Effects of Supplemental Hay and Corn Silage Versus Full-Time Grazing on Ruminal pH and Chewing Activity of Dairy Cows

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ABSTRACT

Grazing young, highly digestible swards with and without supplemental hay or corn silage (5.5 kg of DM/d) offered overnight was tested for its effects on ruminal pH and chewing activity. A double 3 × 3 Latin square arrangement with 6 rumen-cannulated Brown Swiss cows (29 kg/d of milk) was applied. Herbage intake was quantified by controlled-release alkane capsules. Chewing activity was determined using an automatic microcomputer-based system for digital recording of the jaw movements. Except during milking, ruminal pH was measured continuously over 7 d by applying a device consisting of an indwelling pH electrode and a data-recording unit integrated in the cannula's cover. The grazing system had no significant effect on body weight, milk yield or composition (except milk urea), or total DM intake (13.5, 13.8, and 15.7 kg/d with full-time grazing, hay, and corn silage supplementation). No differences occurred for ruminating time per day and time per kilogram of DM intake. Full-time grazing cows spent more time eating per day (+26%) and time per kilogram of DM intake (+31%) than the other cows. Ruminal pH and time with pH < 5.8 at night did not differ. Throughout the day, hay-supplemented cows had a significantly lower pH (−0.23) than full-time grazing cows, and the period of pH < 5.8 was longer compared with corn-silage fed cows (77 vs. 11 min). Nocturnal supplement feeding gave no advantage over full-time grazing, and supplemental hay led to lower daytime pH.

(Key words: pasture, ruminal pH, dairy cow, grazing behavior)

Abbreviation key: C = part-time grazing with supplemental corn silage, G = full-time grazing, H = part-time grazing with supplemental hay.

INTRODUCTION

Low-input strategies have renewed the interest in grass-based milk production systems in grassland dominated regions (Parker et al., 1992). Efficient grazing systems, which allow the cows to cover nutrient and energy requirements, include increasingly young and highly digestible swards which may not provide sufficient physical structure to guarantee the absence of subclinical acidosis (Carruthers et al., 1997). Accordingly, intensively managed pastures are generally high in readily fermentable carbohydrates and low in contents of structural carbohydrates (Reis and Combs, 2000). Subclinical acidosis is a temporarily altered state of the rumen, where the pH is reduced below 5.8 (Maekawa et al., 2002). The consequences include decreased fiber digestion and VFA absorption from the rumen (Owens et al., 1998), inconsistent feed intake, decreased milk fat, diarrhea, and other health disorders (Nocek, 1997). Thus, low ruminal pH has direct negative effects on metabolic energy and protein supply, which are primary factors limiting production of dairy cows on forage-based diets.

Ruminal pH is determined by various factors, but mainly by structural properties of the feed. This directly affects chewing activity and the related buffer flow to the rumen through saliva (Bailey and Balch, 1961). There have been various attempts to describe structural properties of feed or to quantify their effects in the rumen. For instance, minimum dietary contents of NDF and ADF were provided by NRC (1989). However, chemical characteristics are not sufficient to satisfactorily describe structural properties, as this is more a combination of physical properties; other nutrients of the diet may affect the acid production pattern in rumen fluid as well (Krause et al., 2002). Fiber of different origin clearly varies in its effectiveness in stimulating chewing, e.g., because of differences in particle length (Grant et al., 1990). This led to concepts of feed evaluation by their contents of “structural fiber” or “effective fiber” (Mertens, 1997; De Brabander et al., 1999). These systems have limitations as they reflect interactions with other feed components only to a lim-

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ited degree. Milk fat percentage, suggested as an indicator to predict effective fiber content, has not turned out to be a repeatable response variable across different types of diets (Clark and Armentano, 1997) and across different stages of lactation (Allen, 1997). Ruminant pH would be the most meaningful and direct response variable (Allen, 1997), but punctual data obtained from occasionally collected rumen fluid samples are insufficient as they neglect the diurnal fluctuations in ruminal pH. Although methods for the online measurement of ruminal pH are now available (Maekawa et al., 2002; Duffield et al., 2004), there are no investigations to our knowledge on the effects of grazing without supplemental concentrate. Furthermore, chewing activity, which can now be continuously recorded with a special behavior recorder system composed of a sensory noseband and an automatic microcomputer (Rutter et al., 1997), might be a valuable indicator for ruminal pH fluctuations. This relationship needs to be confirmed.

The hypotheses to be tested in the present study were that full-time grazing of a young sward leads to extended periods of unfavorably low ruminal pH in dairy cows and that supplemental feeding of forages with different structural properties might prevent this effect. A combination of methods were applied that allowed us to continuously register ruminal pH and chewing activity, and to quantify feed intake and changes in ruminal fermentation pattern as well as metabolic indicators of subclinical acidosis.

MATERIALS AND METHODS

Experimental Design

Six lactating cows were assigned randomly in a monofactorial design to 3 grazing treatments in a double 3×3 Latin square arrangement. The treatments were full-time grazing with pasture grass as the only feed (**G**), with the cows being off pasture only during milking, and part-time grazing with the cows staying indoors overnight and offered grass hay (**H**) or corn silage (**C**) as supplements. Both supplements were offered at one time at 1700 h in amounts of 5.5 kg of DM/cow per day. Additionally, all cows received 300 g/day of a mineral mix containing per kilogram: 118 g of Ca, 45.5 g of P, 21.6 g of Mg, 89.7 g of Na, 1.24 g of Zn, 475 mg of Cu, 70 mg of Se, 25 mg of I, and 5 mg of Co. Minerals were mixed with 50 g/kg of fat, 511 g/kg of barley, and 7.2 g/kg of wheat middlings to facilitate the pelleting process and to ensure the complete consumption of the supplement. This mixture was provided daily after the morning milking. Each cow completed 3 consecutive 28-d experimental periods. Each period consisted of a 21-d adaptation

period and a 7-d data and sample collection period. The experiment was conducted in accordance with the Swiss guidelines on animal welfare.

Animals, Grazing Management, and Climatic Conditions

Cows were multiparous and of Brown Swiss breed. At the beginning of the experiment, cows on average were 85 (SD 12) DIM, had a BW of 561 (SD 21) kg and produced 29 (SD 2) kg/d of milk. The cows had been surgically fitted with flexible ruminal cannulas of 10 cm diameter (Bar Diamond, Parma, ID) at the end of the previous lactation. The experimental pastures, cultivated as natural meadows, were managed with an average rotation of 3 d, allocating on average 570 (SD 188) m² of space per cow during the experiment. The average sward growth was assessed by an electronic rising plate meter (Farmer tracker, B. M. Butler Computing Ltd., Palmerston North, New Zealand). Cows grazed swards down to mean heights of 12 (6 SD) cm. The average botanical composition, as determined once weekly, was characterized by high proportions of grasses (64%; dominated by *Lolium perenne*, *Poa pratensis*, *Poa trivialis*, *Agrostis alba*, and *Phleum pratense*) and fewer legumes (20%; *Trifolium repens*), and herbs (16%; *Taraxacum officinalis*, *Ranunculus acris*, and *Plantago major*). Ambient temperatures and air humidity, as recorded indoors by an automatic combined temperature and humidity sensor data logger (no. 2001, Escort Messtechnik AG, Aesch, Switzerland) at 0600 and 1900 h, were 20.9 (SD 3.5) and 21.2°C (SD 4.1), and 55.5 (SD 7.1) and 60.2% (SD 15.1), respectively. According to the data of a fixed meteorological station less than 1 km away from the experimental pastures, temperature and air humidity at 1300 and 1900 h were 20.3 (SD 2.5) and 22.0°C (SD 9.9), and 59.2 (SD 1.6) and 60.9% (SD 2.0), respectively.

Data Recording and Sample Collection

In each data collection period, BW and milk yield were determined automatically in the milking parlor at each milking (0530 and 1600 h). Two milk samples were taken per cow during each milking and one extra sample during morning milking. One sample was preserved with Broad Spectrum Microtabs (Gerber Instruments AG, Effretikon, Switzerland) and stored at 5°C for later analysis. The second sample was frozen at -20°C for compositional analysis, and the third (morning only) sample was cooled on ice for immediate pH determination.

Individual herbage intake and digestibility was estimated by the alkane double-indicator technique using

controlled-release capsules (type MCM, Captec Ltd, Auckland, New Zealand) as described and tested by Berry et al. (2000) and Estermann et al. (2001). The capsules were dosed by introducing the capsules through the ruminal cannulas 1 wk before the start of the collecting period. To obtain grass samples satisfactorily reflecting those consumed by the cows, hand-plucked herbage samples were collected daily between 0800 and 0900 h by following and mimicking cow selection, and cutting with a battery grass shearer (No. 7099002, Wolf Geräte GmbH, Betzdorf/Sieg, Germany). As the experimental pastures were far less botanically diverse and grazed at low sward heights, the application of a simpler procedure than described for diverse alpine pastures (Estermann et al., 2001) was considered justified. The herbage collection lasted from 2 d before the start of the actual collecting period to 2 d before the end of that week. In the collecting periods, 2 daily spot samplings of feces per cow, designated for dry and wet sample analysis, were directly obtained from cows defecating indoors between 0600 and 0800 h. The morning feces sampling is considered the best reflection of the average fecal composition (Estermann et al., 2001). The shift between herbage and fecal sampling accounted for the assumed delay in excretion. Hay and corn silage samples were collected daily. Refusals were recorded daily at 0530 h. Forage samples were first stored at 5°C and then pooled over the collection periods. Daily feces samples by cow were frozen at -20°C and composited across the collection period after thawing directly before analysis. At the time of feces collection, urine samples from the first urination were collected in a measuring pitcher and frozen at -20°C (one sample per cow per day). In the collection periods, rumen fluid samples were taken after each milking and preserved with 5% HgCl₂ before being stored at -20°C. Untreated rumen fluid subsamples collected after the morning milking were cooled on ice for immediate determination of rumen microbial counts (only practiced on 3 d of the collection period) or pH (on the other days of the week). Finally, 4 blood samples were taken from the jugular vein directly after milking on d 3 and 4 of the collection week, collected in vacuum tubes, cooled on ice, and centrifuged (Universal 16, Hettich, Tuttlingen, Germany) at 1500 × *g* for 15 min and stored at -20°C. Plasma and serum (only for NEFA analysis) were produced using heparinized vacuettes (Greiner Bioone, Solingen, Germany) or containers without anticoagulant.

Continuous measurement of *in vivo* ruminal pH was performed over the complete collection period, except once daily during milking (half of the cows in the morning, the other half in the evening), using a self-con-

structed device (Figure 1). The central instrument of the device was a pH electrode (Figure 1a; Solitrode-combined LL pH electrode, PP-shaft, Metrohm plug-in head G, no. 6.0220.100, Metrohm, Herisau, Switzerland). The indwelling pH electrode was protected against rumen movement by a plastic barrel (Figure 1b; length = 13 cm; i.d. = 1.6 cm; o.d. = 2 cm) perforated by 16 holes (diameter = 6 mm) equally distributed in 4 lines. The upper end of the electrode was closed in a waterproof way. Finally, to prevent direct contact of the membrane of the indwelling electrode with solid ruminal material, a polyester sampling bag (pore size = 53 mm, 5 × 10 cm, Bar Diamond, Parma, ID), which is commonly used in sacco experiments, was pulled over the plastic barrel and fixed with a binder. A 1-m electric wire (no. 6.2104.020, Metrohm) connected the electrode to a pH adapter (EP-ADP-PH, Escort Messtechnik AG) and a data logger (EX-2E-DDD, Escort), which recorded the ruminal pH at 1-min intervals. To protect the electrical system from rumen fluid, the wire was enclosed in a 35-cm long flexible plastic tube (o.d. = 1.4 cm) and the tube was connected to the indwelling electrode in a waterproof way by a self-constructed sheet-metal box (height = 10.5 cm; i.d. = 1.8 cm; o.d. = 2.2 cm, Inox) and with a plastic pipe (Figure 1e; height = 16 cm; i.d. = 9 cm; o.d. = 10.2 cm) containing the pH adapter and data logger (Figure 1g). Parallel to this first tube, a second tube (Figure 1d) was mounted which ended in a metal cone (height = 7.7 cm; diameter = 1.0 cm) perforated with 11 holes (diameter = 1 mm) per line in 6 parallel lines. When the system was installed in the rumen, rumen fluid could be drawn with this second tube. The pipe taking up the pH measurement equipment was fitted into the flexible rumen cannula. The upper end of the pipe was constructed in a way that allowed removal of the pH adapter and data logger. A waterproof cap was constructed to close the equipment during the pH measurements and a clamp system fastened the entire pipe into the rumen cannula (Figure 1f). A metal weight ring (Figure 1c; length = 4 cm; i.d. = 4 cm; o.d. = 7 cm, Inox) was attached to the upper end of the indwelling pH electrode around both tubes to keep the electrode in the liquid phase in the ventral sac of the rumen. After almost 24 h of measurement, the electrode was removed from the rumen and exchanged by a clean and calibrated electrode. Cleaning was performed using a pepsin solution (50 g/kg of pepsin dissolved in 0.1 M hydrochloric acid) for 1 h and stored in a 3 M potassium chloride solution for at least another 7 h. The electrodes were calibrated using the pH adapter using pH 7 and 4 buffer solutions (Hamilton Duracal, Reno, NV). Along with the replacement procedure, data were transferred from the logger to a computer.

Figure 1. Device for continuous measurement of ruminal pH: a. pH electrode; b. protecting barrel; c. weight ring; d. tube to obtain rumen fluid samples with a cone at the ruminal end and a sealing cap at the opposite end; e. cylinder fitting into rumen cannula; f. cap with clamp system; g. pH adapter for calibration and data logger.

The logger was reprogrammed and the pH adapter recalibrated with a clean electrode for the next 24 h of measurement.

Eating and ruminating behavior was individually recorded by registering jaw movements in the collection period using an IGER Behavior Recorder (Institute of Grassland and Environment Research, North Wyke, UK) as developed by Rutter et al. (1997). At least every 24 h, the recorder had to be removed from the cow to recharge the battery and download the data to a computer. After breaks of about 12 h, recorders were reinstalled on all cows at the same time. Records lasting for more than 22 h were used for analysis and extrapolated to 24 h. The data of the jaw movement recordings were read and analyzed automatically using the Microsoft Windows program "Graze" (Rutter, 2000). From the jaw movement pattern, the program discriminated time spent in eating and ruminating, and time without jaw movement (idle time). In the present study, the definition of idle time included jaw movements that lasted for less than 2 min and were more than 1 min away from the next movement, in addition to the times when no movements were recorded.

Laboratory Analyses

The preserved and refrigerated milk samples were analyzed by infrared spectrometry (Combi-Foss, Gerber Instruments AG) for contents of fat, protein, and lactose (FIL-IDF, 2000; method number 141C). The frozen milk samples were analyzed enzymatically for urea N content (kit no. 61 974, UV 250, BioMérieux, Lyon, France) according to Gutmann and Bergmeyer (1974). Milk pH was measured in untreated and cooled samples 2.5 h after morning milking on 4 d per collecting period with a pH electrode (no. 6.0220.100, Metrohm) and a pH meter (no. 692, Metrohm). The electrode was calibrated with pH 7 and 4 buffer solutions (Hamilton Duracal) before every pH measurement procedure.

Feed and feces samples were lyophilized, milled through a 1.0-mm screen (Brabender mill, no. 880804, Brabender, Duisburg, Germany), heated at 105°C for 3 h and at 550°C for 4 h to determine DM and ash contents, respectively. Contents of crude fiber, NDF, ADF, and acid detergent lignin were analyzed according to standard protocols (Van Soest et al., 1991; Naumann and Bassler, 1997). Nitrogen contents of the feeds were measured using the Dumas method (AOAC,

1990; method number 968.06) on a C/N analyzer (type FP-2000, Leco Instruments, St. Joseph, MI). Contents of n-alkanes in feed and feces were determined on a gas chromatograph (HP-5890 series II, Hewlett Packard, Walbronn, Germany) equipped with a packed column (2-3110, Supelco, Buchs, Switzerland) as described by Berry et al. (2000). In urine, pH was determined by a pH electrode (no. 6.0202.10, Metrohm) attached to a pH meter (EA 940, Orion, Cambridge, MA), and the fractionated net acid-base excretion was analyzed by titration using the recommended formula for calculation as described by Bender et al. (2003).

The treated rumen fluid samples collected after the milkings were thawed and enzymatically analyzed for ammonia concentration (test kit no. 61236 and method of BioMérieux) and for VFA concentration (performed according to Alén et al. (1985) on a gas chromatograph, HP 5890, Hewlett Packard). Rumen fluid ciliates and bacteria were enumerated using 0.1- and 0.02-mm (depth) Bürker counting chambers (Blau Brand, Wertheim, Germany), respectively. Ruminal pH was determined on cooled rumen fluid samples taken directly after milking in the morning on 4 d of every collection period with the same equipment used for determination of milk pH. For the analysis of the ruminal bicarbonate concentration, the thawed samples were first centrifuged (Universal 16, Hettich) at $2000 \times g$ for 20 min at 4°C (Tafaj et al., 1999). Bicarbonate concentration was then determined according to the manual of Mettler Toledo (Greifensee, Switzerland). Briefly, 5 mL of the supernatant was mixed with 45 mL of distilled water and, just before the measurements started, with 5 mL of an initiation solution. This solution was a preparation of 294 g of sodium citrate ($\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 2 \text{H}_2\text{O}$) in 500 mL of distilled water supplemented with 100 mL of hydrochloric acid (32%) and filled up to 1 L with distilled water. Rumen fluid total CO_2 concentration was measured by the combination of a gas-specific electrode (no. 15 232 3000, Mettler Toledo) with a reference electrode (InLab 3200, Mettler Toledo) mounted on the Metrohm pH meter. The results were converted using the average of 2 calibration curves obtained immediately before and after the measurements in the samples. The bicarbonate concentration was calculated by the function of the protonation.

Subsamples of the morning blood samples were subjected to immediate blood gas analysis (partial pressures of O_2 and CO_2 , O_2 saturation, pH, and base excess) using an i-STAT portable analyzer (cartridge EG7, i-STAT Corp., Princeton, NJ). Concentrations of metabolites and minerals in blood plasma were determined enzymatically using the following commercial test kits: glucose, no. 1447513, Roche, Basle, Switzer-

land; fructosamine, no. 1930010, Roche; triglycerides, no. 61 236, BioMérieux; BHBA, Ranbut RB 1008, Randox, Crumlin, UK; NEFA (in blood serum) FA 115, Randox; cholesterol, no. 61 219, BioMérieux; total protein, no. 1553836, Roche; albumen, no. 1553836, BioMérieux; urea, no. 61 974, UV 250, BioMérieux; creatinine, Jaffé, Roche; calcium, no. 1489216, Roche; inorganic phosphorus, no. 1489216, Roche; magnesium, no. 61 411, BioMérieux.

Calculations and Statistical Analysis

The dietary contents of absorbable protein at the duodenum and NE_L were calculated according to RAP (1999). For the estimation of feed intake and digestibility, the average of more than one alkane (here C_{31} and C_{33}) was used to account for analytical errors occurring with each of the alkanes. Formulas given by Mayes et al. (1986) were applied. The known intakes of DM, nutrients, and alkanes with the mineral mix and with hay or corn silage DM, if any, were considered in calculating the grass intakes and diet digestibilities. Times spent in eating and rumination were related to these estimates of intakes of DM and NDF. For evaluation of the continuously obtained pH data, the day was separated into a diurnal (0800 to 1600 h) and a nocturnal (1730 to 0600 h) period. For these periods, minimum, maximum, and mean pH values, and the time pH was below 5.8 (the threshold level for subclinical ruminal acidosis suggested by Maekawa et al., 2002) were calculated. Eating, rumination, and idle time were divided into 2 periods comprising 0600 to 1730 h (here defined as daytime) and 1730 to 0600 h (nocturnal data).

Data were analyzed using the GLM procedure of SAS (SAS Institute, 2002). Data that were estimated on several days per period were averaged over the period before statistical analysis. Multiple comparisons among treatment means were performed by the Tukey procedure (SAS Institute, 2002). Variables determined twice a day were analyzed using the repeated measurement statement of SAS. Mean values and standard deviations (SD) or standard errors of mean (SEM) are presented.

RESULTS

The pasture grass was higher in CP and lower in fiber than the hay (Table 1). Calcium, P, Mg, and K were found in higher concentrations in the grass compared with corn silage, with hay having intermediate levels. Corn silage had the highest calculated NE_L content and the lowest contents of fiber, absorbable protein, and minerals among the 3 forages. Corn silage

Table 1. Composition of the experimental feeds [means of 6 (pasture grass) and of 3 (other feeds) determinations].

	Pasture grass		Hay		Corn silage	
	Mean	SD	Mean	SD	Mean	SD
Analyzed nutrient and mineral composition, g/kg of DM						
DM, g/kg of wet weight	194	33	909	7	361	5
OM	898	7	913	1	964	1
CP	170	53	110	7	76	2
Crude fiber	229	37	262	7	183	11
NDF	459	22	485	7	420	50
ADF	286	21	293	9	215	22
Acid detergent lignin	49.0	16.0	31.1	3.1	23.3	4.4
Ca	6.35	1.18	4.83	0.72	1.49	0.13
P	3.55	0.69	3.16	0.09	1.78	0.15
Mg	2.07	0.29	1.59	0.12	1.06	0.16
Na	0.46	0.17	0.56	0.18	0.30	0.10
K	27.0	6.0	25.2	1.2	11.1	2.2
Calculated ¹ contents, per kg of DM						
Absorbable protein, g	103.3	12.3	84.5	1.4	74.1	2.0
NE _L , MJ	6.23	0.39	5.60	0.06	6.76	0.28
Analyzed n-alkane contents, mg/kg of DM						
C ₃₁	162.7	39.9	196.6	8.2	18.2	3.9
C ₃₂	4.8	0.7	5.3	1.1	2.1	0.1
C ₃₃	54.9	8.1	46.2	3.9	12.0	0.6

¹According to RAP (1999).

also contained less n-alkanes than the grass and the hay.

Cows receiving hay reduced ($P < 0.05$) their grass intake by the same amount as the hay offered (Table 2). Total DM intake as well as fiber, nutrient, and energy intake did not differ among treatment groups. Furthermore, there were no significant differences in OM and fiber digestibilities. Neither BW nor milk yield and composition were modified. This was also true for milk fat content, which is assumed to be highly susceptible to changes in structural fiber content of the diet (Mertens, 1997). Milk urea nitrogen reflected differences in N content of the diets, with low values found with corn silage supplementation and high levels occurring with grass only ($P < 0.05$).

Nocturnal eating time was significantly ($P < 0.001$) reduced by 77 and 86% with hay and corn silage supplementation, respectively, compared with the grass-only group (Table 3). Concomitantly, supplemented cows spent more time ruminating (significant for corn silage supplementation, $P < 0.05$) and more time idle ($P < 0.001$) than full-time grazed cows. In the daytime, eating time increased by 14% with supplemental hay ($P < 0.005$) and by 11% with supplemental corn silage, whereas rumination and idle time did not differ significantly among treatments. Considering the numerically higher DM intakes, overall the corn silage cows required less time to consume 1 kg of diet DM (−33%), total NDF (−31%), and particularly, indigestible NDF (−41%) than the cows subjected to full-time grazing.

The hay-supplemented cows took an intermediate position.

The diurnal evolution of rumen fluid pH showed no clear treatment differences except during daytime, when the hay-supplemented cows developed the largest decline, which was rapidly compensated after supplementing the hay in the afternoon (Figure 2). Overall, this resulted in a significantly lower mean daytime pH in this group compared with the other groups, associated with the longest time with pH values falling below 5.8 (Table 4). Nocturnal pH values were not significantly different among groups. Determinations of pH in rumen fluid samples collected during milking showed the same trends as permanent measurements, although they were not significantly different among groups. The Pearson correlation coefficient between these data and the values of the continuous measurements at the same time was 0.65 ($P < 0.001$). Urine pH showed a trend ($P = 0.06$) toward lower values in the full-time grazed cows. No clear treatment effects were obvious in blood and milk pH.

Supplementation did not affect blood gas profile and rumen fluid CO₂ concentration (Table 5). The same holds true for bicarbonate concentrations in blood and rumen fluid. However, there was a treatment effect in net acid-base excretion and urine base-acid ratio, as both were elevated ($P < 0.05$) with the nocturnal supplementation of hay, compared with full-time grazing. With corn silage supplementation, the net acid-base excretion was not significantly different compared

Table 2. Nutrient intake, digestibility, and performance.

Item	Treatment ¹			SEM	Treatment effect (<i>P</i>)
	G	H	C		
Intake, /cow per d					
Grass DM, kg	13.2 ^a	8.2 ^b	9.7 ^{ab}	1.3	0.048
Total DM, ² kg	13.5	13.8	15.7	1.3	0.46
OM, kg	11.9	12.2	14.2	1.2	0.34
NDF, kg	6.04	6.38	6.87	0.59	0.62
ADF, kg	3.77	3.91	4.04	0.37	0.88
Absorbable protein, ³ kg	1.40	1.31	1.36	0.14	0.90
NE _L , ³ MJ	86.3	81.0	94.3	8.5	0.55
Digestibility					
OM, %	76.8	75.3	77.8	2.2	0.72
NDF, %	81.4	78.4	78.6	2.3	0.59
ADF, %	79.8	77.7	75.4	2.0	0.33
BW, kg	548	555	545	11	0.81
Milk yield and composition					
Yield, kg/cow per d	17.6	18.4	18.9	1.2	0.77
Fat, %	3.79	3.67	3.71	0.14	0.83
Fat, kg/cow per d	0.68	0.67	0.70	0.05	0.91
Protein, %	3.08	3.08	3.08	0.12	1.00
Protein, kg/cow per d	0.54	0.56	0.57	0.02	0.60
Lactose, %	4.62	4.64	4.69	0.06	0.72
Urea N, mg/kg	216 ^a	198 ^{ab}	136 ^b	20	0.035

^{a,b}Means within the same row with different superscripts are significantly different ($P < 0.05$).

¹G = Full-time grazing, H = part-time grazing plus hay, C = part-time grazing plus corn silage.

²Including mineral supplement and, when offered, hay or corn silage.

³Calculated according to RAP (1999).

Table 3. Eating pattern.

Item	Treatment ¹			SEM	Treatment effect (<i>P</i>)
	G	H	C		
Daytime ²					
Eating, min/d	343 ^b	398 ^a	380 ^{ab}	14	0.035
Rumination, min/d	114	87	88	10	0.12
Time idle, min/d	265	235	244	13	0.26
Nocturnal ³					
Eating, min/d	188 ^a	43 ^b	26 ^b	15	<0.001
Rumination, min/d	231 ^b	284 ^{ab}	298 ^a	15	0.017
Time idle, min/d	299 ^b	393 ^a	404 ^a	18	0.001
Average of daytime and nocturnal data					
Eating					
min/d	532 ^a	441 ^b	405 ^b	34	<0.001
min/kg of DM consumed	40.1 ^a	33.9 ^{ab}	27.0 ^b	6.9	0.029
min/kg of NDF consumed	89.1 ^a	73.9 ^{ab}	61.6 ^b	15.0	0.035
min/kg of indigestible NDF consumed	521 ^a	345 ^{ab}	308 ^b	121	0.031
Rumination					
min/d	344	371	386	38	0.25
min/kg of DM consumed	25.6	28.8	25.6	5.7	0.60
min/kg of NDF consumed	57.0	62.7	58.4	12.8	0.77
min/kg of indigestible NDF consumed	336	299	286	94	0.70
Time idle					
min/d	564 ^b	628 ^{ab}	649 ^a	43	0.018

^{a,b}Means within the same row with different superscripts are significantly different ($P < 0.05$).

¹G = Full-time grazing, H = part-time grazing plus hay, C = part-time grazing plus corn silage.

²0600 through 1730 h.

³1730 through 0600 h.

Figure 2. Diurnal fluctuation of ruminal pH of cows grazed full-time (solid line, gray), or receiving nocturnal supplementations of hay (dotted line, gray) or corn silage (solid line, black) ($n = 6$, treatment means and SE as error bars).

with the other treatments, and the base-acid ratio was similar to the full-time grazing group.

Treatment effects on rumen fluid ammonia concentration differed with time of day (interaction, $P < 0.05$; Table 6). In the morning, with generally lower ammonia levels, corn silage supplementation reduced ($P < 0.05$) the concentration further, which was not observed in the afternoon after several hours of grazing, when the hay-supplemented cows expressed the highest ($P < 0.05$) ammonia levels. There were daytime differences in all traits describing VFA concentration and profile, but treatment effects mostly remained insignificant. In variables where a significant interaction between treatment and time was found, treatment differences were more pronounced in the morning, as could be expected from the different nocturnal feeding schedules. Across both sampling times, treatment effects ($P < 0.05$) were found in rumen fluid concentrations of total VFA and acetate such that levels were low with corn silage supplementation (VFA: 56.3 mmol/L; acetate: 37.8 mmol/L) compared with hay supplementation (VFA: 64.3 mmol/L; acetate: 43.8 mmol/L). Overall, the isovalerate concentration in corn silage-supplemented cows (0.33 mmol/L) differed significantly ($P <$

0.05) from hay-supplemented (0.43 mmol/L) and full-time grazed cows (0.43 mmol/L). The average bacteria counts in rumen fluid were 1.97, 2.17, and 1.77×10^{10} /mL for full-time grazing, hay, and corn silage treatments, respectively. The corresponding ciliate protozoa counts were 1.87, 2.1, and 1.83×10^5 /mL (not significant; data not shown).

Supplement feeding increased ($P < 0.05$) blood glucose levels in the morning and in the afternoon (Table 7), and NEFA levels tended to be reduced when supplement was offered. In the morning, BHBA also followed the NEFA trend, but supplemented cows had higher BHBA levels than full-time grazed cows in the afternoon, although levels were under the threshold for subclinical ketosis. Corn silage reduced ($P < 0.05$) plasma urea and creatinine compared with the grass-only group and, occasionally, compared with the hay-supplemented group, too. The other metabolites in blood plasma were not significantly different among treatment groups. The blood plasma levels of P and Mg, but not Ca, showed treatment differences ($P < 0.05$). Corn silage and, to a lesser extent, hay feeding reduced plasma P in the morning but not in the after-

Table 4. Rumen fluid, blood, milk, and urine pH.

Item	Treatment ¹			SEM	Treatment effect (<i>P</i>)
	G	H	C		
Rumen fluid pH (permanent measurement)					
Daytime ²					
Mean	6.46 ^a	6.23 ^b	6.46 ^a	0.06	0.025
Minimum	5.88	5.69	6.00	0.10	0.12
Maximum	6.91	6.74	6.90	0.08	0.24
Time pH<5.8, min/d	51 ^{ab}	77 ^a	11 ^b	15	0.024
Nocturnal ³					
Mean	6.10	6.12	6.21	0.07	0.46
Minimum	5.47	5.33	5.42	0.07	0.40
Maximum	6.61	6.66	6.71	0.05	0.42
Time pH<5.8, min/d	178	121	93	34	0.22
Average of daytime and nocturnal data					
Mean	6.28	6.17	6.34	0.06	0.17
Minimum	5.68	5.51	5.71	0.08	0.40
Maximum	6.91	6.70	6.90	0.05	0.24
Time pH<5.8, min/d	114	99	52	23	0.17
pH in drawn samples					
Rumen fluid ⁴	6.55	6.42	6.62	0.14	0.35
Blood	7.51	7.49	7.47	0.02	0.36
Milk	6.77	6.76	6.76	0.02	0.84
Urine	8.41	8.49	8.48	0.02	0.061

^{a,b}Means within the same row with different superscripts are significantly different ($P < 0.05$).

¹G = Full-time grazing, H = part-time grazing plus hay, C = part-time grazing plus corn silage.

²0800 through 1600 h.

³1730 through 0600 h.

⁴Average of samples drawn at 0600 and 1600 h.

noon. In contrast, cows receiving grass only had the lowest Mg values in blood plasma in the afternoon.

DISCUSSION

Advances in breeding forage plants and their use at an early growth stage have resulted in pasture grass

rich in readily fermentable carbohydrates. The aim of the present study was to investigate whether feeding these grass qualities is associated with a risk of sub-clinical ruminal acidosis and if supplement feeding of hay or corn silage can act preventatively in that regard. Acidosis is of great importance from an economic and an animal welfare point of view.

Table 5. Blood and rumen fluid gas variables, bicarbonate in blood and rumen fluid, and acid-base properties in blood and urine.

Item	Treatment ¹			SEM	Treatment effect (<i>P</i>)
	G	H	C		
Blood gas variables					
Partial O ₂ pressure, mmHg	36.6	37.8	40.9	2.1	0.35
Partial CO ₂ pressure, mmHg	33.5	35.0	32.5	1.9	0.66
O ₂ saturation, %	75.9	76.4	78.2	2.0	0.71
CO ₂ , mmol/L	28.5	27.8	25.3	2.4	0.60
Rumen fluid gas variables					
CO ₂ , mmol/L	12.6	11.4	10.0	1.8	0.59
Bicarbonate, mmol/L					
Blood	27.6	26.8	24.3	2.3	0.58
Rumen fluid	83.1	89.9	106.0	1.8	0.14
Acid-base variables					
Blood base excess, mmol/L	4.50	3.58	0.58	2.59	0.55
Urine net acid-base excretion, mmol/L	155 ^b	229 ^a	187 ^{ab}	14	0.007
Urine base-acid ratio	3.88 ^b	5.52 ^a	3.79 ^b	0.26	<0.001

^{a,b}Means within the same row with different superscripts are significantly different ($P < 0.05$).

¹G = Full-time grazing, H = part-time grazing plus hay, C = part-time grazing plus corn silage.

Table 6. Ruminal fluid variables determined at different times of the day.

Item	Treatment ¹						SEM	Effects ² (<i>P</i>)		
	0600 h			1600 h				T	Time	T × Time
	G	H	C	G	H	C				
Ammonia, mmol/L	6.61 ^{cd}	4.04 ^{de}	1.30 ^e	10.95 ^b	14.22 ^a	9.45 ^{bc}	1.24	<0.001	<0.001	0.03
VFA, mmol/L										
Total VFA	56.6 ^{bc}	56.1 ^{bc}	49.8 ^c	65.5 ^{ab}	72.5 ^a	62.8 ^{abc}	3.9	0.049	<0.001	0.49
Acetate	39.0 ^{ab}	39.9 ^{ab}	34.8 ^b	43.8 ^{ab}	47.8 ^a	40.8 ^{ab}	2.8	0.030	0.002	0.78
Propionate	9.5 ^b	9.2 ^b	8.4 ^b	12.0 ^a	13.3 ^a	12.0 ^a	0.8	0.24	<0.001	0.39
Butyrate	6.62 ^{bc}	5.72 ^c	5.59 ^c	7.87 ^{ab}	9.18 ^a	8.15 ^{ab}	0.49	0.42	<0.001	0.06
Isobutyrate	0.57 ^{cd}	0.48 ^{de}	0.40 ^e	0.69 ^{ab}	0.79 ^a	0.63 ^{bcd}	0.07	0.061	<0.001	0.26
Valerate	0.55 ^{cd}	0.48 ^d	0.37 ^d	0.72 ^{bc}	0.97 ^a	0.81 ^{ab}	0.06	0.076	<0.001	0.018
Isovalerate	0.39 ^{bc}	0.28 ^{cd}	0.22 ^d	0.48 ^{ab}	0.58 ^a	0.45 ^{abc}	0.04	0.021	<0.001	0.038
VFA, molar %										
Acetate	68.4 ^{bc}	71.0 ^a	69.8 ^{ab}	66.9 ^{cd}	65.6 ^d	65.2 ^d	0.7	0.44	<0.001	0.013
Propionate	16.9 ^b	16.5 ^b	17.0 ^b	18.2 ^a	18.4 ^a	18.9 ^a	0.4	0.22	<0.001	0.48
Butyrate	11.9 ^{ab}	10.4 ^c	11.2 ^{bc}	12.0 ^{ab}	13.0 ^a	12.9 ^a	0.5	0.61	0.002	0.065
Isobutyrate	1.05 ^{ab}	0.86 ^{bc}	0.82 ^c	1.08 ^a	1.08 ^a	1.02 ^{ab}	0.10	0.11	0.011	0.29
Valerate	0.99 ^{bc}	0.85 ^{cd}	0.75 ^d	1.08 ^b	1.32 ^a	1.25 ^a	0.05	0.26	<0.001	<0.001
Isovalerate	0.72 ^a	0.51 ^b	0.45 ^b	0.74 ^a	0.79 ^a	0.72 ^a	0.07	0.015	<0.001	0.017
Acetate-to-propionate ratio	4.08 ^a	4.32 ^a	4.13 ^a	3.72 ^b	3.58 ^b	3.48 ^b	0.12	0.29	<0.001	0.12

^{a,b,c,d,e}Means within the same row with different superscripts are significantly different ($P < 0.05$).

¹G = Full-time grazing, H = part-time grazing plus hay, C = part-time grazing plus corn silage.

²T = Treatment.

Methodology

To be able to investigate diurnal pH fluctuation and its relationship to intake pattern, we applied a novel combination of methods not previously used for this type of investigation. Another direct approach is the pH determination by drawing samples from the rumen. However, this method has the disadvantage of a delay until pH measurement takes place, time in which rumen fluid can interact with air CO₂, and microbial activity continues. The measured pH therefore cannot reproduce the correct value of the time point at which the sample was drawn, compared to continuous measurement with an indwelling pH electrode. Accordingly, in the present study, the mean pH during the day as determined by continuous measurement was consistently lower than that determined from rumen fluid samples drawn twice a day. Furthermore, the mean values of the continuous measurements were significantly different among treatments, whereas the values of the spot samples were not, probably due to their higher SEM. Two less invasive techniques for measuring ruminal pH, the rumenocentesis and the oral stomach tube technique, were discussed by Duffield et al. (2004) but were found not to be as sensitive as continuous measurement. In both methods, the site of the rumen where the samples were taken greatly affected the result, and samples obtained orally were less accurate due to contamination with salivary products. The pH electrode used in our study and others (e.g., Krause et al., 2002; Duffield et al., 2004) was

placed in the ventral sac and retained in place with a metal ring to minimize displacement in the rumen. For continuous measurement of ruminal pH, different devices were developed, all introducing a pH electrode as the central component into the rumen through a cannula. Collaborators at the Swiss Federal Research Station conceptually developed the device used in the present study. The device was optimized for pasture experiments by integrating all necessary measurement units into the cover of the rumen cannula thus avoiding the need for devices attached to the back of the cows. The latter were found to be stressful to animals and could be easily removed by them. Other researchers, performing continuous measurements of ruminal pH (Krause et al., 2002; Nocek et al., 2002; Duffield et al., 2004), used devices where the indwelling electrode was connected to a stationary pH meter or directly to a computer next to the animal. Such devices would not be compatible with grazing experiments. To ensure that repeated mechanical readings represent the true ruminal pH and to control the accuracy of the indwelling pH measurement as well as the possible influences of the rumen fluid, Nocek et al. (2002) suggested comparing pre- and postcleaned pH measurements from drawn samples. There were no obvious differences between the values of the pre- and postcleaned pH readings in our study. We cleaned and recalibrated each electrode once daily.

Currently, several indirect methods are in use for the diagnosis of ruminal acidosis. Indicators that are easy to determine would be advantageous due to their

Table 7. Blood plasma concentration of metabolites and minerals, and enzyme activities determined at different times of day.

	Treatment ¹						SEM	Effects ² (<i>P</i>)		
	0700 h			1630 h				T	Time	T × Time
	G	H	C	G	H	C				
Metabolites										
Glucose, mmol/L	2.56 ^c	3.17 ^a	3.37 ^a	2.55 ^c	2.86 ^b	2.82 ^{bc}	0.12	<0.001	0.001	0.035
Fructosamine, μmol/L	274	279	279	268	274	272	8	0.75	0.36	0.99
Triglycerides, μmol/L	411	373	366	359	343	338	52	0.57	0.79	0.93
BHBA, mmol/L	0.64 ^c	0.52 ^c	0.44 ^c	0.94 ^b	1.30 ^a	1.03 ^a	0.07	0.060	<0.001	0.011
NEFA, ³ μmol/L	113.5 ^a	80.0 ^{ab}	77.0 ^{ab}	54.7 ^{ab}	33.9 ^b	30.6 ^b	22.2	0.28	0.005	0.94
Cholesterol, mmol/L	5.49	5.23	5.61	5.16	5.12	5.25	0.34	0.75	0.34	0.92
Total protein, g	85.0	81.6	82.1	82.5	80.6	79.4	3.0	0.50	0.37	0.95
Albumen, g/L	40.0	40.3	41.0	39.3	40.0	39.3	1.0	0.74	0.37	0.89
Urea, mmol/L	6.97 ^{ab}	6.30 ^b	4.21 ^c	8.27 ^{ab}	8.44 ^a	5.82 ^b	0.97	<0.001	<0.001	0.67
Creatinine, μmol/L	77.7 ^b	83.5 ^{ab}	93.4 ^a	85.0 ^{ab}	91.3 ^a	94.3 ^a	4.1	0.020	0.13	0.65
Minerals, mmol/L										
Ca	2.52	2.58	2.52	2.51	2.55	2.58	0.05	0.62	0.88	0.60
P	1.56 ^{ab}	1.41 ^{bc}	1.29 ^c	1.66 ^a	1.41 ^{abc}	1.66 ^a	0.11	0.079	0.033	0.11
Mg	0.90 ^{bc}	0.95 ^{ab}	0.93 ^{abc}	0.88 ^c	0.98 ^a	0.94 ^{abc}	0.03	0.006	0.82	0.59

^{a,b,c}Means within the same row with different superscripts are significantly different ($P < 0.05$).

¹G = Full-time grazing, H = part-time grazing plus hay, C = part-time grazing plus corn silage.

²T = Treatment.

³Determined in blood serum.

simpler and more widespread applicability. Often, milk fat percentage is used as a basis to create an evaluation system predicting the effectiveness of the structural properties of the dietary fiber (Mertens, 1997; De Brabander et al., 1999). In the present study, neither milk yield nor milk composition showed significant responses to the treatments. Thus, milk fat percentage was not sufficient for determining the relatively small differences in ruminal pH across treatments. Another approach, where no rumen fluid would be needed, is the determination of effects on pH of other body fluids such as milk, blood, and urine, where relatively close correlation with ruminal pH can be expected (Owens et al., 1998; Bender et al., 2003), although the body's metabolic buffering systems (Müller-Plathe, 1998) may reduce the effects in magnitude. Measurements of acid-base equilibrium and of blood gas profile might be indicative in that respect. However, none of the variables differed significantly due to treatment by shifts corresponding to those found in ruminal pH fluctuation. On the contrary, blood pH values (>7.45) in all treatments, the partial CO₂ blood pressure (<34.5 mmHg) with full-time grazing and with supplemental corn silage, and a base excess (>3 mmol/L) with full-time grazing indicated a tendency to metabolic alkalosis, which is occasionally observed as a response to ruminal acidosis (Cao et al., 1987). Both urine and milk pH levels were slightly above standard values (urine, 7.8 to 8.4; milk, 6.5 to 6.7), but this may have had methodological reasons such as interactions with air CO₂ during urine storage

and changes in milk until pH measurement 2.5 h after milking. The only variables showing significant treatment differences were the net acid-base excretion via urine and the urine base-acid ratio. The values compared favorably with standard values given by Bender et al. (2003) for the net acid-base excretion (107 to 193 mmol/L) and the base-acid ratio (2.5 to 4.8). Improvements in using indicators to determine potentially detrimental but transient depressions in pH might be given by taking samples more frequently and at times other than only in the morning as was the case in the present study.

A final, indirect assessment of effects on ruminal pH might be given by the determination of chewing activity. The importance of chewing, which increases with increased consumption of structurally effective fiber, to buffer fermentation acids in the rumen by promoting saliva production is well documented (Allen, 1997; Mertens, 1997) and is therefore used for evaluating the structural properties of dietary fiber. The device developed by Rutter et al. (1997) was found to be highly suitable (e.g., Gibb et al., 1997) for determination of chewing activity. It allows for differentiation between grazing and ruminating, and clearly indicates times when cows are idle. Another device uses a microphone to record the sound made by severing of the herbage for determining single bites and chewing activity (e.g., Delagarde et al., 1999). In those methods, some background noises were difficult to distinguish from bite noises and therefore could not be eliminated by filtering; in particular, discrimination between bit-

ing, drinking, and the direct rubbing of hard herbage against the microphone was difficult (Delagarde et al., 1999). Delagarde et al. (1999) calculated that drinking caused less than 1% of misallocations of the total number of bites. We found similar difficulties in our study with the sensor ribbon around the jaw, whereby grazing periods shorter than 2 min could not be clearly distinguished from drinking or short, soft rubbings. Therefore, we used clearly defined criteria to allocate these activities to grazing or idling activities. Visual monitoring of eating and ruminating behavior is another common method (e.g., Krause et al., 2002; Maekawa et al., 2002), in which cows are individually and repeatedly observed each for several minutes. Depending on the frequency of these observations, the estimate for the overall activity over 24 h is either inaccurate or the procedure is extremely laborious.

Effects of Full-Time Grazing vs. Supplemental Feeding

Ruminal pH. In the present study, the grass-alone feeding regimen never caused a critical mean ruminal pH. In the review by Bargo et al. (2003), the mean pH value was between 5.9 and 6.0 in only 2 of 10 studies, and the value was slightly lower (5.75) in only one experiment. In the present study, supplemental feeding had no effect on mean ruminal pH. The mean ruminal pH values were similar to those found in a grazing study of Carruthers et al. (1997), who reported a mean pH level of 6.55. However, differences were observed when looking in more detail at the diurnal fluctuation of ruminal pH, and the times where pH remained below 5.8 [the threshold level given by Maekawa et al. (2002) for subclinical acidosis]. Both at night and during the day, there were extended periods with low pH, and the minimum nocturnal pH found with grazing was 5.47 on average. However, supplemental feeding was not found to improve the situation and hay led to a lower mean pH and extended periods with pH <5.8 during the day. Reis and Combs (2000), by contrast, found mean ruminal pH levels as high as 6.75 during the day and 6.61 at night when cows were grazing a grass-legume pasture and were offered between 0 and 10 kg of a corn-based concentrate. The supplementation with 1.6 kg of straw to grazing cows did not affect diurnal variation of ruminal fluid pH in the study of Wales and Doyle (2003). These researchers found a different fluctuation of ruminal pH with a sharp increase in the afternoon interrupting the general decline over the day. This increase, however, was not observed in studies using a TMR (Maekawa et al., 2002; Duffield et al., 2004), where a roughly continu-

ous forage intake over the day can be assumed, as in our study.

Synthesis of VFA as pH-relevant factor. The concentrations of all VFA in the rumen fluid in the present study were lower compared with a range of different studies with pasture-based diets (e.g., Carruthers et al., 1997; Reis and Combs, 2000). Increasing the concentrations of total VFA is likely to decrease ruminal pH (Owens et al., 1998). In the present study, the total ruminal VFA concentration measured in the morning was lowest with corn silage supplement and, consequently, the nocturnal mean ruminal pH was highest, whereas hay supplementation and grass-only treatments showed the opposite pattern. Furthermore, the high rumen fluid total VFA and acetate concentrations in the hay-fed cows, as determined in the afternoon, were in agreement with the ruminal pH data. Supplementation with concentrated feed is likely to increase the VFA concentration compared with grass-only diets (Bargo et al., 2003), but this is a function of feed intake as this also affects the amount of easily fermentable matter. This may explain the lack of a clear effect of corn silage supplementation.

Chewing activity as pH-relevant factor. In the present study, eating pattern was influenced by treatment and time of day. Cows receiving either 5.5 kg of hay or corn silage in the evening spent less time eating during the night and more time idle (and ruminating) than full-time grazed cows. On the other hand, supplemented cows partly compensated for the reduced nocturnal eating time by increasing this activity in the daytime. On average, the time spent eating per day and time per DMI was 1.9-fold greater than in cows fed with silage and concentrate mixture in other studies (e.g., Soita et al., 2000) and the eating time in the grass-only treatment was 1.3-fold greater than with any supplementation; overall rumination times did not differ among treatments. Rumination times per day and per kilogram of DMI were close to the range determined by Soita et al. (2000). In that study, where the average of total DMI was greater than in our study (20 vs. 14 kg of DM), the effects of decreasing particle size and increasing concentrate proportion, respectively, decreased the times spent eating and ruminating and the times used for total DMI and ruminating per unit of NDF consumed. Approximately one-half of the bicarbonate entering the rumen comes from saliva and is caused by eating and, particularly, by ruminating; the other half enters the rumen in exchange of VFA being absorbed (Owens et al., 1998). Tafaj et al. (1999), who fed hay-based diets varying in particle size to cows, found bicarbonate values of 56 and 31 mmol/L 1 h before and 3 h after feeding, respectively, which was lower than the 93 mmol/L found in the present

study. Because saliva excretion is higher during rumination than during eating (Bailey and Balch, 1961), and cattle tend to ruminate at night and eat during the day (Gibb et al., 1997; present results), this could be an explanation for the high concentration measured early in the morning before feeding. However, the significantly higher nocturnal rumination time observed with cows fed corn silage compared with full-time grazed cows resulted only in a numerical increase in the ruminal bicarbonate concentration. The longer eating times in full-time grazed cows compared with the supplemented cows, therefore, have to be explained by factors other than structural properties. Moreover, content of fiber, particularly indigestible fiber, contributing to gut fill and hampering feed intake, can be ruled out as a major factor because eating times per unit of fiber and indigestible fiber differed among treatments and there was no significant correlation with the NDF content of the diet. This observation is in agreement with that of Soita et al. (2000). Bite size and eating time depend on feed availability, which means that sward height plays an important role for grazing (Gibb et al., 1997). This suggests that sward height (12 cm on average) may not always have been sufficient in the present study, and might have prevented maximum bite sizes. Additionally, the need to walk to get access to feed and the lack of distinct feeding times might have lengthened eating periods per unit of DMI (Cazcarra et al., 1995). Whatever the reason, the increased eating times with full-time grazing have resulted in a more even distribution of the daily feed portion and might have been primarily responsible for the lack of detrimental effects in ruminal pH and the inability of the hay supplementation to improve the situation. This means that the reason was not primarily an increased saliva production, because a prolonged eating time is not necessarily associated with a higher average ruminal pH (Wales and Doyle, 2003), and eating expresses only half of the saliva supporting effect as rumination (Bailey and Balch, 1961). On the other hand, higher eating activity at a lower rate of consumption on pasture, reflecting a smaller bite size, might result in increased saliva secretion rate in relation to units of fermentable carbohydrates ingested and, therefore, in buffering capacity. Supplemented cows, having no access to additional feeds once they had consumed their feed portion in the evening, increased their eating time during the daytime. From the decrease in ruminal pH, especially of hay-supplemented cows, it can be concluded that, after a period of reduced eating activity during the night, supplemented cows ingested pasture grass at a higher rate in the morning resulting in a more intensive fermentation than that occurring in full-time

grazed cows. If this hypothesis is correct, cows grazing young pastures are less prone to subclinical ruminal acidosis than assumed, and efficient forage supplement feeding must ensure a more even intake pattern overnight.

Metabolic effects of supplement feeding. The majority of the blood metabolites and minerals investigated were in the normal range (Radostits et al., 2000); however, BHBA, total protein, albumen, and urea levels were relatively high. Supplement feeding increased blood glucose levels at 0700 and 1630 h. This indicates a slightly better energy supply that was associated with slight trends to higher feed intakes and milk yields. The BHBA levels of full-time grazed cows tended to be higher in the morning and were significantly lower in the afternoon compared with cows fed the supplements. This might be related to the differences in eating pattern, because the grass-only group spent more time eating during the night and less time in the daytime than the other groups. Regardless of treatment, increased eating activity in the daytime, associated with a higher feed intake, would explain the increased plasma urea concentrations in the afternoon. Creatinine, an indicator of muscle protein degradation, was lowest with full-time grazing, but the differences were too small to suggest a serious lack of metabolic protein supply in the supplemented cows (plasma total protein and albumin did not differ from the grass-only group).

Effects of the Type of Supplemental Feed

In the present study, corn silage was superior to hay as a supplemental feed in terms of ruminal pH, especially of diurnal pH. It is likely that the corn-silage supplemented cows, fed in one portion in the evening, had less inclination to ingest pasture grass in the morning than the hay-supplemented cows, and therefore expressed a lower intensity of fermentation. Cows receiving corn silage had numerically shorter eating times than those supplemented with hay. The (presumed) less pronounced structural properties of corn silage could explain the decrease in eating time as was shown with different mixtures of corn silage by Krause et al. (2002), where a reduced particle size decreased eating time. The cows receiving 5.5 kg of DM corn silage per day, which was lowest in fiber content, tended to result in the highest total intake and therefore the highest NDF intake. Corn silage-supplementation clearly reduced rumen fluid ammonia and blood urea concentrations compared with the hay-supplementation and grass-only feeding, demonstrating its ability to counterbalance the excessive dietary supply of rumen-degradable protein.

CONCLUSIONS

Full-time grazing in the present study never caused extended periods of seriously low ruminal pH, but ruminal pH was below 5.8 for almost 2 h/d. Moreover, cows that attain a higher milk yield and are dependent on higher grass intake, perhaps in combination with concentrate, could therefore reach a lower ruminal pH. Nocturnal supplement feeding gave no advantage over full-time grazing. The hay (supposed to be rich in physical structure) given in the evening had no obvious effects in supporting rumination or in stabilizing ruminal pH. Hay supplementation actually markedly decreased average pH during the day compared with the other treatments. The lack of additional feeds during most of the night probably provoked a more intensive ruminal fermentation on pasture over the day. However, more research is needed on pasture-based diets combined with supplementation to understand how the structural properties of grass affect ruminal fermentation and pH fluctuation. This would allow consideration of both fermentation acid production and physically effective fiber when formulating and evaluating the effectiveness of fiber in pasture and fresh grass. Special emphasis should be placed on investigations varying the frequency of supplemental feeding and combinations with concentrate.

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