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in

Baumont R. (ed.), Carrère P. (ed.), Jouven M. (ed.), Lombardi G. (ed.), López-Francos A. (ed.), Martin B. (ed.), Peeters A. (ed.), Porqueddu C. (ed.).
Forage resources and ecosystem services provided by Mountain and Mediterranean grasslands and rangelands

Zaragoza : CIHEAM / INRA / FAO / VetAgro Sup Clermont-Ferrand / Montpellier SupAgro
Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 109

2014
pages 345-348

Effect of growth hormone polymorphisms and of diet on the productive performance and technological quality of Serra da Estrela sheep milk

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Abstract. Sheep milk composition influences, not only cheese yield, but also cheese quality by providing different milk clotting behaviour. This work addresses the effect of the growth hormone (GH) polymorphisms and diet on milk production, composition and technological properties of 83 Serra da Estrela sheep. The daily production and milk properties were analysed at 42, 60 and 90 days of their first lactation. Samples were analysed for pH, and fat, protein and total solids contents. The potential cheese making yield was evaluated and the milk technological properties were assessed by Optigraph, measuring the time to onset of flocculation, casein micelle aggregation rate and gel firmness. There were no differences between the GH genotypes for any of the milk parameters evaluated, which might be due to the low adaptability of primiparous sheep to machine milking. The lighter sheep fed only pasture, hay and silage (group R) showed significantly higher protein, non-fat milk, and solids contents, and also higher values of AR, A40 and lower OK20, especially evident in the genotypes AA and AE.

Keywords. Growth hormone – Sheep milk – Milk technological aptitude**.**

Effet des polymorphismes de l'hormone de croissance et du régime alimentaire sur la performance productive et la qualité technologique du lait de brebis Serra da Estrela

Résumé. *La composition du lait de brebis influence, non seulement le rendement fromager, mais aussi la qualité du fromage en provoquant un comportement du lait différent à la coagulation. Cette étude porte sur l'effet des polymorphismes de l'hormone de croissance (GH) et du régime alimentaire sur la production, la composition ainsi que sur les propriétés technologiques du lait de 83 brebis de la race Serra da Estrela. La production journalière ainsi que les propriétés du lait ont été analysées à 42, 60 et 90 jours pendant la première lactation. Le pH et la teneur en matière grasse, en protéine brute et en solides totaux ont été analysés. Le rendement potentiel de la fabrication de fromage a été analysé et les propriétés technologiques du lait ont été évaluées par Optigraph, en mesurant le temps pour le démarrage de la floculation, le taux d'agrégation des micelles de caséine et la fermeté du gel. Il n'y avait pas de différences entre les génotypes GH pour aucun des paramètres évalués dans le lait, ce qui pourrait être du à la faible capacité d'adaptation des brebis primipares à la traite mécanique. Les brebis plus légères alimentées avec du pâturage, du foin et de l'ensilage (groupe R) ont présenté des teneurs en protéines, en lait écrémé, et en solides totaux significativement plus élevés, de même que des valeurs plus élevées de l'AR et A40 et des valeurs inférieures de OK20, particulièrement pour les brebis des génotypes AA et AE.*

Mots-clés. *Hormone de croissance – Lait de brebis – Propriétés technologiques du lait.*

I – Introduction

Growth hormone (GH) is one of the main hormones involved in mammary development both in puberty and gestation (Sejrsen *et al.*, 1999) in ruminants. During lactation, GH takes part in the modeling of the mammary gland towards a higher milk synthesis rate and an increase of persistency of the lactation curve (Etherton and Bauman, 1998). The biological effects of the GH are mediated by GHR-JAK2-STAT-IGF-1 pathway (Carter-Su *et al.*, 2000). Signal transducer and activators of transcription (STATs) play an important role in the GH-regulated mammary modulation. Specific mammary activation of STAT5A regulates the development, function, and survival of mammary epithelial cells by inducing distinct subsets of target genes in mammary epithelial cells, including whey acid proteins and caseins (Clarkson *et al.*, 2006), that might lead to differences in milk composition, and thus in processing performance of the milk (Bencini and Pulina, 1997), i.e., its capability to be transformed into high quality dairy products.

In our previous work we also found significant associations between GH genotypes and milk production and composition in Serra da Estrela ewes (Marques *et al*., 2006). Differences in composition of milk, rather than influencing only the cheese making yield, may also influence the cheese quality by providing different milk clotting behaviour and thereby the subsequent stages, curd draining and cheese ripening. Decision makers should be aware of the milk clotting properties when deciding the introduction, in genetic programmes of selection markers, aiming to improve milk yield, and thus advising cheese makers of the need to adapt processing to overcome the differences that may influence the quality of the cheese (Martins *et al.*, 2009).

This work addresses the effect of the growth hormone (GH) polymorphisms and feeding regime at puberty (referred as growth rate) on milk production, composition and technological properties of Serra da Estrela sheep throughout the first lactation.

II – Materials and methods

Six SNPs [X12546: g.649C>G (F2L), g.668C>T (R9C), g.704C>G (L21V), g.1057A>G (S63G), g.1062G>C (K64N), g.1852G>A (G160S)] at the GH2-Z copy was genotyped by a single-base extension (SNaPshot) method (Marques *et al.*, 2012). Eighty-three ewe lambs carrying GH2-Z copy genotypes AA (R9R/S63S; n = 28), AB (R9C/S63S; n = 28), and AE (R9R/S63G; n = 27) were reared during pre-pubertal phase and gestation under two different feeding regimes to obtain a restricted growth rate of 79 g/day (group R - 42 ewe lambs) and a normal growth rate of 106 g (group N - 41 ewe lambs). The ewe lambs rotationally graze a pasture consisting of *Lolium perene*, *Festuca arundinacea*, *Tripholium* spp., and *Medicago* spp., and also received hay and corn silage. The lambs of group N had also access to a supplement of corn grain/sunflower meal. During lactation all sheep grazed the same pasture and received a supplement covering dairy sheep requirements to produce 1.5 L milk/day.

Sheep were milked twice a day after weaning of their lambs at 42 days of lactation. The daily production and milk properties were analysed at 42, 60 and 90 days of lactation. Proportionate samples representing the two daily milking were analysed for fat, protein, and total solids contents (Milko Scan 113B, Foss, Denmark), and pH (Metrohm 713, Switzerland). The evaluation of the cheese making yield (CY) was carried out according to Remeuf *et al.* (1989) and the milk technological properties were assessed by Optigraph (Ysebaert, France) as described by Martins *et al.* (2009), for the following parameters: R (clotting time, min), firmness measures (Volt) after 20 minutes (A20) or 40 minutes (A40) of trial, and after a 2R (2 x clotting time, AR) period and OK20 (rate of firming, min).

Data were analysed using the MIXED procedure of SAS for repeated measures (SAS, 2008) with sheep nested within genotype and growth rate, using an unstructured covariance matrix. A multiple comparison of means was performed using Tukey's test ($P<0.05$).

III – Results and discussion

There were no differences between the GH genotypes for any of the parameters evaluated (Table 1). The lack of differences between genotypes might be due to the low adaptability of primiparous sheep to machine milking since the value of 471 ml/day obtained at the first week after weaning, is well below the 1200 ml/day of suckled milk which, according to Degen *et al.* (2003) corresponds to the average growth of lambs of 230 g/day up to weaning.

The sheep raised at the lowest growth rate (R group) showed a significantly higher protein ($P<0.01$) and non-fat solids ($P<0.001$) contents in milk, which influenced its technological characteristics, i.e., significantly higher values of AR ($P<0.05$), A40 ($P<0.01$) and lower OK20 ($P<0.05$).

Throughout lactation, the milk of all primiparous sheep showed the expected significant increase in the values of their chemical composition ($P<0.001$), especially after 60 days of lactation. Technological parameters followed the evolution of the chemical composition, with higher values of cheese yield and firmness of the curd achieved in less time, as lactation day and the protein content of milk increased (Table 1).

Table 1. Effect of *GH* genotype (GH), growth rate (GR) and lactation day on milk yield, chemical composition and clotting properties in Serra da Estrela primiparous sheep

		Milk (ml/ day)	Fat (% w/w)	Protein (% w/w)	Non-fat solids (% w/w)	CY (g DM/ 100ml)	pH	R (min.)	AR (V)	A_{20} (V)	A_{40} (V)	0K20 (min.)
GH	AA	482	6.99	6.24	12.40	16.86	6.62	14.48	10.07	4.59	13.70	9.88
	AB	387	6.82	6.03	12.17	16.45	6.62	14.10	9.56	4.92	13.65	9.08
	AE	453	6.86	6.07	12.24	16.46	6.64	13.78	9.21	5.12	13.45	9.82
GR	R	420	6.87	6.30 ^{b}	12.46 ^{b}	16.60	6.62	14.11	10.45 ^{b}	5.34	14.88 ^{b}	8.45 ^{a}
	N	453	6.91	5.93 ^{a}	12.08 ^{a}	16.61	6.64	14.21	9.00 ^{a}	4.49	12.64 ^{a}	10.49 ^{b}
Day	42	471 ^{b}	5.62 ^{a}	5.77 ^{a}	12.20 ^{a}	14.59 ^{a}	6.65 ^{b}	13.36 ^{a}	7.69 ^{a}	4.34 ^{a}	11.55 ^{a}	12.53 ^{b}
	60	482 ^{b}	6.79 ^{b}	5.89 ^{a}	12.06 ^{a}	16.23 ^{b}	6.66 ^{b}	14.11 ^{ab}	8.75 ^{a}	4.46 ^{a}	12.74 ^{a}	9.76 ^{ab}
	90	370 ^{a}	8.26 ^{c}	6.69 ^{b}	12.54 ^{b}	18.95 ^{c}	6.56 ^{a}	14.89 ^{b}	12.40 ^{b}	5.83 ^{b}	16.51 ^{b}	6.48 ^{a}

Note: In each column, different letters mean significant differences ($P<0.05$).

Although interaction genotype × day was not significant for milk production (not shown), it was observed that sheep from AA genotype gave more milk at days 42 and 60 of lactation than at day 90. The decrease in milk production was less noticed in AB and AE genotypes, which might indicate an increased persistency of lactation for those genotypes.

The interaction genotype × growth rate tended to be significant for protein content ($P<0.10$), and was significant for AR ($P<0.05$) and 0K20 ($P<0.05$). Milk from R group sheep tended to be richer in protein content, in AA and AE genotypes; which is reflected in AR and OK20 parameters, namely for AA sheep where those differences are significant (Fig. 1).

It was also observed a slightly significant interaction genotype * day of lactation for protein content ($P<0.10$) and a significant one for total protein ($P<0.05$). At 42 days of lactation total protein was respectively 19.5, 25.8 and 30.7 g/day for AB, AE and AA sheep ($P<0.05$), having not varied throughout lactation except for the sheep of AA genotype in which decreased from day 60 to 90 of lactation (31.5 *vs* 24.6 g/day; $P<0.05$). These findings are in agreement with possible effects of GH in the expression of milk protein genes (Clarkson *et al.*, 2006).

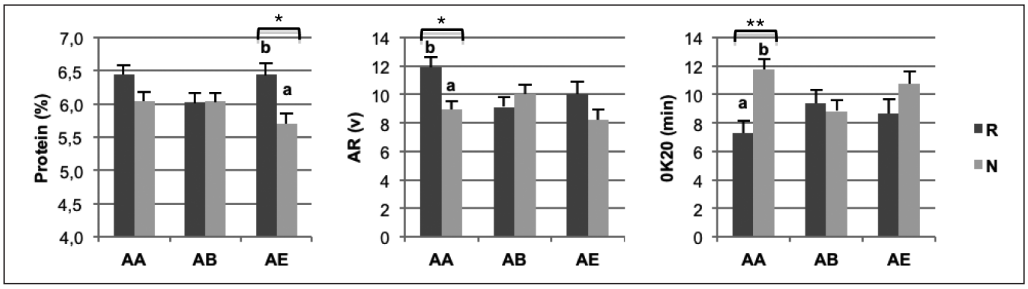

Fig. 1. Effects of the interaction genotype (AA, AB or AE) * growth rate (R or N) on milk protein content, firmness of the cloth (AR) and rate of firming (0K20) Serra da Estrela primiparous sheep. Note: * - P<0.05; ** - P<0.01.

IV – Conclusions

There were no differences between the GH genotypes for any of the milk parameters evaluated. Results indicate that the sheep of AA genotype produce more milk in early lactation while AB and AE seem to have greater persistency of lactation. However the assessment of milk production was affected by poor adaptability of primiparous sheep to mechanical milking.

The lighter sheep fed only pasture, hay and silage (group R) showed significantly higher protein and non-fat milk in higher solids contents, and also higher values of AR, A40 and lower OK20, especially evident in the genotypes AA and AE.

Acknowledgments

This work is funded by national funds through FCT – Foundation for Science and Technology under the project PTDC/CVT/112054/2009.

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