ml)	18.84 ± 0.14	18.95 ± 0.12	18.34 ± 0.25
SNF (g/100 ml)	11.0 ± 0.12	11.15 ± 0.10	10.86 ± 0.20
Ash (g/100 ml)	0.98 ± 0.00	0.98 ± 0.00	0.98 ± 0.00
Casein number (percent)	77.0 ± 0.00	76.0 ± 0.00	78.4 ± 0.02

^{A,B}p<0.01; ^{a,b}p<0.05.

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