# Effect of goat's alpha-s1 casein genotype on diet selection in a free choice feeding system

**Avondo M., Pagano R., Guastella A.M., Criscione A., Di Gloria M., Valenti B., Lutri L., Pennisi P.**

*in*

**Papachristou T.G. (ed.), Parissi Z.M. (ed.), Ben Salem H. (ed.), Morand-Fehr P. (ed.).
Nutritional and foraging ecology of sheep and goats**

**Zaragoza : CIHEAM / FAO / NAGREF**
**Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 85**

**2009**
pages 367-371

# Effect of goat's alpha-s1 casein genotype on diet selection in a free choice feeding system

**M. Avondo*, R.I. Pagano*, A.M. Guastella*, A. Criscione*, M. Di Gloria*, B. Valenti*, L. Lutri** and P. Pennisi***

*Dipartimento Scienze Agronomiche, Agrochimiche e delle Produzioni Animali (DACPA), University of Catania, via Valdisavoia 5, 95123 Catania (Italy)
**Istituto Sperimentale Zootecnico per la Sicilia, via Roccazzo 85, 90136 Palermo (Italy)

---

**Abstract.** In goats, alpha-s1 casein polymorphism is related to different rates of protein synthesis. Two genetic variants, A and F, have been identified as strong and weak alleles based on a production of 3.5 and 0.45 g/l of alpha-s1 casein per allele. The aim of the trial was to test if goats can self-regulate their diet as a function of the genetic aptitude to produce milk at different casein levels. Two groups of 8 animals, homozygous for strong (AA) or weak (FF) alleles were selected from a flock of 120 Girgentana lactating goats. Animals were housed in individual pens. Using a manger subdivided into 5 separate containers, the goats were offered, daily for 3 weeks, 1.5 kg of alfalfa pelleted hay, two sources of starch (0.7 kg of whole barley, rapidly degradable and 0.7 kg of whole maize, slowly degradable) and two sources of protein (0.7 kg of whole faba bean, more rapidly degradable and 0.7 kg of pelleted sunflower cake, less rapidly degradable). Individual intake for each feed was measured daily; individual feeding behaviour was measured for 7 days using a 24 hour video surveillance system; milk characteristics were measured every three days. As expected, protein and casein were significantly higher in AA group than in FF group (protein: 3.50 ± 0.17% *vs* 3.18 ± 0.16%; casein: 2.70 ± 0.14% *vs* 2.40 ± 0.17%; $P < 0.01$), whereas milk urea was higher in FF group (59.7 ± 8.0 *vs* 48.8 ± 4.4 mg/dl; $P < 0.01$). Total DM intake and chemical composition of the selected diets were similar between groups. However, goats carrying AA alleles had a tendency to reduce the concentrate/forage ratio (1.68 ± 0.28 *vs* 1.97 ± 0.34) and to select less faba bean (9.0 ± 1.9% *vs* 12.8 ± 3.3%; P = 0.016) and more maize (22.9 ± 2.6% *vs* 19.9 ± 2.5%; P = 0.03) than goats carrying the FF alleles. This self regulation of the diet could partially explain the differences in milk composition.

**Keywords.** Goats – Alpha-s1 casein genotype – Free-choice feeding – Milk quality – Diet selection.

***Effet de la caséine alpha-s1 de la chèvre sur la ration sélectionnée dans un système d'alimentation de libre choix***

***Résumé.*** *Chez les caprins le polymorphisme de la caséine alpha-s1 est lié aux différents taux des protéines synthétisées. Deux variantes génétiques A et F ont été identifiées comme fort et faible allèles sur la base de la production de 3,5 et 0,45 g/l de caséine alpha-s1 par allèle. Le but de l'essai est de tester si la chèvre est capable d'autoréguler son régime en fonction de l'aptitude génétique pour la production de lait à différents taux de caséine. Deux groupes de 8 animaux homozygotes pour les allèles fort AA et faible FF, caractérisés par isoelectric focusing et réaction en chaîne de la polymérase spécifique, ont été sélectionnés d'un troupeau de 120 chèvres laitières Girgentana. Tous les animaux ont été logés dans des boxes individuels avec des mangeoires subdivisées en 5 récipients séparés ; les aliments distribués aux animaux durant 3 semaines ont été : 1,5 kg de foin de luzerne, deux sources d'amidon (0,7 kg de grains d'orge entiers rapidement dégradables et 0,7 kg de maïs entier caractérisé par une dégradation lente) et deux sources de protéines (0,7 kg de grain de fèverole qui se dégrade rapidement et 0,7 kg de tourteau de tournesol qui se dégrade moins rapidement). L'ingestion individuelle de chaque aliment a été mesurée quotidiennement. Le comportement alimentaire individuel a été contrôlé durant 7 jours en utilisant le système de surveillance par vidéo pendant 24 heures ; les caractéristiques du lait ont été déterminées tous les 3 jours. Comme prévu, les protéines et les caséines ont été nettement plus élevées chez le groupe AA que chez le groupe FF (protéine : 3,50 ± 0,17%* vs *3,18 ± 0,16% ; caséine : 2,70 ± 0,14%* vs *2,40 ± 0,17% ; P < 0,01), alors que l'urée dans le lait a été plus élevée chez le groupe FF (59,7 ± 8,0* vs *48,8 ± 4,4 mg/dl ; P < 0,01). La MS totale ingérée et la composition chimique des régimes sélectionnés sont similaires pour les deux groupes. Cependant, les chèvres portant l'allèle AA ont tendance à réduire le rapport concentré/fourrage (1,68 ± 0,28* vs *1,97 ± 0,34) et à sélectionner moins de fèverole (9,0 ± 1,9%* vs *12,8 ± 3,3% ; P = 0,016) et plus de maïs (22,9 ± 2,6%* vs *19,9 ± 2,5% ; P = 0,03) que* *les chèvres portant l'allèle FF. Cette auto-régulation du régime peut expliquer partiellement la différence dans la composition du lait*.

***Mots-clés.*** *Caprins – Génotype de la caséine alpha-s1 – Libre choix alimentaire – Qualité du lait – Sélection du régime.*

## I – Introduction

The genotype of individuals greatly affects the quantitative level of casein: animals homozygous for strong alleles at alpha-s1 casein locus (A/A) will produce milk with alpha-s1 casein content of ca. 7 g/l or 2.2 g/l respectively, whereas animals homozygous for weak alleles (F/F) will have alpha-s1 casein content of ca. 0.9 g/l (Martin *et al.*, 1999). Milk protein synthesis in the mammary gland takes place by processing the amino acids deriving from digestion of microbial and by-pass proteins. It could be hypothesised that the genetic type that determines the milk casein level changes the efficiency of use of feeds and/or food selection by goats. Moreover, this efficiency is strongly influenced by the combination of various nutrients.

Therefore the aim of the trial was to investigate the feeding self-regulation capacity of lactating goats with different genetic propensity to casein production, in a "free choice" feeding system.

## II – Materials and methods

Sixteen lactating goats homogeneous for milk production (1.94 ± 0.24 kg/d) and body weight (37.6 ± 5.1 kg) were selected from a flock of 120 Girgentana lactating goats. Animals were divided into two groups, eight homozygous for strong (AA) and eight homozygous for weak (FF) alleles, as characterized by isoelectric focusing (Erhardt *et al.*, 1998) and allele specific-polymerase chain reactions at the CSN1S1 *locus* for the strong A, B and C and weak F alleles (Leroux *et al.*, 1992). All the animals were housed in individual pens. Using a manger subdivided into 5 separate containers, the goats were offered, daily for 3 weeks, 1.5 kg of alfalfa pelleted hay, two sources of starch (0.7 kg of whole barley, rapidly degradable and 0.7 kg of whole maize, slowly degradable) and two sources of protein (0.7 kg of whole faba bean, more rapidly degradable and 0.7 kg of pelleted sunflower cake, less rapidly degradable). In each box water and salt were always available. Individual intake of each feed was measured daily. For each goat, individual feeding behaviour was continuously measured for 7 days using a 24 hours video surveillance system. Every three days, individual milk production was measured and milk samples were collected from the morning and afternoon milking. Milk samples, consisting of proportional volumes of morning and evening milk productions, were measured for lactose, fat and protein by infrared method (Combi-foss 6000, Foss Electric, Illerød, Denmark). Total nitrogen (TN), non-protein nitrogen (NPN) and non-casein nitrogen (NCN) were determined by FIL-IDF standard procedures (FIL-IDF, 1964). From these nitrogen fractions, total protein (TN × 6.38) and casein {[TN – (NCN × 0.994)] × 6.38} were calculated. Milk urea content was determined by differential pH-metry (CL10, Eurochem, Italy). Fat-corrected milk at 3.5% fat (FCM) was calculated according to the equation of Pulina *et al.* (1991). Three samples for each feed were collected and analysed for dry matter, crude protein and NDF content (AOAC, 1990).

Individual data of milk production and composition, intake and diet composition were analysed using the GLM procedure for repeated measures of SPSS (SPSS for Windows, SPSS Inc., Chicago, IL).

## III – Results and discussion

### 1. Feeding behaviour and intake

Great differences are evident between feeds (Table 1), in terms of crude protein, NDF and PDI (protein digestible in the small intestine) obtained from INRA tables (Andrieu *et al.*, 1988). Cereals grains, maize and barley, respectively with slow and fast starch degradability (Offner *et al.*, 2003), had similar crude protein content (respectively 9.2 and 10.9% DM) but different levels of structural carbohydrate (11.4 and 22.8% DM). The protein-rich feeds, sunflower cake and faba bean, were characterized by protein contents equal to 27 and 32% DM, respectively and a different NDF percentage (equal to 44 and 20% DM respectively). The PDIN and PDIE (that represent, respectively, the nitrogen or the energy limiting protein digestible in the small intestine) show different values for the 5 feeds and a marked imbalance between PDIN and PDIE contents, above all in the higher protein feeds.

**Table 1. Chemical composition and PDI content of the feeds supplied (% DM)**

| | Dry matter | Crude protein | NDF | PDIN | PDIE |
|---|---|---|---|---|---|
| Alfalfa hay | 93.3 | 15.0 | 52.6 | 9.5 | 8.3 |
| Maize | 86.4 | 9.2 | 11.4 | 8.2 | 12.0 |
| Barley | 89.6 | 10.9 | 22.8 | 7.9 | 10.2 |
| Faba bean | 86.1 | 27.0 | 20.4 | 17.5 | 10.4 |
| Sunflower cake | 89.4 | 31.9 | 44.3 | 21.8 | 11.1 |

Dry matter intake (Table 2) was high and similar between the two genotypes. Both groups showed a marked preference for corn and barley. Moreover, these grains were generally consumed within minutes of administration. This normally leads to the excess of starch in the rumen and to the consequent high production of volatile fatty acids which can be potential harmful. However, the goats did not show any evident ill-effects. Moreover, blood parameters related to lipidic and protein metabolism and hepatic functionality stayed within the normal range. The feeding behaviour observed during the 24 hours could explain the good tolerance showed by goats to the high energy inputs. Hay was consumed constantly, even in little quantity, compared to concentrate feeds, thus reaching, in both groups, the highest levels of intake. It seems that the ingestion of hay have exercised a "curative" effect that enabled the goats to continue to take in very high levels of energy and protein, without manifesting metabolic disorders.

**Table 2. DM intake (g/d) and percentages of each feed in the selected diets (% DM)**

| | AA | FF | P |
|---|---|---|---|
| DM intake | 2526.4 ± 129.2 | 2654.1 ± 115.3 | 0.133 |
| Alfalfa hay | 38.0 ± 2.6 | 34.8 ± 3.3 | 0.043 |
| Barley | 18.4 ± 4.1 | 21.2 ± 1.2 | 0.127 |
| Faba bean | 9.0 ± 2.0 | 12.8 ± 3.3 | 0.016 |
| Maize | 22.9 ± 2.6 | 19.9 ± 2.5 | 0.030 |
| Sunflower cake | 10.4 ± 2.2 | 11.3 ± 5.7 | 0.667 |

Food selection was different between the two genotypes. The AA goats selected more hay and maize and less faba bean, compared to FF goats. Surprisingly, this behaviour has no effect on the composition of the diet ingested by each group (Table 3), even if each group was selecting a different combination of feeds. More surprising, the values of PDIN and PDIE, apart form being very similar between groups and periods, were also highly balanced in the selected diets. The French PDI system, designed to optimise microbial protein synthesis in the rumen, recommend that the PDIN and the PDIE to be as similar as possible (Vérite and Peyraud, 1988). In our experimental conditions, the feeds strongly unbalanced in terms of PDIN and PDIE with values that respectively oscillate between 8.0% to 24.5% and from 9.4% to 12.8%, however, the goats, throughout a selective behaviour, were able to build a diet in which the levels of the two parameters were equal.

**Table 3. Proximal analysis of the food consumed (% DM)**

| | AA | FF | |
|---|---|---|---|
| Crude protein | 15.7 ± 0.6 | 16.5 ± 0.8 | 0.068 |
| NDF | 33.5 ± 0.6 | 32.8 ± 1.3 | 0.217 |
| PDIN | 10.9 ± 0.4 | 11.3 ± 0.6 | 0.103 |
| PDIE | 10.0 ± 0.1 | 10.0 ± 0.1 | 0.419 |

## 2. Production and composition of milk

The normalized milk production was significantly higher in group AA (Table 4). To our knowledge, there are no reports in literature of similar productive differences between AA and FF goats. As all the animals, at the beginning of the trial, were homogeneous in terms of milk production and lactation stage, it is possible that during the experimental period the efficiency of food transformation by FF group worsened, as a consequence of the very high intakes. Moreover, it must be highlighted that the percentage of fat, in both groups, was very low. The low fat content in the milk could be related to the very high proportion of concentrated feed in the diet (on average 76% of the dry matter). In this regard, according to INRA guidelines, to maintain a good level of milk fat, cereals should not represent more than 50% of the total dry matter and in any case forage should never be less than 40% of the ration (Morand-Fehr and Sauvant, 1988). The percentage of fat was higher in the FF group, whereas opposite results are reported in literature (Grosclaude *et al.*, 1994; Barbieri *et al.*, 1995; Chilliard *et al*., 2006). As milk fat represents a component closely linked to production levels, it is more likely that the higher percentage observed is the result of a "concentration" effect in the FF group milk, rather than causes of genetic origin.

**Table 4. Composition and normalized milk production†**

| | AA | FF | P |
|---|---|---|---|
| FCM (g/d) | 1057.7 ± 182.6 | 736.8 ± 139.8 | 0.001 |
| Fat (%) | 2.4 ± 0.1 | 2.8 ± 0.2 | <0.001 |
| Protein (%) | 3.5 ± 0.2 | 3.2 ± 0.2 | 0.005 |
| Lactose (%) | 4.4 ± 0.1 | 4.6 ± 0.1 | 0.009 |
| Urea (mg/dl) | 48.8 ± 4.4 | 59.7 ± 8.0 | 0.008 |
| Casein (%) | 2.7 ± 0.1 | 2.4 ± 0.2 | 0.006 |

†Milk corrected for 3.5% fat (Pulina *et al.*, 1991).

Lactose was significantly greater in the FF group, although the differences were small. The statistical significance was most probably related to the low individual variability that is typical for this parameter (Pulina *et al.*, 2005). It would be difficult to justify such small differences based on genetic characteristics or diet.

The percentages of protein and casein, as expected, were higher in AA group than in the FF group. In fact, strong alleles, such as A, are associated to high levels of alpha-s1 casein (3.5 g/l per allele), whereas weak alleles, such as F, are associated with lower levels (0.45-0.60 g/l per allele; Martin *et al.*, 1999). Therefore, the AA goats should have produced milk with a casein content of about 0.6

percentage points more than the FF goats. In our experimental conditions, we observed only a 0.3 percentage points higher in group AA. The difference between the expected and observed values could be related to a "concentration" effect of lower milk productions observed in group FF. Important differences between groups were observed for urea levels, significantly greater in FF milk.

## IV – Conclusions

The AA goats produced more milk, containing more casein and less urea, compared to FF goats, unless the identical DM intake and chemical composition of the diets. Our findings lead us to hypothesize that the better performances of AA goats should be linked to a more efficient feed utilization at ruminal level, reached modulating their behaviour to optimise the starch and protein input, probably improving the synthesis of microbial protein. In our experimental conditions, goats with a different genotype at the alpha-s1 casein locus, seem able to adapt their feeding behaviour to the aptitude to produce different quantities of casein.

## References


**Andrieu J., Demarquilly C. and Sauvant D., 1988.** Table de la valeur nutritive des aliments. In: Jarrige R. (ed.). *Alimentation des Bovins, Ovins et Caprins*. Paris, France: INRA. p. 351-465.

**AOAC, 1990.** *Official Methods of Analysis*, 15th edn. Arlington, VA, USA: Assoc. Off. Anal. Chem.

**Barbieri M.E., Manfredi E., Elsen J.M., Ricordeau G., Bouillon J., Grosclaude F., Mahé M.F. and Bibé B., 1995.** Influence du locus de la caséine αs1 sur les performances laitières et les paramètres génétiques des chèvres en race Alpine. In: *Gen. Sel. Evol.*, 27. p. 437-450.

**Chilliard Y., Rouel J. and Leroux C., 2006.** Goat's alpha-s1 casein genotype influences its milk fatty acid composition and delta-9 desaturation ratios. In: *Anim. Feed Sci. Technol.*, 131. p. 474-487.

**Erhardt G., Juszczak J., Panicke L. and Krick-Saleck H., 1998.** Genetic polymorphism of milk proteins in Polish Red Cattle: A new genetic variant of β-lactoglobulin. In: *J. Anim. Breed. Gen.*, 115. p. 63-71.

**Grosclaude F., Ricordeau G., Martin P., Remeuf F., Vassal L. and Bouillon J., 1994.** Du gène au fromage: Le polymorphisme de la caséine $\alpha_{s1}$ caprine, ses effets, son évolution. In: *INRA Prod. Anim.*, 7. p. 3-19.

**FIL-IDF, 1964.** *Determination of the Casein Content of Milk*. FIL-IDF Standard No. 29. Brussels, Belgium: International Dairy Federation.

**Leroux C., Mazure N. and Martin P., 1992.** Mutation away from splice site recognition sequences might cis-modulate alternative splicing of goat αs1-casein transcripts structural organization of the relevant gene. In: *J. Biol. Chem.*, 267. p. 6147-6157.

**Martin P., Ollivier-Bousquet M. and Grosclaude F., 1999.** Genetic polymorphism of caseins: A tool to investigate casein micelle organization. In: *Intern. Dairy J.*, 9. p. 163-171.

**Morand-Fehr P. and Sauvant D., 1988.** Alimentation des caprins. In: Jarrige R. (ed.). *Alimentation des Bovins, Ovins et Caprins*. Paris, France: INRA. p. 281-304.

**Offner A., Bach A. and Savant D., 2003.** Quantitative review of *in situ* starch degradation in the rumen. In: *Anim. Feed and Sci. Technol.*, 106. p. 81-93.

**Pulina G., Battacone G. and Nudda A., 2005.** La produzione di latte nei caprini. In: Pulina G. (ed.). *L'Alimentazione della Capra da Latte*. Bologna, Italy: Avenue Media. p. 9-25.

**Pulina G., Cannas A., Serra A. and Vallebella R., 1991.** Determinazione e stima del valore energetico di latte di capre di razza Sarda. In: *Atti XLV Congresso SIS Vet.*, Altavilla Milicia (Palermo, Italy). p. 1779-1781.

**Vérite R. and Peyraud J.L., 1988.** Nutrition azotée. In: Jarrige R. (ed.). *Alimentation des Bovins, Ovins et Caprins*. Paris, France: INRA. p. 75-93.