



# Effect of corn and beet pulp based concentrate on ruminal parameters in wethers fed with fresh forage

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## ABSTRACT

Four wethers housed in individual pens and fitted with ruminal cannulae were grouped (two wethers per group) and fed fresh forage (Italian ryegrass, *Lolium multiflorum*) with either beet pulp (BP) or corn based concentrate (C) as supplements, using a crossover experimental design. The experiment was carried out during two experimental periods: the growing phase (early spring, G) and the reproductive phase (late spring, R) of the ryegrass. The diet of each animal was changed as the grass moved from the vegetative phase to the reproductive phase. The intake, the chemical composition of the diet and ruminal parameters such as pH, ammonia and volatile fatty acid were determined. Total dry matter intake (DMI) decreased from early to late spring (1081 vs 767 g/d;  $P < 0.0001$ ). Herbage DMI in particular decreased more (811 vs 543 g/d;  $P < 0.05$ ) than concentrate DMI (271 vs 224 g/d;  $P < 0.054$ ). There were marked differences in the chemical composition of the diet in wethers fed with the forage from early or late spring. This was because of changes in the characteristics of the forage. In detail, DM, NDF and ADF increased from early to late spring while CP, and EE decreased ( $P < 0.05$ ). Ruminal pH was lower in G than in R (6.24 vs 6.57,  $P < 0.05$ ). The concentrate used had no effect on the pH value. The ammonia concentration in the rumen fluid decreased from early to late spring (10.79 vs 6.68 mg/100 ml,  $P < 0.05$ ) and it was not influenced by the source of carbohydrates from the concentrate. VFA rumen fluid concentration increased ( $P < 0.05$ ) after feeding and decreased before the second meal. In the rumen fluid, the maximum total VFA concentration and the lowest pH were observed 2 h after the morning meal. Acetate and butyrate concentrations in the rumen fluid were influenced by the period ( $P < 0.05$ ) but not by the concentrate source. By contrast, the concentrate source significantly influenced the rumen fermentation patterns of propionate and VFA ratios, with higher propionic acid and lower acetate to propionate ratios in C than BP. This highlights the importance of the carbohydrate source in supplements for pasture based rations which varied according to the proportion of beet pulp and corn in the ingested diet.

*Key words:* Ryegrass, Concentrate, pH, Volatile fatty acid, Ammonia.

## RIASSUNTO

## EFFETTO SUI PARAMETRI RUMINALI DELLA INTEGRAZIONE CON MANGIMI COSTITUITI DA POLPE DI BIETOLE O MAIS IN MONTONI ALIMENTATI CON FORAGGI VERDI

Su quattro montoni castrati muniti di fistola ruminale è stata condotta una prova per valutare l'effetto dell'integrazione di due mangimi a base di mais (C) o polpe di bietole (BP) su una dieta costituita da erba di loiessa (*Lolium multiflorum*). La prova è stata svolta in due periodi: fase vegetativa (inizio primavera G) e fase riproduttiva (fine primavera, R) del loglio in un disegno sperimentale di tipo "crossover". I parametri considerati sono stati: composizione chimica della dieta, ingestione di SS, pH e livello di acidi grassi volatili ruminali. L'ingestione totale di SS giornaliera è diminuita quando gli animali ingerivano un foraggio allo stadio riproduttivo rispetto alla fase vegetativa (1081 vs 767 g/d;  $P < 0,0001$ ) e in particolare è diminuita l'ingestione relativa al foraggio (811 vs 543 g/d;  $P < 0,05$ ) mentre la quota relativa ai concentrati rimaneva costante (271 vs 224 g/d;  $P < 0,054$ ). Anche la composizione della dieta variava in funzione dello stadio fenologico dell'erba. In particolare il tenore in SS, NDF e ADF aumentava mentre le PG e l'EE diminuivano ( $P < 0,05$ ) col passaggio dallo stadio vegetativo a quello riproduttivo. Il pH ruminale è stato influenzato dal periodo, con valori bassi in G rispetto a R (6,24 vs 6,57;  $P < 0,05$ ). La fonte glucidica dei concentrati non ha manifestato effetti sui valori di pH ruminale. La concentrazione di ammoniaca ruminale è diminuita durante la somministrazione di foraggio in fase riproduttiva (10,79 vs 6,68;  $P < 0,05$  mg/100 ml), mentre nessun effetto ha fatto registrare la fonte di carboidrati. Il livello massimo di acidi grassi volatili totali e il minor valore di pH è stato riscontrato la mattina a due ore dalla distribuzione del pasto. La concentrazione di acido acetico e acido butirrico nel rumine è stata influenzata dal periodo ( $P < 0,05$ ) mentre nessun effetto è stato riscontrato da parte della fonte dei carboidrati. Il gruppo C ha manifestato valori superiori di acido propionico ed un inferiore rapporto acetato/propionato, sottolineando come la fonte glucidica del concentrato possa modificare le fermentazioni ruminali in ovini alimentati con foraggi verdi.

Parole chiave: Loglio, Concentrato, pH, Acidi grassi volatili, Ammoniaca.

## Introduction

The sheep feeding system in the Mediterranean countries is marked by seasonal fluctuations in pasture quality and feed supply. Chemical analysis has shown that the largest seasonal differences in pasture composition are in the CP and NDF levels (Molle *et al.*, 2004). As the proportion of NDF in the diet increases and that of CP decreases, ruminal fermentation and passage rate decrease. This has a negative impact on the intake (Van Soest, 1982). Maas *et al.* (2001) reported that differences in the growth rates of sheep may be due to differences in the chemical composition of spring and autumn pastures. For grazing cattle, protein nutrition of growing cattle is of utmost importance, because a deficiency of 1 g of protein/d can reduce the gain rate by 10 g/d (NRC, 1984). The milk production of gra-

zing cows may be reduced by an imbalance of rumen fermentable carbohydrate and RDP, because in this case as much as 30% of the nitrogen may be lost before it reaches the intestines (Beever *et al.*, 1986), or because the cow is unable to consume enough pasture DM to satisfy the nutrient requirements necessary for higher milk production (Holden *et al.*, 1994). Several studies such as, for example, Tamminga in 1992, have shown that efficient use of dietary N by dairy cows for milk protein synthesis averaged about 20%. More information is needed on how grazing animals use nutrients, if milk is to be produced more efficiently. For example, well managed pasture may provide a highly digestible, highly palatable forage with a high N content and in optimal conditions milk production may be up to 29 kg/d (Kolver and Muller, 1998). However, there is little information available on ruminal

fermentation and nutrient flow in grazing sheep. Corn, wheat and barley are the most frequently used high-starch supplements. Beet and citrus pulps or soy hulls are the most important sources of highly fermentable NDF or pectins, but their effects on rumen fermentation and the total digestion of pasture by grazing sheep have not been thoroughly evaluated. The aim of the present study was to assess the impact on nutrient use by wethers fed on fresh forage of dietary supplements with either corn or beet pulp.

## Material and methods

### *Animals and experimental design*

Four wethers (BW  $58.40 \pm 4.01$  Kg, means  $\pm$ SE) fitted with ruminal cannulae were grouped (two wethers per group) and fed with either beet pulp (BP) or corn-based concentrate (C) as supplements to fresh forage (Italian ryegrass, *Lolium multiflorum*, Lam. cv Teanna). Using the crossover design, wethers were fed a similar energy content of two concentrates containing beet pulp (40% DM of beet pulp, BP) or corn (60% DM of corn, C) as shown in Table 1.

The experiment was carried out for two experimental periods of 21 days each (17 d for adaptation followed by 4 d for sample collection). These were the vegetative phase (early spring, G) and reproductive phase (late spring, R) of the ryegrass. The dietary supplement given to each animal was switched from the vegetative to the reproductive phase. The wethers were housed in individual pens (1m x 1.5m), and cared for according to the guidelines laid down by the Council of the European Communities (86/609/EEC).

### *Experimental feed*

The forage was mechanically mown twice daily, the sward being cut at 4-6 cm stubble height, measured by a weighed square grass-

meter (Holmes, 1974). The sward height at cutting was kept within a range of 300-400 mm during the experimental periods.

The beet pulp (BP) or corn (C) concentrates were formulated so that there was a consistent difference in the carbohydrate source. The composition of the experimental concentrates is given in Table 1. The concentrates were offered to each wether in a iso-energetic amount corresponding to 300 g/d and 320 g/d for C and BP, respectively.

The wethers were fed 52 g/kg BW, which corresponded to 90% of ad libitum intake (57 g/kg BW). This had been determined in the pre-experimental period. The forage and concentrates were given in two feeds (08.00 h and 14.00 h) and refusals were collected and weighed daily. The chemical composition of the composite samples (per wether, concentrate and period) were analyzed. Each wether had continuous access to water.

### *Measurements and chemical analyses*

The dry matter intake (DMI) of herbage and concentrate were measured daily by weighing the amounts offered and the correspondingorts. Forage and orts samples were taken daily, and kept frozen at  $-20^{\circ}\text{C}$  until processed for further analysis. Frozen samples were freeze-dried and then ground through a 1 mm screen before analyses. The crude protein (CP) and ether extract (EE) content were determined (AOAC, 1980). NDF, ADF and ADL were determined with an Ankom Fiber Analyzer using the Filter Bag Technique (Ankom technology Corp, Fairport, NY) and the in vitro DM digestibility (IVDMD) according to the method by Boever *et al.* (1986). The protein fractions determined were non-protein nitrogen (NPN), true protein (TP), and buffer soluble protein (BSP). Total soluble protein (SP) content was obtained by summing NPN and BSP (Licitra *et al.*, 1996). Ruminal fluid was collected by suction on d 18 and d 21 of each

Table 1. Ingredient content and chemical composition of the experimental concentrates.

		Concentrate	
		BP	C
Ingredients of concentrate (% DM):			
Soybean meal		19.50	19.50
Corn		36.50	60.00
Sugar beet pulp		39.65	16.15
Molasses		4.00	4.00
Mineral/vitamin		0.35	0.35
Chemical analysis:			
DM	%	87.56	87.03
OM	% DM	95.47	96.31
CP	"	17.46	17.07
EE	"	1.91	2.62
NDF	"	28.21	21.22
ADF	"	11.44	5.35
ADL	"	0.66	0.55
NSC	"	47.89	55.40
WSC	"	8.90	6.48
Starch	"	35.79	50.38
Protein fractions (% CP):			
TP		85.05	86.17
NPN		11.68	11.48
BSP		2.46	2.93
SP		14.14	14.41
IVDMD	%	88.60	90.00

NSC: calculated as  $100 - (\text{NDF} + \text{CP} + \text{EE} + \text{ash})$ ; WSC: water soluble carbohydrates; TP: true protein; NPN: non protein nitrogen; BSP Buffer soluble nitrogen; SP: soluble protein calculated as  $(\text{NPN} + \text{BSP})$ ; IVDMD: in vitro dry matter digestibility.

experimental period from each of the four wethers, using a pipe inserted into the ventral sac just before feeding (0 h) and 2, 4, 6, 8, and 10 h after feeding.

Rumen fluid pH was recorded immediately after sampling. The samples were then centrifuged at 4000.g for 30 min at 4°C, in order to obtain a clear supernatant. The supernatant was frozen until analysis for rumen ammonia N, using the phenol-hypochloride procedure (Broderick and Kang,

1980). Volatile Fatty Acid (VFA) concentrations were analysed by gas chromatography (Fussel and McCalley, 1987).

#### *Statistical analysis*

The data on the chemical composition of the fresh forage was analysed by ANOVA with period as the main factor. The data on chemical composition of diet and intake were analysed by ANOVA with period, concentrate type and their interaction as fixed factors.

Data on ruminal parameters were analysed by ANOVA with animal, treatment, period and sampling time as main factors and animal x treatment x period x sampling time interaction. Differences between treatments were compared by the Tukey test and significance was determined at  $P < 0.05$ .

## Results and discussion

The effect of the season on the chemical composition of fresh pasture in early and late spring are shown in Table 2. The period affected all the chemical components. DM, NDF and ADF increased ( $P < 0.05$ ) from early (G) to late spring (R), while CP and

EE decreased ( $P < 0.05$ ). In detail, NDF increased from 50.42 (G) to 60.23% DM (R) ( $P < 0.05$ ), while CP decreased from 14.71 (G) to 11.13% DM (R) ( $P < 0.05$ ).

This seasonal shift in forage quality affected the chemical composition of the diet (Table 3). It affected, among other components, the carbohydrate and CP content and their partitioning. To give one example, the BSP (as % of CP) content decreased during the reproductive phase of the forage ( $P < 0.05$ , Table 3).

The phenological phase of the forage had also an overriding effect on the intake of forage and concentrate. From early to late spring the forage dry matter intake decrea-

Table 2. Chemical composition of the Italian ryegrass herbage during the growing (G) and reproductive phase (R).

		G	R	SEM	Significance
DM	%	15.22	29.24	2.70	*
OM	% DM	87.12	91.70	0.98	*
CP	"	14.71	11.13	0.72	*
EE	"	2.87	2.07	0.16	*
NDF	"	50.42	60.23	1.88	*
ADF	"	26.19	32.41	1.20	*
ADL	"	1.47	3.12	0.32	*
NSC	"	19.14	18.27	0.61	ns
WSC	"	11.77	11.31	0.65	ns
Protein fraction:					
TP	% CP	62.53	58.62	1.67	ns
NPN	"	37.47	41.41	1.67	ns
BSP	"	5.53	3.18	1.14	ns
SP	"	43.00	44.58	1.79	ns
IVDMD	%	81.29	63.47	3.41	*

NSC: calculated as  $100 - (\text{NDF} + \text{CP} + \text{EE} + \text{ash})$ ; WSC: water soluble carbohydrates; TP: true protein; NPN: non protein nitrogen; BSP Buffer soluble nitrogen; SP: soluble protein calculated as  $(\text{NPN} + \text{BSP})$ ; IVDMD: in vitro dry matter digestibility.

\*:  $P < 0.05$ ; ns: not significant.

Table 3. Intake and diet composition of sheep fed with growing (G) or mature (R) fresh ryegrass supplemented with either a beet pulp (BP) or a corn-based concentrate (C).

		G		R		SEM	Significance		
		BP	C	BP	C		P <sup>1</sup>	C <sup>2</sup>	PxC
Chemical composition of diets:									
CP	% DM	15.28	15.41	13.12	12.78	0.24	*	ns	ns
NDF	"	45.40	42.49	50.13	49.60	0.79	*	ns	ns
ADF	"	22.83	20.52	25.83	25.06	0.53	*	*	ns
Starch	"	8.69	13.78	11.18	13.56	0.74	ns	*	ns
EE	"	2.54	2.66	1.97	2.24	0.08	*	ns	ns
Ash	"	12.56	9.98	7.16	7.07	0.44	*	*	*
NSC	"	24.21	29.45	27.62	28.30	0.69	ns	*	*
WSC <sup>3</sup>	"	10.91	10.16	10.56	10.01	0.09	*	*	ns
Starch+WSC	"	19.60	23.93	21.74	23.57	0.67	ns	*	ns
F/C <sup>4</sup>	ratio	24.27	27.34	31.25	26.92	1.76	ns	ns	ns
TP <sup>5</sup>	% CP	69.32	69.53	69.63	68.19	0.57	ns	ns	ns
NPN <sup>6</sup>	"	29.96	29.10	28.89	30.68	0.46	ns	ns	ns
BSP <sup>7</sup>	"	4.87	4.61	2.70	2.63	0.20	*	*	ns
SP <sup>8</sup>	"	34.84	33.71	31.59	33.31	0.52	*	ns	ns
Dietary ingredient composition:									
Herbage	%	75.73	72.66	68.75	73.08	1.46	ns	ns	ns
Soybean meal	"	4.73	5.33	6.09	5.25	0.29	ns	ns	ns
Corn	"	8.86	16.41	18.75	9.83	1.06	ns	ns	*
Sugar beet pulp	"	9.62	4.42	5.05	10.67	0.68	ns	ns	*
Molasses	"	0.97	1.09	1.25	1.08	0.06	ns	ns	ns
Mineral/vitamin	"	0.08	0.10	0.11	0.09	0.01	ns	ns	ns
Intake:									
DMI - concentrate	g/d	280	261	242	206	11.93	*	ns	ns
DMI - forage	"	884	738	543	544	38.25	*	ns	ns
DMI - total	"	1164	999	785	749	43.33	*	ns	ns
NDF - concentrate	"	79	55	68	44	3.56	*	*	ns
NDF - forage	"	451	375	326	325	18.15	*	ns	ns
DMI - tot./ kg MW	g/kg	54.93	48.15	37.89	35.49	2.15	*	ns	ns
	BW <sup>0.75</sup>								
DMI - tot./kg LW	% LW	1.99	1.75	1.38	1.29	0.08	*	ns	ns

<sup>1</sup>period; <sup>2</sup>concentrate type; <sup>3</sup>water soluble carbohydrates; <sup>4</sup>forage/concentrate ratio; <sup>5</sup>true protein; <sup>6</sup>non protein nitrogen; <sup>7</sup>Buffer soluble nitrogen; <sup>8</sup>soluble protein calculated as (NPN+BSP).

NSC: calculated as 100-(NDF+CP+EE+ash).

\*:  $P < 0.05$ ; ns: not significant.

sed by about 33% (811 vs 543 g/d;  $P < 0.05$ ) while concentrate intake decreased by about 17% (271 vs 224 g/d;  $P < 0.05$ ). Wethers fed the early spring pasture supplemented with C concentrate tended to have a lower forage intake than the ones supplemented with BP ( $P < 0.05$ ), while in late spring the type of concentrate had no detectable effect on forage DMI. The NDF intake from the forage was influenced by the period. During the early season (G) corn concentrate depressed the forage-NDF intake by about 17% (451 vs 375; ns) more than the BP concentrate did, while during late spring (R) the forage NDF intake was similar for the diets.

The total intake in g/kg BW<sup>0.75</sup> and also as a percent of BW was affected by the period (Table 3).

Ruminal parameters were clearly influenced by the period, with a lower ruminal pH (6.24 vs 6.57;  $P < 0.05$ ) and a higher NH<sub>3</sub> (10.79 vs 6.68 mg/100ml;  $P < 0.05$ ) in G than R (Table 4). Acetate, propionate and butyrate (expressed as mg/100 ml) in the rumen fluid were also influenced by the period but not by the concentrate source. Propionate was the only VFA affected by the carbohydrate source of the supplement, with higher levels in C than BP ( $P < 0.05$ ).

Acetate/propionate and (acetate+butyrate)/propionate ratios were all influenced at a statistically significant level by both the period and the concentrate source. They decreased when wethers were fed a corn concentrate supplement, particularly during the reproductive phase (interaction between concentrate source and period:  $P > 0.05$ ).

The results in Figure 1 are means for the two sampling dates during each experimental period. Concentrate type had no effect on the pattern and magnitude of the post-feeding pH decrease in the rumen ( $P > 0.05$ , Figure 1, a).

Ruminal ammonia at 2 and 10 h after the morning meal was higher in G than in R ( $P < 0.05$ , Figure 1, b).

A period x concentrate x sampling time interaction ( $P < 0.05$ ) was observed for acetate, propionate and butyrate (Figure 1, d, e and f).

Total VFA concentration was higher (1000 vs 556 mg/100 ml;  $P < 0.05$ ) when wethers were fed forage from early spring (the vegetative phase) rather than late spring (reproductive phase).

The trends in Table 2 for NDF and CP in forage are similar to those reported by Molle *et al.* (2002) and Molle *et al.* (2003) for ryegrass-based pastures and by Coblenz *et al.* (2002) for wheat-based pasture.

Maturity, and hence the high NDF content of the herbage, had a negative effect on total DMI (Table 3). This agrees with Molle *et al.* (2004) results, which showed that there was a seasonal difference in the DM intake of lactating ewes fed herbage in early or mid spring, with lower levels in the latter period.

It is well known that there is a negative relationship between forage NDF, DMI and rumen fill (Demarquilly *et al.*, 1981; Mertens, 1992; Varga *et al.*, 1998).

In our case (Mediterranean climatic conditions) as the season moves to late spring (the reproductive phase), annual grasses react by starting to produce seed heads. There is also a change in the plant structure and, as the temperature increases, rapid maturation and lignification of the plant tissues (Fick *et al.*, 1988).

In our experiment the NDF content of the forage increased by 19% and the ADF content by 24% from early to late spring. The forage lignin content probably decreases the NDF rate and the fiber degradation rate in the rumen (Jung and Deetz, 1993) by reducing the rumen passage rate and the DMI. Overall, the phenological stage of the fresh forage also affected the DM intake from concentrates. This tended to decrease (-17%,  $P = 0.0506$ ) from the G to R phase, in particular for corn concentrate (-21%,  $P = 0.098$ ). As

Figure 1. Effect of the sampling time on rumen pH (a) ruminal ammonia (b) total VFA (c), acetate (d), propionate (e) and butyrate (f) of wethers fed with growing (G) or mature (R) fresh ryegrass supplemented with either a beet pulp (BP) or a corn-based concentrate (C).

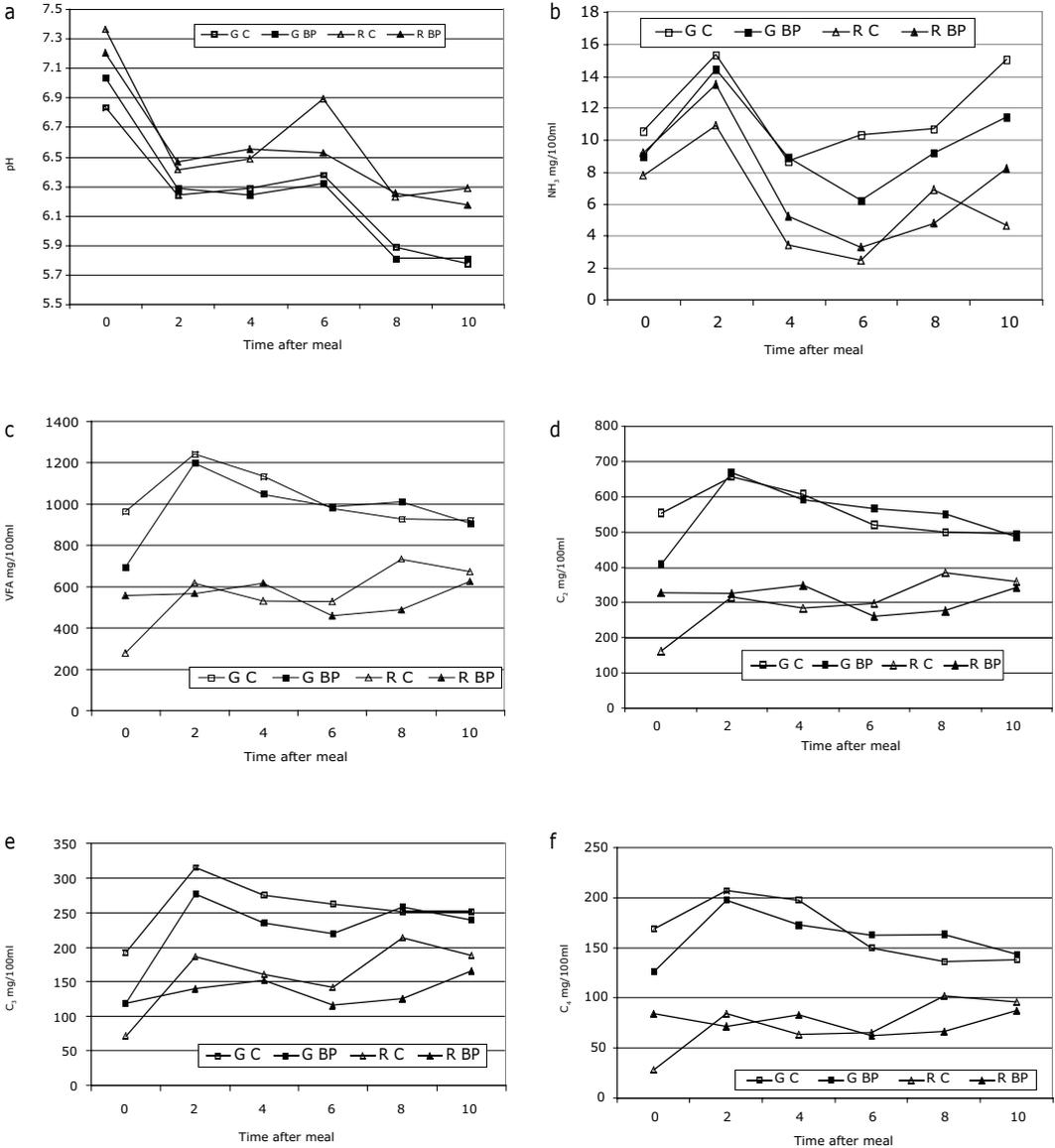


Table 4. Composition of rumen fluid in wethers fed with growing (G) or mature (R) fresh ryegrass supplemented with either a beet pulp (BP) or a corn-based concentrate (C).

		G		R		SEM	Significance		
		BP	C	BP	C		P	C	PxC
pH		6.25	6.23	6.53	6.61	0.05	*	ns	ns
NH <sub>3</sub>	mg/100 ml	9.84	11.74	7.36	6.01	0.61	*	ns	ns
Acetate	mg/100 ml	547	556	314	300	18.96	*	ns	ns
Propionate	"	224	258	137	160	8.63	*	*	*
Butyrate	"	161	167	76	73	6.61	*	ns	ns
Isobutyrate	"	11	12	7	6	0.45	*	ns	*
Valerate	"	17	20	11	11	0.78	*	ns	ns
Isovalerate	"	12	15	9	8	0.65	*	ns	*
Total isoacid	"	42	47	25	26	1.66	*	ns	ns
Total VFA	"	973	1,027	552	560	34.83	*	ns	ns
C <sub>2</sub> /C <sub>3</sub>	ratio	2.54	2.21	2.39	1.98	0.06	*	*	ns
(C <sub>2</sub> +C <sub>4</sub> )/C <sub>3</sub>	ratio	3.30	2.87	2.97	2.44	0.08	*	*	ns

$C_2/C_3$ =acetate/butyrate ratio;  $(C_2+C_4)/C_3$ =(acetate+ butyrate)/propionate ratio.

a result there is a change in the proportions of corn and sugar beet pulp in the diet. The protein fractioning of the diet was not influenced by period and type of concentrate, with the exception of the BSP fraction, which was slightly higher in the C than in the BP diet, and, as expected, decreased in the late season when compared to the early season ( $P<0.05$ ).

Ruminal pH was influenced by the experimental period, with lower values in G than in R (6.24 vs 6.57,  $P<0.05$ ). During late spring the higher pH values indicate that there was a high buffering capacity. This was probably due to the animals ruminating and chewing more effectively because of the higher NDF and DM content of the forage (Table 2). Concentrate type had no effect on the pattern and magnitude of the post-feeding pH decrease in the rumen ( $P>0.05$ ).

A marked drop in post-feeding pH after

the second meal agrees with the results by Soto Navarro *et al.* (2000) for cows fed twice daily. The pH values for the last two sampling times were below 6.10, critical for ruminal microbial activity, only during early spring.

This was probably due to the lower lignification of the cell wall of the growing rather than maturing forage, as was also observed by Coblenz *et al.* 2002.

The ruminal ammonia concentration decreased from early to late spring (10.79 vs 6.68 mg/100 ml;  $P<0.05$ ) and it was not influenced by concentrate carbohydrate source (Table 4). The pattern over time for ammonia was similar to that for pH (Figure 1, a and b), but peak concentrations during the day were 2 h after the morning meal. The ruminal NH<sub>3</sub> concentration may be due to the protein in the mature forage being less degradable. The forage soluble protein

(BSP) dropped by about 40% ( $P>0.05$ ) in late spring when compared to early spring. This agrees with the results of McCracken *et al.* (1993), who found a decrease in ruminal ammonia when steers were moved from spring to autumn pasture. During early spring a higher ruminal ammonia content was observed 2 and 10 h after the morning meal than in late spring, because of the lower CP content in the diet (15.35 vs 12.95 mg/100 ml;  $P<0.05$ ).

VFA concentration increased ( $P<0.05$ ) after feeding and decreased before the second meal. The maximum total VFA concentration and the lowest rumen fluid pH values were observed 2 h after the morning meal. Acetate, propionate and butyrate in the rumen fluid were influenced by the period but not by the concentrate type (Table 4). A period  $\times$  concentrate  $\times$  sampling time interaction ( $P<0.05$ ) was observed for acetate, propionate and butyrate (Figure 1). Acetate, propionate and butyrate levels were high during early spring, when fiber digestibility is high, as reported by McCracken *et al.* (1993). The type of energy source supplemented by the concentrate had a statistically significant effect on only propionate production. The corn based concentrate (C) produced a higher propionate concentration in the rumen fluid in both G and R.

Acetate/propionate and (acetate+butyrate)/propionate ratios were all statistically significantly influenced by both period and concentrate source.

The starch based concentrate produced lower  $C_2/C_3$  and  $(C_2+C_4)/C_3$  ratios, indicating the greater neoglucogenic potential of

the ration. By contrast, the cellulose and hemicellulose based concentrate (BP) concentrate produced higher  $C_2/C_3$  and  $C_2+C_4/C_3$  ratios, which means that there was more cellulolytic activity in the rumen, and probably a more effective synthesis of microbial protein. The effect of starchy vs hemicellulose based concentrates on ruminal parameters was similar in wethers fed with either immature or mature ryegrass forage.

## Conclusions

Early (vegetative) and late spring (reproductive) fresh ryegrass forage fed to wethers had marked differences in its chemical composition, depending on the season and its stage of maturity. Forage maturation had negative effects on DMI and the chemical composition of the wethers' diets. Wethers fed with BP concentrate consumed slightly more than those fed the C concentrate.

The carbohydrate source in the concentrate significantly influenced rumen fermentation patterns, producing a higher propionate level and lower acetate to propionate ratio in C than BP diets. This highlights the importance of the carbohydrate source in the supplements for wethers fed on fresh forage or pasture.

More information is needed on the optimal level of starch and digestible fiber in supplements for dairy sheep fed on pasture so that strategies to prevent ruminal acidosis can be implemented and also so that energy in the diet is converted more efficiently into those milk solids which are used for cheese-making.

## REFERENCES

- AOAC, 1980. Official Methods of Analysis. 13<sup>th</sup> ed. Association of Official Analytical Chemists, Washington, DC, USA.
- Beever, D.E., Siddons, R.C., 1986. Digestion and metabolism in grazing ruminants. In: L.P. Milligan, W.L. Grovum and A. Dobson (eds.) Control of digestion and metabolism in ruminants. Prentice-Hall, Banff, Canada, pp 479-497.

- Boever, J.L., Cottyn, B.G., Buysse, F.X., Wainman, F.W., Vanacker, J.M., 1986. The use of an enzymatic technique to predict digestibility, metabolizable and net energy of compound feedstuffs for ruminants. *Anim. Feed Sci. Tech.* 14:203-214.
- Broderick, G.A., Kang, J.H., 1980. Automated simultaneous determinations of ammonia and total amino acids in ruminal fluid and in vitro media. *J. Dairy Sci.* 63:64-75
- Coblentz, W.K., Coffey, K.P., Turner, J.E., Scarbrough, D.A., Skinner, J.V., Kellogg, D.W., Humphry, J.B., 2002. Comparisons of in situ dry matter disappearance kinetics of wheat forages harvested by various techniques and evaluated in confined and grazing steers. *J. Dairy Sci.* 85:854-865.
- Demarquilly, C., Andieu, J., Weiss, P., 1981. L'ingestibilité des fourrages verts et des foin et sa prévision. INRA ed., Paris, France.
- Fick, G.W., Holt, D.A., Lugg, D.G., 1988. Environmental physiology and crop growth. In: A.A. Hanson (ed.) *Alfalfa and alfalfa improvement*. Agron. Monogr. 29. ASA, CSSA, and SSSA publ., Madison, WI, USA, pp 163-194.
- Fussell, R.J., McCalley, D.V., 1987. Determination of volatile fatty acids (C2-C5) and lactic acid in silage by gas chromatography. *Analyst* 112:1213-1216.
- Holden, L.A., Muller, L.D., Fales, S.L., 1994. Estimation of intake in high producing Holstein cows grazing grass pasture. *J. Dairy Sci.* 77:2332-2340.
- Holmes, C.W., 1974. The Massey grass meter. *Dairy farming annual*. Massey University ed., Palmerston North, New Zealand.
- Jung, H.G., Deetz, D.A., 1993. Cell wall lignification and degradability. In: H.G Jung, D.R Buxton, R.D. Hatfeld and J. Ralph (eds.) *Forage cell wall structure and digestibility*. American Society of Agronomy publ., Madison, WI, USA, pp 315-346.
- Kolver, E.S., Muller, L.D., 1998. Performance and nutrient intake of high producing Holstein cows consuming pasture or a total mixed ration. *J. Dairy Sci* 81:1403-1411.
- Licitra, G., Hernandez, T.M., Van Soest, P.J., 1996. Standardization of procedures for nitrogen fraction of ruminant feeds. *Anim. Feed Sci. Tech.* 57:347-358.
- Maas, J.A., Wilson, G.F., McCutcheon, S.N., Lynch, G.A., Burnham, D.L., France, J., 2001. The effect of season and monensin sodium on the digestive characteristics of autumn and spring pasture fed to sheep. *J. Anim. Sci.* 79:1052-1058.
- McCracken, B.A., Krys, L.J., Parks, K.K., Holcombe, D.W., Judkins, M.B., 1993. Steers Grazing Endophyte-Free Tall Fescue: Seasonal Changes in Nutrient Quality, Forage Intake, Digesta Kinetics, Ruminal Fermentation, and Serum Hormones and Metabolites. *J. Anim. Sci.* 71:1588-1595.
- Mertens, D.R., 1992. Nonstructural and structural carbohydrates. In: H.H. Van Horn and C.J. Wilcox (eds.) *Large dairy herd management*. ADSA Publ., Champaign, IL, USA, pp 219-235.
- Molle, G., Decandia, M., Cabiddu, A., Kruger, M., Ligios, S., Fois, N., Sitzia, M., 2002. Feeding value of self-seeding Mediterranean forages grazed by dairy ewes. *Proc. 19<sup>th</sup> Gen. Meet. of the European Grassland Federation*, La Rochelle, France, 7:144-145.
- Molle, G., Decandia, M., Fois, N., Ligios, S., Cabiddu, A., Sitzia, M., 2003. The performance of Mediterranean dairy sheep given access to sulla (*Hedysarum coronarium L.*) and annual ryegrass (*Lolium rigidum Gaudin*) pastures in different time proportions. *Small Ruminant Res.* 49:319-328.
- Molle, G., Decandia, M., Ligios, S., Fois, N., Treacher, T.T., Sitzia, M., 2004. Grazing management and stocking rate with particular reference to the Mediterranean environment. In: G. Pulina and R. Bencini (eds.) *Dairy sheep nutrition*. CABI Publishing, Oxon, UK, pp 191-211.
- NRC, 1984. *Nutrient Requirements of Beefcattle*. National Academy Press 6<sup>th</sup> ed., Washington, DC, USA.
- Soto-Navarro, S.A., Krehbiel, C.R., Duff, G.C., Galyean, M.L., Brown, M.S., Steiner, R.L., 2000. Influence of feed intake fluctuation and frequency of feeding on nutrient digestion, digesta kinetics, and ruminal fermentation profiles in limit-fed steers. *J. Anim. Sci.* 78:2215-2222.

- Tamminga, S., 1992. Nutrition management of dairy cows as a contribution to pollution control. *J. Dairy Sci.* 75:345-357.
- Van Soest, P.J., 1982. Nutritional Ecology of the Ruminant. O&B Books, Corvallis, OR, USA.
- Varga, G.A., Dann, H.M., Ishler, V.A., 1998. The use of fiber concentrations for ration formulation. *J. Dairy Sci.* 81:3063-3074.