EFFECT OF SUBSTITUTION OF BARLEY AND TICKBEAN TO MAIZE AND EXTRUDED SOYBEAN IN THE DIET ON MILK AND CHEESE FROM EWES GRAZING UNDER TWO DIFFERENT STOCKING RATES

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1 Abstract

This experiment aimed to investigate the effects of a concentrate supplement based on barley and tickbean (BT), compared with a concentrate of maize and extruded soybean meal (MS), on milk and cheese yield and composition of ewes grazing with different stocking rate (SR). Thirty two Comisana ewes, divided into four groups, for 50 days in spring were allowed to graze plots of a mixed sward of Italian reygrass and berseem clover with a low (LSR, 23 ewes/ha) or a high SR (HSR, 38 ewes/ha) and fed BT or MS. Herbage allowance *per* ewe was more than double at LSR compared to HSR ($P<0.001$), and daily milk yield was 10 to 15% higher ($P<0.01$) in the LSR-BT ewes than in the other groups. Milk composition, cheese yield and composition were not influenced by treatments. Curd firming time (k_{20}) was higher ($P<0.05$) for LSR-BT milk. Milk fatty acid composition of ewes fed MS, compared to BT, had a higher level of C18:0 and a lower level of C16:0 and C18:3 n-3. Milk *trans*-10 C18:1 and *trans*-13 C18:1 fatty acids were higher ($P<0.05$) at HSR and with BT, while *trans*-12 C18:1 was higher at LSR ($P<0.05$). LSR increased ($P<0.05$) the cheese content of odd and branched chain fatty acids compared to HSR. The results suggest that both concentrate type and pasture availability influenced milk and cheese composition, whereas milk yield was positively affected by the interaction LSR*BT.

2 Keywords

Ewes, concentrate, pasture, stocking rate, milk, cheese, fatty acid composition.

3 Introduction

In sheep productive system, grazing pasture provides the main feeding resources, integrated by concentrate and/or conserved forage when the grass availability or composition is inadequate to support animal nutritional needs. Among the techniques to develop a rational grazing management, the adoption of a correct stocking rate positively impacts animal productivity (Animut *et al.*, 2005; Bonanno *et al.*, 2007). On the other hand, the recourse to appropriate amount and type of concentrate supplement is necessary during the periods of forage shortage, and generally the supply of concentrate, increasing energy intake, increases milk production (Morand-Fehr *et al.*, 2007). Both stocking rate and concentrate supply, modifying feeding behaviour and milk composition of grazing animals, can have an important effect on milk properties for cheese-making ability, and cheese nutritional and safety characteristics. Particularly, the amount and composition of herbage offered to grazing ewes can affect fatty acid composition of milk. High amounts of pasture in the diet of ewes, in fact, favour the accumulation of conjugated linoleic acid and α-linolenic acid in milk (Antongiovanni *et al.*, 2003). Moreover, some concentrate components such as barley and tickbean, being safer feed sources than maize, at risk of aflatoxins contamination, and soybean, at

GMO risk, can be conveniently proposed in diet as alternative feed sources for achieving safe dairy products, either in organic or conventional production systems.

Therefore, this experiment aimed to verify the impact of feeding a concentrate supplement composed by barley and tickbean, compared to that based on maize and extruded soybean meal, on milk and cheese yield and composition of ewes grazing at different stocking rates.

4 Methodology of the study

The experimental site was the “Pietranera farm” of the University of Palermo situated in a hilly semi-arid area of Sicily (S. Stefano Quisquina, Agrigento, 37°37’N; 13°29’E; 178 m a.s.l.). In the trial four feeding treatments were compared, in relation to stocking rate (SR) at pasture and concentrate type (CT) offered.

Thirty two Comisana ewes, initially averaging 62±4 days in milk and 46.5±6.6 kg of live weight, were divided into four groups. The groups were allowed to graze a mixed sward of Italian ryegrass (*Lolium multiflorum* Lam. subsp. *Westerwoldicum, var.* Elunaria) and berseem clover (*Trifolium alexandrinum* L., var. Lilibeo) at a low (LSR, 23 ewes/ha) or a high stocking rate (HSR, 38 ewes/ha) and fed a concentrate supplement (BT) based on barley (B) and tickbean (T), or a concentrate (MS), of maize (M) and extruded soybean meal (S). Each feeding treatment was duplicated using two sward randomised blocks, each divided into four plots, two of 1046 m^2 and two of 1720 m^2. For 50 days, starting on 11^{th} April 2006, each plot was continuously grazed by 4 ewes during the daytime (09:00-16:00). All ewes were supplemented daily an equal amount of crude protein (CP) (69 g/day) and net energy for lactation (NE_L) (0.33 Mcal/day) from roughly-ground concentrate, receiving 550 g of BT (B and T in the ratio 80:20 in weight; CP 14.5%, NE_L 2.0 Mcal/kg of dry matter (DM)) or 500 g of MS (M and S in the ratio 82:18 in weight; CP 15.9%, NE_L 2.2 Mcal/kg of DM). In addition, all ewes were offered 300 g/day of sulla hay (CP 11.6%, NDF 46.9%, NE_L 1.1 Mcal/kg of DM). Ewes were hand milked twice in a day (7:30 and 16:30).

Milk yield *per* plot was recorded daily from. The available herbage mass at pasture was estimated weekly by clipping 3 sample areas per plot at ground level. Pasture allowance (kg of DM/day *per* ewe) was determined by dividing the herbage mass on offer by the number of grazing ewes. Measurements and sampling of bulk milk and cheese were executed three times over the experiment (20^{th} April, 12^{th} and 25^{th} May). The row bulk milk produced by each group during 48 hours was processed in line with the standard method for making “PDO Pecorino Siciliano” cheese.

Bulk milk samples were analysed for pH and titrable acidity (°SH/50 ml); lactose, fat, protein, casein and somatic cells count (SCC) by infrared method (Combyfoss 6000, Foss Italy); urea by enzymatic method using difference in pH (CL10 instrument, Eurochem, Italy); total bacterial count (TBC) (Bacto-scan 8000S, Foss Italy); clotting time (r, min), curd firming time (k_{20}, min) and curd firmness (a_{30}, mm) (Formagraph instrument, Foss Italy). Cheese samples cut after 30 days of cheese ripening were analysed for DM, fat, total and soluble nitrogen, and ash. Milk fat extraction was performed according to Secchiari *et al.* (2003), whereas cheese fat was extracted according to Folch *et al.* (1957). Methyl esters of fatty acids were prepared by the alkali-catalyzed trans-methylation described by Christie (1982), using a nonadecanoic acid methyl ester (Sigma Chemical Co., St. Louis, MO, USA) as the internal standard. Fatty acid composition was determined by gas chromatography (GC) using a ThermoQuest (Milan, Italy) gas-chromatograph equipped with an FID and a high polar fused silica capillary column (Chrompack CP-Sil 88 Varian, Middelburg, the Netherlands; 100 m × 0.25 mm internal diameter; film thickness 0.20 μm). Helium was used as the carrier gas at a flow of 1 $mL\cdot min^{-1}$. The split ratio was 1:80. An aliquot of the sample was injected under the following GC conditions: the oven temperature was taken to 150°C and held at that level for 1 min; it was then increased to 185°C at a rate of 5°C·min^{-1}, and held at that level for 20 min, before being increased to 188°C at 0.3°C·min^{-1} and held for 1 min, and then to 230°C at a rate of 3°C·min^{-1}, at which temperature it was held for 15 min. The injector temperature was set at 270°C, while the detector temperature was set at 300°C.

Data were statistically analysed by GLM procedure of SAS 9.1.2 software. The models included the effects of replicate, SR, CT and interaction SR*CT for pasture allowance and daily milk yield; the effects of SR, CT and SR*CT for bulk milk and cheese parameters. The SCC and TBC values were transformed logarithmically ($\log_{10}$). Treatment differences were assessed by Student "*t*" test.

5 Results and discussion

On average, forage allowance *per* ewe at pasture was more than double at LSR compared to HSR (85 *vs.* 38 kg of DM/day; P<0.001). The supply of BT concentrate corresponded to a higher pasture allowance than MS (68 *vs.* 54 kg of DM/day; P<0.05), especially at HSR in comparison with LSR (Table 1). This result can be presumably linked also to a lower grass intake of ewes receiving 50 g higher amount of BT concentrate.

The SR and the interaction between SR and CT affected daily milk yield (Table 1). Individual daily milk yield was increased by the LSR (1239 *vs.* 1153 g/ewe; P<0.01) but, in direct relation to pasture allowance, was higher in the LSR-BT group than in the other treatments. In contrast, the daily milk yield per hectare was higher at HSR (28.8 *vs.* 44.1 kg/ha; P<0.001), but was improved with BT concentrate at LSR. Based on these results, the LSR-BT treatment seems to have allowed the ewes to better balance the grass selected at pasture with the concentrate being more degradable in the rumen.

Table 1. Effect of stocking rate and concentrate type on pasture allowance and daily milk yield during the grazing period (50 days) (least square means)

Stocking rate (SR)	HSR (38 ewes/ha)		LSR (23 ewes/ha)		SEM	Significance (1)			Root MSE
Concentrate type (CT)	BT	MS	BT	MS		SR	CT	SR*CT	
Pasture allowance, kg of DM/day *per* ewe	46.7	28.4	89.9	79.5	6.8	***	*	NS	42.9
Daily milk yield, g/ewe	1130^{B}	1175^{B}	1300^{A}	1178^{B}	28.1	**	NS	**	281
Daily total milk yield, kg/ha	43.2^{Aa}	45.0^{Aa}	30.2^{Bb}	27.4^{Bc}	0.89	***	NS	**	8.9

(1) *$P\leq0.05$; **$P\leq0.01$; ***$P\leq0.001$; NS: not significant. A, B: $P\leq 0.01$; a, b, c: $P\leq 0.05$

Milk composition and rennet properties (Table 2) were not influenced by SR, whereas the BT concentrate increased lactose (4.83 *vs.* 4.72%) and reduced SCC (706 *vs.* 1959 cells x 1000/ml). Curd firming time (k_{20}) was higher in LSR-BT milk than in other treatments, linked to the lower, but not significantly, milk casein. The cheese yield and chemical composition (Table 2) was similar among the four experimental groups most likely as a consequence of the lack of differences in milk composition.

Since the fatty acid composition of cheese substantially reflected the composition of milk, Table 3 reports only the milk fatty acid composition. The effect of the type of concentrate on milk fatty acid composition resulted in a higher level of C18:0 and a lower level of C16:0 for the ewes fed

MS. The C18:3 n-3 content is higher in milk for the ewes fed BT, probably due to the higher levels of C18:3 n-3 in barley and tickbean compared to maize and extruded soybean meal. *Trans*-10 C18:1, *trans*-12 C18:1 and *trans* 13 C18:1 content of milk was higher when ewes were fed at HSR, probably as a consequence of a lower allowance of herbage. Increasing proportions of *trans* C18:1 isomers different to *trans*-11 C18:1 are reported, in fact, with decreasing forage:concentrate ratios (Bauman and Griinari, 2003). Interestingly, LSR increased (+ 8%; P<0.05) the milk and cheese content of odd and branched chain fatty acids (OBCFA) compared to HSR. According to literature, total OBCFA in rumen bacteria and in milk decrease with decreasing forage:concentrate ratio. In particular, the forage proportion is strongly related to the concentration of anteiso C15:0, iso C14:0 and iso C16:0 (Vlaemink *et al.*, 2006a, b). In our experiment the higher level of these fatty acids have been observed in milk from ewe fed at LSR, when the herbage allowance *per* ewe was more than double, if compared to HSR (Table 1).

Table 2. Effect of stocking rate and concentrate type on bulk milk composition and rennet properties, and cheese yield and composition (least square means)

Stocking rate (SR)		HSR (38 ewes/ha)		LSR (23 ewes/ha)		SEM	Significant effects (1)	Root MSE
Concentrate type (CT)		BT	MS	BT	MS			
Milk	Lactose, %	4.85	4.73	4.82	4.71	0.04	CT	0.07
	Fat, %	6.38	6.56	6.33	6.23	0.24		0.42
	Protein, %	5.27	5.44	5.22	5.32	0.10		0.17
	Casein, %	4.07	4.17	3.99	4.08	0.08		0.14
	Urea, mg/dl	52.7	51.4	52.7	53.8	3.65		6.33
	CCS (*1000/ml)	818	2362	595	1555	478	CT	0.38
	TBC, cfu*1000/ml	100	260	120	165	93		0.57
	pH	6.59	6.64	6.65	6.62	0.02		0.04
	Titratable acidity, °SH/50ml	4.41	3.89	4.23	4.26	0.16		0.27
	Clotting time (r), min	21.1	23.1	23.4	22.1	3.00		5.20
	Curd firming time (k_{20}), min	1.92[b]	2.27[ab]	2.98[a]	1.92[b]	0.33	SR*CT	0.57
	Curd firmness (a_{30}), mm	41.5	37.7	32.7	43.1	9.64		16.7
Cheese at 30 days	Yield (kg/100 kg milk)	13.5	13.5	12.6	13.4	0.36		0.63
	Dry matter (%)	70.6	71.9	71.2	73.0	1.69		2.93
	Fat (%)	20.1	22.6	24.0	22.7	1.42		2.47
	Protein (nitrogen x 6.38) (%)	45.0	44.8	44.7	44.9	0.72		1.26
	Soluble nitrogen (%)	0.8	1.1	0.9	1.1	0.24		0.43
	Ash (%)	5.5	5.4	5.6	5.8	0.81		1.47

(1) $P \leq 0.05$. a, b: $P \leq 0.05$

6 Conclusion

The LSR, increasing herbage allowance, improved individual milk production but lowered the milk yield per hectare. A productive improvement emerged at LSR when ewes were offered BT concentrate. Both stocking rate and concentrate type affected milk and cheese fatty acids composition. Particularly, the BT concentrate increased the C18:3 n-3 content in milk, whereas pasture availability significantly affected OBCFA probably as a consequence of differences in herbage intake that modified the forage:concentrate ratio of the diets.

7 References

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Table 3. Effect of stocking rate and concentrate type on milk fatty acid composition (g/100 g fat) (least square means)

Stocking rate (SR)	HSR (38 ewes/ha)		LSR (23 ewes/ha)		SEM	Significant effects (1)	Root MSE
Concentrate type (CT)	BT	MS	BT	MS			
C8:0	1.79	1.69	1.75	1.58	0.11		0.20
C10:0	7.23	6.87	6.79	5.72	0.39		0.68
C11:0	0.06	0.08	0.07	0.07	0.01		0.01
C12:0	3.65	3.97	3.96	3.32	0.21		0.37
C13:0	0.07	0.09	0.08	0.08	0.01		0.01
C14:0 *iso*	0.07	0.08	0.09	0.09	0.01	SR	0.01
C14:0	10.00	10.07	10.75	9.23	0.50		0.86
C14:1 *cis*-9	0.16	0.17	0.16	0.17	0.01		0.02
C15:0 *anteiso*	0.49	0.47	0.52	0.51	0.03	SR	0.06
C15:0	0.91	0.88	0.99	1.04	0.06	SR	0.09
C16:0 *iso*	0.21	0.21	0.25	0.24	0.02	SR	0.03
C16:0	25.45	23.50	25.22	21.88	1.08	CT	1.86
C16:1 *cis*-9	1.07	1.04	1.11	1.04	0.07		0.11
C17:0 *anteiso*	0.54	0.53	0.57	0.51	0.03		0.05
C17:0	0.54	0.49	0.56	0.49	0.02	CT	0.03

C18:0	6.74	7.68	7.47	7.76	0.24	CT	0.41
C18:1 *trans*-6 - *trans*-8	0.22	0.21	0.20	0.20	0.02		0.03
C18:1 *trans*-9	0.26	0.29	0.26	0.26	0.01		0.02
C18:1 *trans*-10	0.45	0.42	0.42	0.36	0.01	SR, CT	0.02
C18:1 *trans*-11	2.33	2.52	2.54	2.60	0.12		0.21
C18:1 *trans*-12	0.52	0.56	0.52	0.46	0.02	SR	0.03
C18:1 *trans*-13+ *trans*-14	0.47	0.49	0.49	0.40	0.01	SR, CT	0.02
C18:1 *cis*-9	12.81	14.17	13.53	13.44	0.47		0.81
C18:1 *cis*-11	0.25	0.28	0.26	0.23	0.01		0.02
C18:1 *cis*-12	0.18	0.26	0.24	0.20	0.04		0.07
C18:1 *cis*-13	0.19	0.19	0.20	0.16	0.01		0.01
C18:1 *cis*-14	0.53	0.58	0.58	0.53	0.09		0.16
C18:2 n-6 *cis*-9, *cis*-12	1.51	1.58	1.49	1.36	0.09		0.15
C18:2 n-6 *trans*-9, *trans*-12	0.24	0.27	0.24	0.21	0.01		0.02
C18:2 *cis*-9, *trans*-11	1.14	1.18	1.19	1.28	0.06		0.11
C18:3 n-3	0.94	0.87	0.95	0.71	0.05	CT	0.08

(1) $P \leq 0.05$