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Effect of nutrition-genotype interaction on protein and casein synthesis in goat milk of the Malagueña breed

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SUMMARY – An experiment was carried out using twelve goats of the Malagueña breed. Six of these animals belonged to the high capability (three BB and three AB) and six to the low capability (three FF and three EF) genotype for α_{s1} -casein synthesis. They were fed two different diets according particularly to their crude protein content: 13.6% (D1) and 17.7% (D2). Within each genotype, the animals were allocated to a 2x2 Latin square design with three repetitions. Together with the metabolizable energy intakes ($\text{kJ/kg}^{0.75}\cdot\text{d}$), the milk protein ($\text{g/kg}^{0.75}\cdot\text{d}$) and casein ($\text{g/kg}^{0.75}\cdot\text{d}$) synthesis were determined. From the corresponding results it was first inferred that the milk protein and casein synthesis depended on the metabolizable energy intake, these quantities being higher for the high capability genotype for α_{s1} -casein animals and for the higher protein content in the diet. The effects of genotype and protein content in the diet were mainly independent. So, one way of exploiting the genetic potential expression for milk protein and casein synthesis, could be to make use of diets with a high content of crude protein.

Keywords: Genotype, α_{s1} -casein, diet protein content, milk protein synthesis, goat.

RESUME – "Effet de l'interaction nutrition-génotype sur la synthèse de protéine et de caséine dans le lait de chèvres de race Malagueña". Nous avons réalisé une expérience avec douze chèvres de race Malagueña. Six d'entre elles appartenaient à un génotype de haute capacité et six à un génotype de faible capacité pour la synthèse de α_{s1} -caséine. Les animaux furent alimentés selon deux régimes différents quant au niveau de protéines : 13,6% (D1) et 17,7% (D2). Dans chaque génotype, les animaux furent répartis selon un carré latin 2x2 se répétant trois fois. En même temps que l'ingestion d'énergie métabolisable ($\text{g/kg}^{0.75}\cdot\text{jour}$) on a déterminé la synthèse de protéines ($\text{g/kg}^{0.75}\cdot\text{jour}$) et de caséines ($\text{g/kg}^{0.75}\cdot\text{jour}$) dans le lait. On a déduit des résultats obtenus comment la synthèse de protéines et de caséines dans le lait dépendait en premier lieu de l'ingestion d'énergie métabolisable, qui était plus élevée chez les animaux du groupe à haute capacité de synthèse de α_{s1} -caséine et faible consommation du régime le plus riche en protéine. Les effets du génotype et le niveau protéique du régime se trouvaient être des effets indépendants. Par conséquent, une manière d'exploiter le potentiel génétique concernant la synthèse de protéines et de caséines dans le lait, pourrait être d'utiliser un régime ayant une grande teneur en protéines.

Mots-clés : Génotype, α_{s1} -caséine, teneur en protéines du régime, synthèse de protéines du lait, chèvre.

Introduction

It is well known that goats show a genetic polymorphism, at the locus CSN1S1, coding for α_{s1} -casein which is associated to differences in protein, total casein and α_{s1} -casein contents in milk (Grosclaude and Martin, 1997; Martin *et al.*, 1999). On the other hand, there is considerable information with regard to the use of different sources and levels of protein in the diet of dairy goat (Hadjipanayiotou *et al.*, 1987; Morand-Fehr *et al.*, 1991; Romero *et al.*, 1994; Sanz Sampelayo *et al.*, 1999). All this provides an ideal model to study the nutrition-genetic interaction from which would be possible to obtain a specific information about the importance of nutrition on the potential expression genetic factors.

With this in mind and taken into account that this topic has not been previously studied in goats, an experiment was carried out using goats of the Malagueña breed with genotypes associated to high and low levels of α_{s1} -casein synthesis. The animals were fed on two different diets according to their crude protein content (\cong 14 and 18% DM). Within each genotype, milk protein and casein synthesis was estimated for each level of crude protein.

Material and methods

Experimental design and procedure

Twelve goats of the Malagueña breed midway through their second, third or fourth lactation were used. Six of these animals had genotypes (three BB and three AB) associated to high level of α_{s1} -casein synthesis and the other six had genotypes (three FF and three EF) associated to low level of this casein. The animals were fed on two diets differing on their protein content: 13.6% crude protein DM (D1) and 17.7% crude protein DM (D2). The proportion of degradable protein in the rumen was 85.0% for D1 and 81.8% for D2. Within each genotype, the animals were allocated to a 2x2 Latin square design with three repetitions. Each goat received at the same time and in separate containers, a daily ration consisting of 1.0 kg of forage and 1.0 kg of concentrate; the specific N and energy requirements of this species were considered in the dietary formulation (Aguilera *et al.*, 1990). The forage fraction of the diet consisted of long alfalfa hay (700 g and 800 g per animal per day for D1 and D2, respectively) and cereal straw (300 g and 200 g per animal per day for D1 and D2, respectively). Table 1 shows the composition of the concentrates and of the two types of forage supplied. The total duration of each assay was 21 days. The first 14 days were for adaptation and the last seven days constituted the main trial period. Goats were then housed individually in metabolism cages. At 09.00 h every day, once the orts from the ration that was offered the previous day had been collected, the goats were hand-milked. Subsequently, the daily rations were distributed. Water was available at all times. After milking, goats were weighed on the 1st, 14th and 21st day of the experimental period. Feed intake and milk production were monitored daily.

Table 1. Composition of concentrate mixtures used (g/kg), their chemical composition and that of lucerne hay and cereal straw (g/kg DM)

| | Concentrates | | | |
|----------------------------|--------------|-------|-------------|--------------|
| | D1 | D2 | Lucerne hay | Cereal straw |
| Ingredients | | | | |
| Oats | 293 | 150 | | |
| Corn | 390 | 75 | | |
| Beans | 240 | 335 | | |
| Bypass fat | 37 | - | | |
| Corn gluten feed | - | 200 | | |
| Cottonseed | - | 200 | | |
| Mineral-vitamin complement | 40 | 40 | | |
| Chemical composition | | | | |
| Dry matter (%) | 89.05 | 89.52 | 91.80 | 89.37 |
| Organic matter (% DM) | 92.06 | 91.70 | 92.51 | 94.84 |
| Crude protein (% DM) | 12.68 | 19.56 | 18.59 | 4.74 |
| Fat (% DM) | 6.83 | 6.46 | 0.99 | - |
| Gross energy (MJ/kg DM) | 18.55 | 18.49 | 19.02 | 18.20 |

Measurements and analyses

Every day during the main trial period, samples were taken of the forage and concentrate provided, of the food refused and of the faeces produced. These samples were maintained at -20°C until required for analysis. Similarly, samples of milk with no added preservatives were stored at -30°C until analysis.

The DM and N contents of the samples of feedstuffs, orts and milk were analysed in fresh samples. All other analyses were performed on dried samples. The DM of the feedstuffs and orts was determined by oven drying at 100±2°C for 24 h. The N contents of the feedstuffs and milk were measured using the Kjeldahl method. For feedstuffs the results were converted to crude protein by multiplying N by a factor of 6.25.

Protein N content of the milk samples was calculated as differences between total N and non-protein N (NPN); total N was determined from whole milk samples, and NPN was determined from a filtrate of whole milk after precipitation with 12% (wt/vol) trichloroacetic acid (Martín-Hernández *et al.*, 1988). The casein N content was calculated as the differences between the protein N and the whey N. The whey N was calculated as the difference between non-casein N and NPN; non-casein N was determined from filtrates of whole milk after precipitation at pH 4.6 with buffer solution of 10% (wt/vol) acetic acid plus 1N sodium acetate. Protein and casein N values were converted to protein and casein by multiplying by a factor of 6.38.

Statistical procedure

Effects of animal genotype and type of diet consumed on dry matter intake (g/day) and milk protein (g/kg^{0.75}.day) and milk casein (g/kg^{0.75}.day) contents and the interaction between both factors were tested using the general linear model procedure of SAS (1987). As the dry matter intake was not different between groups, it was possible to use these values as covariance factor. Where the effect of interaction was not significant (P<0.05), the least square means were calculated from the model omitting this term (Steel and Torrie, 1984).

Results and discussion

Table 2 gives the values for dry matter intake and milk protein and milk casein contents, for the two diets assayed and the two animal genotypes. Milk protein and casein contents were dependent of dry matter intake (P<0.05). Significant effect of genotype and diet on protein and casein contents were also observed (P<0.05). Non significant interaction between both factors (P>0.05) was detected.

Table 2. Dry matter intake (g/day) and milk protein and milk casein produced (g/kg^{0.75}.d). Effects of α_{s1} -casein genotype and diet consumed

| | Diet 1 | | Diet 2 | | RSD | Level of significance | | | |
|--------------|--------|--------|--------|--------|-------|-----------------------|----------|--------------|-----|
| | HC | LC | HC | LC | | Covariance | Diet (D) | Genotype (G) | DxG |
| DM intake | 1378.2 | 1360.2 | 1465.9 | 1358.5 | 208.3 | - | NS | NS | NS |
| Milk protein | 2.359 | 1.853 | 2.852 | 2.170 | 0.172 | *** | *** | *** | NS |
| Milk casein | 1.996 | 1.507 | 2.100 | 1.769 | 0.193 | *** | * | *** | NS |

Diet 1 and 2: with 14 and 18% of crude protein DM, respectively; HC and LC: High and low capability genotype for protein and casein synthesis, respectively; RSD: Residual standard deviation; NS: Not significant; Covariance: metabolizable energy intake.

*:P≤0.05; ***:P≤0.001.

In goats, energy intake is the most significant factor determining milk composition (Giger *et al.*, 1987; Sauvant *et al.*, 1987; Sanz Sampelayo *et al.*, 1998). It is also well known that together with this, the protein content of the diet is an important factor determining milk protein content (Hadjipanayiotou *et al.*, 1987; Morand-Fehr *et al.*, 1991; Romero *et al.*, 1994). Together with this, providing diets containing sources of protein with a lower degradability at the ruminal level is a commonly used strategy for achieving milk with a higher protein concentration. The fraction of dietary protein that escapes ruminal fermentation may, in virtue of its amino acid composition, supplement the protein of microbial origin in the duodenum. In this way, the protein content of the milk can be increased (Chandler, 1995; Santos and Huber, 1996; Sanz Sampelayo *et al.*, 1999). The results obtained here are in good agreement with those described above. Milk protein and casein synthesis depended on the energy intake, resulting at the same time higher for the animals fed on D2, that with higher crude protein content and higher proportion of undegradable protein in the rumen.

Goat species shows a genetic polymorphism at the CSN1S1 locus, coding for α_{s1} -casein. Up to 15 alleles have been described (Martin and Addeo, 1996). Different alleles at the locus CNS1S1 are

associated to different levels of synthesis of α_{s1} -casein and protein in milk. Boulanger *et al.* (1984) on the basis of their different electrophoretic mobility, classified these alleles into four groups. First group was formed by the "strong" alleles, A, B and C, which were reported to have an average level of 3.6 g of α_{s1} -casein per kg of milk. Second group was constituted by only one "intermediate" allele, E, associated to an average level of 1.6 g/kg of this casein. The third group, with "weak" alleles D and F had an average effect of 0,6 g/kg. Fourth group is that of the "null" allele, which determines the absence of this casein in milk (Grosclaude *et al.*, 1987). More recent works, some of them carried out at field level (Barbieri *et al.*, 1995) and some other realised at an experimental station (Grosclaude and Martin, 1997; Martin *et al.*, 1999), have established the correlation between these α_{s1} -casein levels and total casein content and protein content in the milk and confirmed the relative ranking of genotypes in French breeds.

In Spanish breeds, four main variants A, B, E and F have been identified. Goats with alleles A, B, E and F are characterized with high (A, B) intermediate (E) and low (F) level of α_{s1} -casein in milk. Some of these effects are quantitatively different from those described for French breeds (Analla *et al.* 2000) but the ranking of genotypes with respect to α_{s1} -casein, total casein and protein contents in milk are similar than those (Angulo *et al.*, 2002; Serradilla, 2003; Agüera *et al.*, 2005).

Conclusion

As indicated in the introduction, the objective of this study was to investigate the possible interaction between the level of crude protein in the diet and the genotype in CSN1S1 locus α_{s1} -casein. As discussed before, both factors showed significant effects on milk protein and casein contents. Furthermore, they were independent to each other. So, it is concluded that in this case, there was an interaction between nutrition-genotype in relation to the synthesis of protein and casein in milk.

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