Influence of ovine β-casein phenotype on milk production and composition from Merino ewes

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SUMMARY
The aim of this study was to evaluate the different variants of β-casein and their relationship with milk composition in a population of Merino ewes from the research institute *La Orden-Valdesequera* in the South West of Spain. After lambing, milk samples were collected monthly to evaluate milk composition and one of the samples were use to determine the different β-casein variants. After isoelectric precipitation at pH 4,6, the casein fractions were individually submitted to IEF at pH 2,5-6,5, in polyacrilamide gels in the presence of urea. Three variants, Type 1, Type 2 and Type 5 were identified for β-casein in 2,96%, 96,45% and 0,59%, of the ewes studied, respectively. There was an influence of β-casein variants on milk composition. Significant differences ($P<0,05$) were found in the percentage protein, dry matter and non-fatty solids. Type 1 showed to have higher percentages of protein, dry matter and non-fatty solids than Type 2.

1 INTRODUCTION

The possible effect that milk protein polymorphisms may have in milk production and composition is very interesting in animal production not only for the potential application in the technological properties of milk but also for the potential benefits in animal breeding [2].
There have been some research on milk protein polymorphisms in sheep, and some were based in the application of techniques like electrophoresis and isoelectric focusing [4][2]. In this study, by using electrophoretic the aim was to identify the β-casein variants present in the milk of a Merino ewes population, and to find any association between milk production and milk composition. The objective of studying the best phenotype to produce sheep milk will improve the cheese production.

2 MATERIAL AND METHODS

A group of 176 ewes from a population of 742 Merino ewes from the research institute *La Orden-Valdesequera* in the South West of Spain was used for this study. After lambing, ewes were milked, and milk samples were collected monthly to evaluate milk composition (by using a MilkoScan; Foss Electric, Denmark) and to determine the different β-casein variants. The casein fractions were separated from the whole milk samples by isoelectric precipitation at pH 4,6 [3]. Then, every casein sample, were individually submitted to IEF at pH 2,5-6,5, in polyacrilamide gels (260 x 100 x 1mm) in the presence of urea. The 2,5 – 6,5 pH gradient of the gels was achieved by using three different carrier ampholytes: 2,5-5,0 (Pharmalyte), 4,5-5,4 (Pharmalyte) and 4,0-6,5 (Pharmalyte). IEF was performed in a horizontal electrophoresis apparatus (Pharmacia LKB, Multiphor II), with the following carrier conditions: 1200V, 16W and 15 mA and constant 12°C. There was a pre-focalisation of the gel in the first hour, than were applied 12 µl of lyophilized sample of casein on the Whatman nº 1 paper (10 x 5 mm) near the anode. Focalization occurred during two more hours.

To test the relationship between the β-casein variants and milk composition, 2690 results from the composition samples collected in spring and fall lactations were obtained from these ewes between 1999 and 2006. The phenotype effect on milk composition was considered a fixed effect, and was analysed using a mixed model including the various composition results of the same lactation as repeated measures using PROC MIXED of SAS (SAS Institute, 1998). Were included in the model fixed effects like the season-year of lambing (15: since Autumn of 1999 until Spring of 2006), type of birth (2: one lamb or two lambs), milking hour (2: morning and afternoon), number of lambins (1 to 3) and phenotypes. Ewe and residual effects were included as random effects. The model was:

$$\mathbf{Y_{ijklmn} = \mu + X_i + T_j + P_k + NP_l + Phen_m + Ov_n + \varepsilon_{ijklmn}}$$

3 RESULTS AND DISCUSSION

Three different isoelectrophoretic patterns were observed. Those patterns corresponded to Type 1, Type 2 and Type 5 identified by Chianese et al. 1995, and, therefore, here, the nomenclature of these authors is maintained. Type 1 was found in 2,96%, Type 2 in 96,45% and Type 5 was found only in 0,59%, of the ewes studied. When analysing the statistical result to know the β-casein variants influence in milk composition, significant differences (P<0,05) were found in the percentage protein, dry matter and non-fatty solids, due to the differences in the β-casein variants. Type 1 showed to have higher percentages of protein, dry matter and non-fatty solids than Type 2. Type 5 was excluded from the statistical analyses because it was only found in one sheep (Table 1).

These results cannot be compared with others authors because, until now, no research in this area has been done. In fact there are some doubts on the origin of β-casein variants. Factors like stage of lactation, health, age of individuals, and an altered phosphate availability may change the level of phosphorylation of β-casein, and, therefore, determine different variants [4]. According to this theory, the presence of any β-casein variant would be explained, not by genetic factors, but by factors that also affect milk composition during a female life. Furthermore, new studies of β-casein variants will indicate whether associations between β-casein variants and production traits will be suitable for performance improvement.

4 CONCLUSION

We believe that the research on this protein must continue, to understand if these protein variants are really genetically determined or if they are products of posterior reactions at Golgi apparatus [5] and influenced by the nutrient intake to mammary gland. It is very important to understand how protein polymorphism affect sheep milk production and composition, and how we can use it to produce better and more suitable milks for cheese production.

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Table 1. Least square means (LS Means) and standard error (SE) for fat, protein, lactose, dry matter and non-fatty solids of ovine β-casein variants.

		β-Casein	
		Type 1	Type 2
Fat	n	56	2634
	LSMeans ± SE (%)	8,14 ± 0,27^a	7,74 ± 0,07^a
Protein	n	56	2634
	LSMeans ± SE (%)	6,86 ± 0,25^a	6,42 ± 0,06^a
Lactose	n	40	2184
	LSMeans ± SE (%)	4,40 ± 0,09^a	4,41 ± 0,02^a
Dry matter	n	56	2634
	LSMeans ± SE (%)	12,31 ± 0,17^a	11,80 ± 0,04^b
Non-fatty solids	n	56	2634
	LSMeans ± SE (%)	20,17 ± 0,35^a	19,29 ± 0,09^b

* n: number of samples; ** ^{a,b} – different letters indicate significant differences to P< 0,05.