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SUMMARY – To study whether selectivity for plants or parts of plants affect the fatty acid composition of milk fat, two groups of five mid lactation ewes were used: the "Grass" group – not supplemented, and the "Maize" group – supplemented with 600 g of crushed maize/day. Five paddocks were successively grazed, each containing five strips of the following species seeded separately: *Trifolium repens*, *Lolium perenne*, *Medicago sativa*, *Festuca arundinacea* and *Trifolium fragiferum*. Milk composition and fatty acid (FA) composition of milk fat was determined at the second and at the last day in each paddock. At the beginning of the grazing period, when selectivity was higher, the contents of long chain FA, monounsaturated FA, branched-chain and odd-chain FA and the $C_{18:2}/C_{18:3}$ ratio was significantly lower and the contents of $C_{12:0}$-$C_{15:1}$ FA, saturated FA, polyunsaturated FA and conjugated linoleic acid (CLA) were significantly higher. Discriminant analysis showed that the FA profile was efficient to allocate milk samples to feeding groups accurately.

Key words: Dairy ewes, grazing, milk production, milk composition, milk fatty acids.

RESUME – *"Composition des acides gras du lait de brebis laitières au pâturage". Deux groupes de 5 brebis (le groupe "Herbe" – non supplémenté et le groupe "Maïs" – supplémenté avec 600 g de maïs aplati) ont été utilisées pour étudier l'effet de la sélectivité sur la composition de la matière grasse du lait en acides gras (AG). Cinq parcelles de* Trifolium repens*,* Lolium perenne*,* Medicago sativa*,* Festuca arundinacea *ou de* Trifolium fragiferum*, ont été pâturées successivement. La composition du lait et de la matière grasse du lait ont été évaluées au 2ème et au dernier jour pour chaque parcelle. Les teneurs du lait en AG à chaîne longue, les AG monoinsaturés, les méthyl-AG et les AG à chaîne impaire et le rapport $C_{18:2}/C_{18:3}$ ont été significativement inférieurs et celles en AG $C_{12:0}$-$C_{15:1}$, AG saturés, AG polyinsaturés et l'acide linoléique conjugué (ALC) ont été significativement plus élevés au début de la période de pâturage. L'analyse du profil des AG a permis de faire correspondre d'une manière précise les échantillons de lait au régime alimentaire.*

Mots-clés : *Brebis laitières, pâturage, production du lait, composition du lait, acides gras du lait.*

Introduction

Physical and chemical characteristics of herbage, and the type of supplement offered to dairy ruminants affect milk quality, namely fatty acids (FA) composition of milk fat (Bauman and Griinari, 2003; Chilliard *et al*., 2003). Dairy ewes, with high energy and protein requirements for milk production should receive high quality herbage with high digestibility and low in fibre and high in crude protein. When herbage quality decreases, milk production also decreases, and milk composition changes. The introduction of energetic supplements in the diet of lactating ewes may prevent milk production to decrease, but it affects fat content and its FA composition (Marques and Belo, 2001b). The control of grass and legume palatability on dairy ewes under rotational grazing conditions (Marques and Belo, 2001a) allowed to conclude that legumes were preferred regardless of supplementation, and that feeding activity increased from 65 to 95% of time spend grazing between the 1st and the 5th day in each paddock, along with the decrease in leaves availability of selected plant species.

In several works the effect of supplementation upon FA composition of milk fat produced by grazing ruminants was studied, but to our knowledge there are no studies on the effect of the variation of pasture quality under grazing upon milk yield and composition.

The objective of this work was to check whether selectivity for plants or parts of plants could affect milk yield and FA composition of milk fat in grazing dairy ewes with or without supplementation.

Materials and methods

During the month of June, 10 "Serra da Estrela" ewes were observed for 23 days during which they successively grazed on five 385 m^2 paddocks, each containing five strips of the following species seeded separately: white clover (*Trifolium repens*, cv. Ladino and Pitau, WC), ryegrass (*Lolium perenne*, cv. Ariki, RG), lucerne (*Medicago sativa*, cv. Aurora, LUC), fescue (*Festuca arundinacea*, cv. Clarine, FES) and strawberry clover (*Trifolium fragiferum*, cv. Palestine, SC). Ewes entered paddocks when the mean height of LUC was 25-30 cm. In average, ewes spent 5 days on each paddock. The dry matter (DM) availability of each species and their chemical composition was determined when animals entered (1st day) and when they left each paddock (5th day). Two random samples were collected from each species by throwing a 0.125 m^2 iron ring. The DM percentage, the quantity of ashes, total nitrogen and neutral detergent fibre (NDF), DM and organic matter (OM) *in vitro* digestibility, and metabolised energy were determined in these samples.

Ewes grazed from 8:00 to 12:00 and from 16:00 to 20:00. They were divided into two groups of five animals: the "Grass" group – ewes with no supplementation and the "Maize" group – ewes supplemented with 600 g of crushed maize/day. In average, ewes were in their 60th day of lactation when the trial began and were milked twice a day at 7:30 and 20:00. Daily milk production was recorded. Samples were collected on the 2nd and last day (5th day) that ewes spent in each paddock to determine fat, protein, lactose, and total solids contents by Milk-O-Scan, and FA composition of milk fat by gas chromatography as described by Marques and Belo (2001b).

Milk production, milk composition, and FA composition of milk fat were analysed by ANOVA using a factorial in a split-plot in time design to test repeated measures in the same individual. Milk FA profiles were analysed using a canonical discriminant analysis. It was carried out by a forward stepwise method of Statistical Software (Statsoft, 1995) with the objective of evaluating the reliability of milk FA profiles to allocate it to a given feeding system.

Results and discussion

Herbage biomass available the first day of grazing averaged 54.8 kg DM, this corresponds to a mean availability of 1.1 kg DM/day/ewe and a grazing pressure of 18.25 ewes/100 kg DM. Mean chemical composition of grazed species at the 1st and the 5th days is showed in Table 1.

Table 1. Dry matter (DM), crude protein (CP), neutral detergent fibre (NDF), dry matter digestibility (DMD) and metabolised energy of the grazed species

Specie†	DM (%)		CP (%)		NDF (%)		DMD (%)		Metabolised energy (MJ)	
	1st day	5th day	1st day	5th day	1st day	5th day	1st day	5th day	1st day	5th day
RG	13.35	16.67	21.26	14.05	62.19	65.08	68.88	59.58	9.46	8.01
FES	16.01	20.31	17.08	9.91	64.34	70.21	68.28	52.66	9.40	7.19
LUC	12.43	18.87	22.05	15.19	43.99	58.61	69.75	58.20	9.51	7.82
WC	10.18	11.05	25.82	26.63	35.23	36.71	78.75	78.98	10.84	10.76
SC	10.98	11.85	26.56	25.74	35.12	39.13	77.95	71.00	10.60	9.79

†RG – ryegrass; FES – fescue; LUC – lucerne; WC – white clover; SC – strawberry clover.

Between the 1st and the 5th day, DM content increased in LUC, in RG, and in FES (Table 1). Simultaneously, for the same species, CP content decreased by seven percentage points and DMD and organic matter digestibility (OMD) decreased by sixteen percentage points, approximately (Table 1). In the LUC, NDF content increased considerably from the 1st to the 5th day, providing evidence of the strong increase of the proportion of stems observed throughout the grazing period, once LUC and its leaves were preferentially consumed at the 1st day (Marques and Belo, 2001a). It was also observed that, in clover species, DM, CP and NDF contents remained constant during all period, and its DMD and OMD were high throughout the study (Table 1).

Milk production, showed in Fig. 1, was significantly higher (P < 0.01) in the "Maize" group ewes, than in the "Grass" group ewes (750 and 550 ml/day, respectively). Milk production remained constant throughout the 1st and the 2nd days but decreased significantly thereafter especially in "Maize" group' ewes. This may indicate that the quality of the grass affected the intake and was not enough to sustain milk production levels for each treatment.

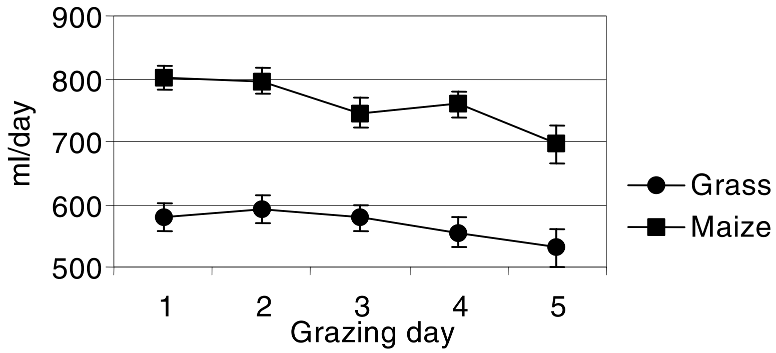

Fig. 1. Milk production (ml/day) from "Grass" and "Maize" groups' ewes during the grazing period.

Milk composition is showed at Table 2. Fat, protein and total solids contents were significantly higher in "Grass" group ewes than in "Maize" group ewes (7.92, 6.10 and 19.24% vs 6.87, 5.56, 17.85% respectively). Lactose content was not affected by maize supplementation. Similar results to those obtained for fat, lactose and total solids contents were reported by Susin *et al.* (1995), but an increase of protein content was expected in ewes supplemented with maize. From the 2nd to the 5th day in the paddocks, milk fat and total solids contents decreased in both groups and protein and lactose contents were similar. The increase of milk fat on the 5th day was unexpected, once the percentage of fibre available in each species was higher, however the increased feed intake in the beginning of the period could have led to a higher total intake of fibre.

The introduction of energetic supplements and the increase of fibre in the diet of dairy ewes along with the grazing period can change rumen fermentation, and consequently the FA composition of milk fat. While $C_{4:0}$-$C_{10:1}$ and $C_{12:0}$-$C_{15:1}$ synthesised *de novo* in the mammary gland can increase during the beginning of the grazing period, the level of long chain fatty acids (LCFA) derived from diet or from adipose tissue mobilization tended to be higher in the period of less feed availability.

Milk fat from the "Maize" group ewes was higher in $C_{4:0}$-$C_{10:1}$ (13.00 vs 10.59%; P < 0.05) and lower in LCFA (43.96 vs 46.56%; P < 0.10) than the milk fat from "Grass" group ewes (Table 2). This could be a consequence of an increase in total feed intake by "Maize" group ewes, achieved only through the supplement intake once "Maize" group ewes spent 6% less time in grazing activity (Marques and Belo, 2001a). Therefore, this resulted in a decrease of total intake of fibre, which might stimulated the propiogenic fermentation in the rumen and the hepatic neoglucogenesis. Thereby more energy was available for the *de novo* synthesis of FA, and adipose tissue mobilization was reduced.

In the "Maize" group it was also observed a significant decrease in the content of polyunsaturated fatty acids (PUFA – from 5.58 to 5.00%; P < 0.05), linolenic acid ($C_{18:3}$ – from 1.42 to 1.00%; P < 0.05), conjugated linoleic acid (CLA – from 1.54 to 1.21%; P < 0.05), branched chain FA (BCFA – from 2.96 to 2.57%; P < 0.01), and odd-chain FA (OCFA – from 2.72 to 2.33%; P < 0.01) (Table 2).

In the beginning of grazing in each paddock (2nd day; Table 2), when the selectivity was maximum and the leaves were the preferred part of plants consumed by ewes, milk quality was better, mainly through its significantly low contents of MUFA (26.69 vs 28.12%; P < 0.001), and high levels of PUFA (5.46 vs 5.11%; P < 0.001), $C_{18:3}$ (1.28 vs 1.14; P < 0.001), and CLA (1.48 vs 1.26; P < 0.001). These latter components are important in preventing coronary heart diseases and have anticarcinogenic activity (Parodi, 1999).

Table 2. Milk yield, milk composition and fatty acids composition of milk fat (mol/100mol of total FA)

	Supplementation group (S)		Day in the paddock (D)		MSE[††]	Significance		
	Grass	Maize	2nd day	5th day		S	D	S*D
Milk yield (ml/day)	550^a	750^b	688^b	611^a	67	**	***	ns
Fat (%)	7.92^b	6.86^a	7.60^b	7.19^a	0.53	*	**	ns
Protein (%)	6.10^b	5.55^a	5.83	5.82	0.17	*	ns	ns
Lactose (%)	4.51^a	4.75^b	4.62	4.63	0.08	†	ns	ns
Total solids (%)	19.21^b	17.83^a	18.75^b	18.28^a	0.46	*	***	ns
$C_{4:0}$-$C_{10:1}$	10.59^a	13.00^b	12.05	11.53	2.56	*	ns	ns
$C_{12:0}$-$C_{15:1}$	16.97	17.67	17.50^b	17.14^a	1.24	ns	*	ns
C_{16}	25.88	25.37	26.24	25.01	1.14	ns	***	***
LCFA[†]	46.56	43.96	44.20^a	46.32^b	3.33	ns	***	ns
SFA[†]	63.01^a	66.92^b	65.60^b	64.33^a	2.26	†	***	**
MUFA[†]	28.92	25.90	26.69^a	28.12^b	2.00	ns	***	**
PUFA[†]	5.58^b	5.00^a	5.46^b	5.11^a	0.40	*	***	ns
$C_{18:2}$	2.63	2.78	2.69	2.72	0.20	ns	ns	ns
$C_{18:3}$	1.42^b	1.00^a	1.28^b	1.14^a	0.20	*	***	ns
CLA[†]	1.54^b	1.21^a	1.48^b	1.26^a	0.20	*	***	ns
BCFA[†]	2.96^b	2.57^a	2.66^a	2.88^b	0.25	**	***	ns
OCFA[†]	2.72^b	2.33^a	2.49^a	2.55^b	0.20	***	*	ns
$C_{18:2}/C_{18:3}$	1.94^a	2.89^b	2.23^a	2.60^b	0.31	**	***	***

[†]LCFA – long chain FA; SFA – saturated FA; MUFA – monounsaturated FA; PUFA – polyunsaturated FA; CLA – conjugated linoleic acid; BCFA – branched-FA; OCFA – odd-chain FA.
[††]MSE – Mean Standard Error.
[a,b]Means with different superscripts differ significantly.
*P < 0.05; **P < 0.01; ***P < 0.001; ns – P > 0.10; †P < 0.10.

The $C_{18:2}/C_{18:3}$ ratio was recently proposed as a valorisation factor for fats. The World Health Organization (WHO, 1998) recommended for a human diet a $C_{18:2}/C_{18:3}$ ratio lower than 5:1. In this study, this ratio was significantly affected by the interaction between supplementation group and days in the paddock. The $C_{18:2}/C_{18:3}$ ratio raised with supplementation and with grazing time in each group, i.e. ewes in the "Grass" group exhibited a $C_{18:2}/C_{18:3}$ ratio of 1.85 at the 2nd day and a 2.03 ratio at the 5th day. "Maize" group ewes had a $C_{18:2}/C_{18:3}$ ratio of 2.62 at the 2nd day and of 3.17 at the 5th day. These results showed that it is possible, in grazing ewes, to obtain ratios as low as 2:1, and reinforce the importance of an efficient grazing management.

To better understand the feeding system, a discriminant analysis was performed. This analysis generated three significant uncorrelated linear combinations of $C_{18:2}/C_{18:3}$ ratio and 12 FA (C17:0, iC15:0, CLA, C18:0, C18:2, C18:3, C15:1, C18:1, aC16:0, C16:1, C14:0 and C4:0). The discriminant analysis allowed allocating properly 90% of samples (Fig. 2). The percentage of correct allocation was 90, 92, 88 and 90%, for groups "Grass" – 2nd day, "Maize" – 2nd day, "Grass" – 5th day, "Maize" – 5th day, respectively. Discriminant functions 1 and 2 had a higher discriminant power than function 3, accounting for 63.8 and 31.9% of the total variation between groups (Table 3). This methodology is especially accurate to discriminate between supplementation groups, once only two samples of the "Maize" group were misallocated in the "Grass" group.

Conclusions

This study showed clearly the effect of grazing management on milk production in dairy ewes. Rotational grazing had the advantage of allowing the persistence of forage species in pastures, namely lucerne. Moreover, the conduction of dairy ewes under rotational grazing system allowed the production of milk with better nutritional quality, especially because of its richness in PUFA and CLA and its lower content in LCFA. Furthermore, milk from ewes grazing pasture with high leaves content has a lower $C_{18:2}/C_{18:3}$ ratio, than milk from ewes grazing a low quality pasture or receiving supplementation. The effectiveness of milk FA profiles to assign milk samples to a feeding system, to discriminate among ewes feed with or without supplements was also demonstrated in the current work.

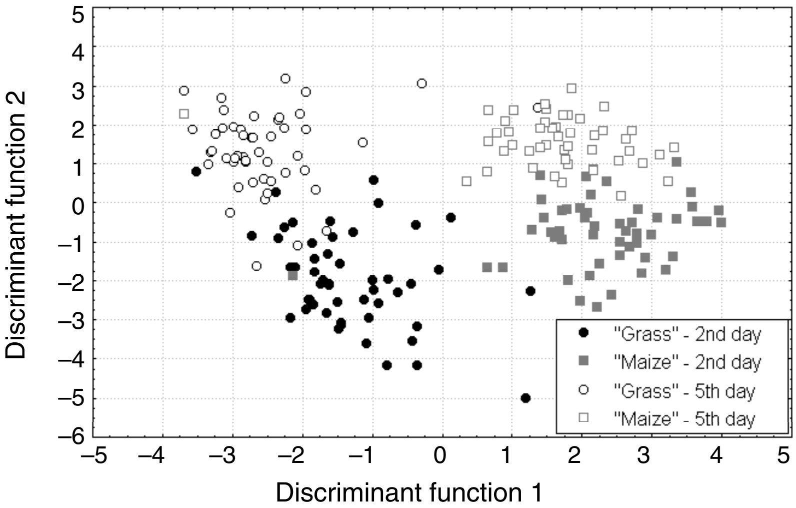

Fig. 2. Spatial distribution of fatty acids profile from four groups in the plane defined by the discriminant function 1 and 2.

Table 3. Statistics for each discriminant function

Discriminant function	Canonical R	Eigen value	Cumulative proportion	Wilks' Lambda	P-level
1	0.900	4.257	0.638	0.047	***
2	0.825	2.133	0.957	0.248	***
3	0.472	0.287	1.000	0.777	***

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