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Marques M.R., Belo C.

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M.R. Marques and C.C. Belo
Departamento de Ovinicultura e Caprinicultura, Estação Zootécnica Nacional,
Fonte Boa, 2000-763 Vale de Santarém, Portugal

SUMMARY – One hundred and eight (108) grazing dairy 'Serra da Estrela' ewes were used in two lambing seasons (Autumn and Spring) to evaluate the effects of 4 levels of crushed corn (0, 300, 600 and 900 g) on milk composition and fatty acid (FA) composition of milk fat at 40, 60, 80 and 100 days of lactation. Milk fat and protein content did not change significantly with increasing levels of crushed corn. In Autumn, linolenic acid ($C_{18:3}$), conjugated linoleic acid (CLA), branched-FA (BCFA) and odd-chain FA (OCFA) decreased significantly with supplementation. In Spring, medium chain FA ($C_{12:0}$-$C_{15:1}$) and saturated FA (SFA) significantly increased and $C_{18:3}$ and OCFA decreased with increasing supplementation. Short chain FA ($C_{4:0}$-$C_{10:1}$), $C_{12:0}$-$C_{15:1}$ and $C_{18:3}$ decreased significantly throughout the Spring lactation period, while long chain FA (LCFA), monounsaturated FA (MUFA), linoleic acid ($C_{18:2}$), BCFA and OCFA increased.

Key words: Grazing dairy ewes, corn supplementation, milk composition, milk fatty acids.

RESUME – *"Composition des acides gras du lait de brebis 'Serra da Estrela' au pâturage supplémentées avec quatre niveaux de maïs aplati". Cent huit (108) brebis 'Serra da Estrela' au pâturage ont été utilisées à deux époques de mise bas (Automne et Printemps) pour évaluer l'effet de 4 niveaux de maïs aplati (0, 300, 600 et 900 g) sur la composition du lait et de la matière grasse en acides gras (AG) à 40, 60, 80 et 100 jours d'allaitement. Les taux butyreux et protéique du lait n'ont pas changé significativement avec l'augmentation du niveau de maïs aplati. En automne, les acides linolénique ($C_{18:3}$) et linoléique conjugué (ALC), les méthyl-AG et les AG à chaîne impaire ont diminué significativement avec la supplémentation énergétique. En mars, les AG à chaîne moyenne ($C_{12:0}$-$C_{15:1}$) et les AG saturés (AGS) ont augmenté significativement et le $C_{18:3}$ et les AG à chaîne impaire ont diminué avec l'augmentation du niveau de supplémentation. Les AG à chaîne courte ($C_{4:0}$-$C_{10:1}$), les AG $C_{12:0}$-$C_{15:1}$ et le $C_{18:3}$ ont diminué significativement pendant la période de l'allaitement en automne, tandis que les AG à chaîne longue (AGCL), les AG monoinsaturés, l'acide linoléique ($C_{18:2}$), les méthyl-AG et les AG à chaîne impaire ont augmenté.*

Mots-clés : *Brebis au pâturage, supplémentation énergétique, composition du lait, acides gras du lait.*

Introduction

The type and level of concentrate, the herbage quality and its availability and the stage of lactation have significant effects on milk yield and quality and on the cheese making properties of milk produced under grazing dairy systems. This evidence is particularly important for ewe milk, which is exclusively used in dairy products. Traditionally, 'Serra da Estrela' ewes were not supplemented with concentrates, although on recent years this practice has become common. Concentrate diets promote milk production and milk protein content (Susin *et al.*, 1995) both factors contributing to increased cheese-making efficiency. However, this type of diets often results in milk fat depression. High levels of concentrate changes ruminal fermentation, with decreasing ruminal pH modifying microbial population and consequently the proportion of volatile fatty acids in the rumen: acetate and butyrate levels fall and propionate levels rise. A rise in propionate usage in hepatic neoglucogenesis, increases levels of glucose in blood which promotes pancreatic releases of insulin with a consequent inhibition of adipose tissue lipolyses (McGuire *et al.*, 1995). Milk fat precursors are acetate and -hydroxibutyrate (-HBA) from ruminal fermentation and long chain fatty acids (LCFA) from diet or adipose tissue mobilization (Dils, 1986). So, increasing levels of concentrate may contribute to changes in fatty acids (FA) composition of milk fat as well as in cheese (Ha and Lindsay, 1991), since there are no significant alterations during ripening (Buchin *et al.*, 1998).

The objective of this work was to evaluate the effect of 4 levels of crushed corn (0, 300, 600 and 900 g) on milk production, milk composition and FA composition of milk fat at 40, 60, 80 and 100 days of lactation in grazing 'Serra da Estrela' dairy ewes.

Materials and methods

Milk production and composition were measured on lactating 'Serra da Estrela' ewes in two lambing seasons (Autumn and Spring). Thirteen ewes were allocated to each supplementation group considering the homogeneity in time of lambing, number of nursing lambs and body condition score (BCS). The lambs were nursed till the 21^{st} day and ewes were milked once a day between the 21^{st} and the 42^{nd} day. From the 42^{nd} day on, ewes were milked twice a day. After the 21^{st} day of lactation, ewes were offered a supplementation of 0, 300, 600 or 900 g of crushed corn (corn with 14.2 MJ ME/kg DM) while grazing a pasture of *Lolium perene*, *Dactylis glomerata*, *Medicago* spp. and *Tripholium fragiferum* in both lambing seasons. Chemical composition and nutritive value of pasture was the following: dry matter content (DM) – 21.4, 20.0, 21.4 and 25.6%; acid detergent fibre (ADF) – 20.9, 28.8, 25.4 and 32.1%; dry matter digestibility (DMD) – 71.8, 73.8, 64.2 and 63.0; and crude protein (CP) – 19.5, 21.8, 13.2, for the 21^{st} to 42^{nd} day of lactation and for the 42^{nd} day to the end of lactation periods in Autumn and for the same periods in Spring, respectively.

Body weight (BW) and BCS of the ewes were recorded on the 21^{st}, 42^{nd} and 100^{th} days of lactation. Milk samples of bulk milk were collected at days 40, 60, 80 and 100 of lactation, and milk fat, protein, lactose and total solids content were determined by Milk-O-Scan.

FA composition of milk fat was evaluated by gas chromatography. Total lipids extraction was performed by modified Folch method (Sperry and Brand, 1955). For the preparation of FA methyl esters (FAME) of milk fat, 120 l of fat was dissolved in 4.5 ml of n-heptane, and 2.5 ml of 2*N* potassium hydroxide in methanol (Christopherson and Glass, 1969). FAME solution (1 l) was analysed in a Hewlett-Packard 5890 SERIES II gas chromatograph with a fused silica capillary column [SGE 25QC2/BP20 0.25; phase BP20 (polar); 25 m x 0.22 mm x 0.33 m film thickness] and a Hewlett-Packard HP 3396 SERIES II integrator. The injector and the detector were maintained at 230∝C and 280∝C, respectively. The column temperature programme used was: 70∝C for 2.5 min, then increased 8∝C/min to 190∝C and held for 40 min. Carrier gas was nitrogen (17.4 ml/min).

Data was analysed by ANOVA using a factorial in a split-plot in time design to test repeated measures in the same ewes.

Results and discussion

BW and BCS were not influenced by the level of crushed corn in the diet. In Autumn, BCS increased from the 42^{nd} day to the end of lactation (2.19 *vs* 2.68; P<0.05). In Spring, BCS increased from the 21^{st} to the 42^{nd} day of lactation (2.23 *vs* 2.53; P<0.05) and remained constant afterwards.

Milk production tended to be higher in ewes supplemented with 600 g of crushed corn in Autumn, and with 300 g in Spring (Table 1). However, milk composition did not change with supplementation, results that are in agreement with those of Avondo *et al*. (1995). Over the lactation period, milk yield and lactose content decreased significantly and fat (very low on the 40^{th} day of lactation), protein and total solids contents increased significantly throughout the lactation period in both lambing seasons.

Values for FA composition of milk fat presented in Table 1 follow the same trends reported for ewes (Muir *et al*., 1993) and goats (Alonso *et al*., 1999) milk fat. The $C_{4:0}$-$C_{10:1}$, the $C_{12:0}$-$C_{15:1}$ and the SFA levels increased with the energetic supplementation in both seasons, but only significantly in Spring for $C_{12:0}$-$C_{15:1}$ and SFA (P<0.05). In Autumn, $C_{18:3}$, conjugated linoleic acid (CLA), BCFA and OCFA levels decreased significantly with supplementation. Levels of $C_{18:3}$ and OCFA also decreased significantly with supplementation in Spring.

Over the lactation period, in Autumn, $C_{4:0}$-$C_{10:1}$, $C_{12:0}$-$C_{15:1}$, $C_{18:3}$, $C_{18:2}$ and OCFA levels remained constant, while in Spring the first three decreased and $C_{18:2}$ and OCFA levels increased significantly (P<0.05). SFA decreased significantly in both seasons and C_{16} only decreased in Autumn. On both seasons, MUFA, CLA and BCFA increased, but in Spring CLA and BCFA decreased their levels between the 80^{th} and the 100^{th} days.

Table 1. Milk yield, milk composition and fatty acid (FA) composition of milk fat (mol/100 mol of total FA)†

	Supplementation group – S (g)				Lactation day – D				MSE	Significance		
	0	300	600	900	40	60	80	100		S	D	S*D
Autumn season												
Milk yield (ml/day)	509^{a}	610^{ab}	738^{b}	617^{b}	902^{c}	648^{b}	491^{a}	433^{a}	11		***	
Fat (%)	7.3	7.2	7.0	6.8	3.4^{a}	7.7^{b}	8.8^{c}	8.4^{c}	0.2	ns	***	ns
Protein (%)	6.4	6.4	6.5	6.7	5.7^{a}	6.4^{b}	6.8^{c}	7.0^{c}	0.0	ns	***	ns
Lactose (%)	4.4	4.3	4.5	4.2	5.0^{c}	4.3^{b}	4.0^{a}	4.1^{a}	0.0	ns	***	ns
T. Solids (%)	18.8	18.7	18.7	18.5	14.8^{a}	19.1^{b}	20.5^{c}	20.2^{c}	0.2	ns	***	ns
$C_{4:0}$-$C_{10:1}$	11.4	11.5	12.9	12.9	12.7	12.4	12.2	11.5	0.3	ns	ns	ns
$C_{12:0}$-$C_{15:1}$	16.8	17.1	17.6	19.0	17.7	17.2	17.7	17.9	0.1	ns	ns	ns
C_{16}	24.8	25.2	24.8	25.7	26.6^{c}	24.2^{a}	25.2^{b}	24.6^{ab}	0.2	ns	***	ns
LCFA	46.9	46.1	44.7	42.4	43.0	46.2	44.8	46.0	0.4	ns	ns	ns
SFA	61.6	63.0	64.0	65.5	65.5^{b}	63.2^{a}	63.1^{a}	62.2^{a}	0.3	ns	*	ns
MUFA	27.2	26.6	26.1	25.2	25.2	26.6	26.2	27.1	0.3	ns	ns	ns
$C_{18:2}$	2.4	2.6	2.5	2.5	2.6	2.5	2.5	2.5	0.0	ns	ns	ns
$C_{18:3}$	1.4^{c}	1.2^{bc}	1.1^{ab}	0.8^{a}	1.0	1.2	1.1	1.1	0.0	*	ns	ns
CLA	1.5^{b}	1.3^{ab}	1.1^{a}	1.0^{a}	1.0^{a}	1.3^{b}	1.2^{b}	1.3^{b}	0.0		**	ns
BCFA	4.3^{c}	4.2^{bc}	3.8^{ab}	3.6^{a}	3.6^{a}	4.1^{b}	4.0^{b}	4.2^{b}	0.0	*	**	ns
OCFA	2.6^{b}	2.5^{b}	2.3^{a}	2.2^{a}	2.4	2.3	2.4	2.4	0.0	**	ns	ns
Spring season												
Milk yield (ml/day)	509^{a}	669^{b}	542^{ab}	609^{ab}	741^{d}	650^{c}	544^{b}	394^{a}	12		***	ns
Fat (%)	6.7	6.5	6.6	6.8	3.3^{a}	7.9^{b}	7.8^{b}	7.5^{b}	0.2	ns	***	ns
Protein (%)	5.9	5.9	5.9	5.9	5.2^{a}	5.9^{b}	6.2^{c}	6.3^{c}	0.0	ns	***	**
Lactose (%)	4.6	4.7	4.5	4.6	4.8^{c}	4.4^{a}	4.6^{b}	4.5^{ab}	0.0	ns	***	ns
T. Solids (%)	15.8	17.8	16.1	18.0	14.1^{a}	17.7^{b}	17.8^{b}	18.1^{b}	0.2	ns	***	ns
$C_{4:0}$-$C_{10:1}$	8.6	9.8	10.5	11.2	11.3^{b}	10.3^{b}	10.5^{b}	.0^{a}	0.2	ns	***	ns
$C_{12:0}$-$C_{15:1}$	15.1^{a}	16.1^{ab}	16.4^{bc}	17.5^{c}	17.1^{b}	16.0^{a}	16.1^{a}	16.0^{a}	0.1	*	**	ns
C_{16}	26.4	27.2	27.1	26.1	26.3	26.7	26.6	27.1	0.2	ns	ns	ns
LCFA	49.8	46.8	46.0	45.2	45.2^{a}	47.0^{a}	46.8^{a}	48.8^{b}	0.3	ns	**	ns
SFA	60.5^{a}	63.0^{b}	64.0^{b}	64.1^{b}	65.8^{b}	62.4^{a}	62.0^{a}	61.4^{a}	0.2	*	***	ns
MUFA	28.6	27.1	26.5	26.3	24.7^{a}	27.4^{b}	27.3^{b}	29.2^{c}	0.2	ns	***	ns
$C_{18:2}$	3.1	2.9	3.0	3.0	2.9^{a}	2.9^{a}	.2^{b}	3.0^{ab}	0.0	ns	**	ns
$C_{18:3}$	1.4^{b}	1.1^{ab}	1.0^{a}	0.9^{a}	1.1^{b}	1.2^{b}	.2^{b}	0.9^{a}	0.0	*	***	ns
CLA	1.2	1.1	1.1	1.1	1.0^{a}	1.3^{b}	.3^{b}	0.9^{a}	0.0	ns	***	ns
BCFA	4.2	3.8	3.7	3.8	3.5^{a}	4.1^{b}	4.2^{b}	3.8^{a}	0.0	ns	***	ns
OCFA	2.8^{c}	2.6^{b}	2.4^{a}	2.4^{a}	2.5^{a}	2.5^{a}	2.5^{a}	2.7^{b}	0.0	**	**	ns

†LCFA: long chain FA; SFA: saturated FA; MUFA: monounsaturated FA; CLA: conjugated linoleic acid; BCFA: branched-FA; OCFA: odd-chain FA; MSE: Mean Standard Error.

[a,b,c,d]Means with different superscripts differ significantly; ns: P>0.10; P<0.10; *P<0.05; **P<0.01; ***P<0.001.

The $C_{4:0}$-$C_{10:1}$ and $C_{12:0}$-$C_{15:1}$ FA levels suggest that ewes receiving increasing levels of crushed corn would probably have had a greater feed intake, since these FA are originated in "*de novo*" synthesis. Results for LCFA suggest the hypothesis that ewes fed with higher levels of supplement had lower needs of fat tissue mobilization, which can be corroborated by the results of BCS. Moreover, supplementation tended to induce milk fat richer in SFA and poorer in $C_{18:3}$, CLA, BCFA and OCFA, strongly suggesting a substitution effect of corn. The results obtained can be supported by the following references: (i) the decrease on the content of $C_{18:3}$ with supplementation is related with the results of Doreau *et al.* (1987) and Carlier *et al.* (1991) that indicated levels of $C_{18:3}$ between 47 and 79% for grass and 0.9% for corn, respectively; (ii) the increase on the levels of C_{16} and SFA seems to be related with the pre-formed FA from diet (corn supplementation) since the other possible source (fat tissue mobilization) was small over the lactation period; (iii) the content of CLA in milk fat rises with pasture intake (Kelly *et al.*, 1998); and (iv) the lower content of $C_{4:0}$-$C_{10:0}$ for ewes lambing in Spring can be linked to a decrease in pasture intake due to its lower nutritive value during this season (Table 1).

Conclusions

Under the conditions of this study, supplementation with crushed corn did not improve significantly milk production and did not change milk composition. Supplementation seems to have a detrimental effect on FA composition of milk fat, since SFA increased with increasing levels of supplementation and $C_{18:3}$, CLA, BCFA and OCFA decreased. FA composition of milk fat seems to reflect the nutritive value of the pasture and was influenced by its intake level.

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