Anim. Res. 54 (2005) 321–335

DOI: 10.1051/animres:2005032

Original article

Influence of supplementing hay to grass once or three times per day on the effectiveness of the fibre as determined by changes in ruminal pH, chewing activity and milk composition of cows

Christoph M. GRAF[a], Michael KREUZER[b], Frigga DOHME[a]*

[a] Agroscope Liebefeld-Posieux, Swiss Federal Research Station for Animal Production and Dairy Products (ALP), 1725 Posieux, Switzerland
[b] Institute of Animal Science, Animal Nutrition, Swiss Federal Institute of Technology (ETH), 8092 Zurich, Switzerland

(Received 12 July 2004; accepted 24 May 2005)

Abstract – Pasture grass in combination with or without 6 kg supplemental hay provided either once at 18:00 h or in three equal portions per day (07:00, 13:00, 17:00 h), respectively, were tested in a 3 × 3 Latin square design in cows for its influence on the effectiveness of fibre through modifications of ruminal pH fluctuation, chewing activity and milk constituents. Six rumen cannulated cows (Brown Swiss: Holstein = 1:1, 32.9 kg milk·day^{-1}) were tested in three periods consisting of 14 days of adaptation and 7 days of intensive data and sample collection. Chewing activity was recorded with the IGER Behavioural Recorder. Ruminal pH was measured continuously over 7 days by using an indwelling pH electrode and a data-recording unit, the latter being integrated in the cover of the cannula. The experimental treatment had no significant effect on milk yield and composition as well as ruminal pH fluctuation. The cows fed only grass spent 11% more time eating per day and 22% more time eating per kg neutral detergent fibre (NDF) than hay-supplemented cows, but no differences were observed in the time spent ruminating and ruminating time per kg dry matter and NDF. Across the complete day, the ruminal pH was non-significantly decreased by 0.1 when hay was supplied at once compared with the other treatments. Hay supplementations gave no advantage over grass-alone feeding with respect to variables assumed to respond to the effectiveness of fibre. By contrast, supplementing hay only once per day even seems to be inferior in maintaining a sufficiently high pH.

ruminal pH fluctuation / chewing behaviour / feeding frequency / grass / fibre

Résumé – **Influence de la distribution une ou trois fois par jour d'un complément de foin à une ration d'herbe sur l'efficacité des fibres définie par l'évolution du pH ruminal, les mouvements de mâchoire et la composition du lait chez la vache laitière.** L'effet d'une ration d'herbe, avec ou sans complément de foin (6 kg distribués en une portion à 18h00 ou en 3 portions à 7h00, 13h00 et 17h00), sur l'efficacité des fibres a été étudié selon un dispositif en carré latin 3 × 3 sur des vaches laitières en mesurant l'évolution du pH ruminal, les mouvements de mâchoire et la composition du

* Corresponding author: frigga.dohme@alp.admin.ch

lait. Six vaches laitières porteuses d'une fistule du rumen (Brown Swiss : Holstein = 1:1, 32,9 kg lait·jour^{-1}) ont été utilisées durant 3 périodes constituées chacune de 14 jours d'adaptation et 7 jours de collecte des échantillons et des données. Les mouvements de mâchoire ont été mesurés par un enregistreur comportemental développé par l'IGER. L'évolution du pH ruminal a été enregistrée en continu pendant 7 jours avec une électrode de pH placée dans le rumen et un enregistreur fixé dans le couvercle de la fistule. Les traitements n'ont pas eu d'effet significatif sur la production et la composition du lait ainsi que sur l'évolution du pH ruminal. L'herbe seule a exigé un temps d'ingestion plus long de 11 % par jour et de 22 % par kg de parois (NDF) que l'herbe complémentée avec du foin. Mais aucune différence n'a été observée dans la durée de rumination journalière ou la durée de rumination par kg de MS et par kg de parois. Sur l'ensemble de la journée, le pH ruminal a baissé de manière non significative de 0,1 unité avec le complément de foin donné en une portion par rapport aux autres traitements. Par rapport à l'herbe seule, le complément de foin n'a pas amélioré les variables prises en compte pour définir l'efficacité des fibres. Il semble au contraire que le complément de foin donné une fois par jour soit moins capable de stabiliser le pH ruminal dans une zone adéquate.

évolution du pH ruminal / comportement alimentaire / mode de distribution / herbe / fibres

1. INTRODUCTION

Grass when utilised efficiently is by far the cheapest feed available on the farm [10, 34]. In a grass-based milk production system maximising net energy intake of dairy cows on pasture is central to economic competitiveness [27]. However, in order to meet the energy requirements of the cow, grazing has to be carried out in a young growth stage of the grass when it is rich in readily fermentable carbohydrates and poor in the physical property of fibrousness. This could result in a reduced chewing activity during intake and a shorter rumination time and, consequently, in a decreased saliva production. Cows secret more saliva during chewing than when being idle [2]. Saliva contributes to approximately half of the bicarbonates entering the rumen [30], which helps buffer the acids produced during fermentation. Insufficient neutralisation of the fermentation products could cause a decrease in ruminal pH below 5.8 as the threshold where the risk of subclinical acidosis arises [25]. The consequences of subclinical acidosis are reduced fibre digestion, inconsistent feed intake, diarrhoea, decreased milk fat content, laminitis, and other health disorders [29].

Since there are various determinants such as the type of fibre, particle size and size distribution of the fibre [11, 26], the effectiveness of fibre to induce chewing and thus to maintain favourable ruminal fermentation conditions is difficult to predict. Wales and Doyle [43] observed that ryegrass straw, where seeds had been harvested before cutting, was ineffective in influencing ruminal pH when supplemented to pasture grass both with and without cereal. These findings are in line with our previous results where part-time grazing supplemented with hay at night even caused a lower average pH during the daytime compared with full-time grazing without supplementation [16]. In the latter study, however, cows were observed to consume the hay in a very short time, which left most of the night and the early morning without feed while intake of full-time grazing cows was much more evenly distributed over the 24 h.

The aim of the study was to compare the effectiveness of the fibre of a diet containing only grass with that supplemented with hay and to investigate whether or not the effectiveness of the fibre of supplemental hay can only be fully expressed when hay is offered more frequently than once. These aspects were investigated in cows by continuously recording ruminal pH fluctuations and chewing activity. In addition, the effects of ruminal fermentation, fibre digestion and milk composition were followed.

2. MATERIALS AND METHODS

2.1. Experimental design

The experiment was based on a monofactorial design where six cows were randomly assigned to three treatments in a double 3 × 3 Latin square arrangement. The treatments were fresh grass fed ad libitum (G) as well as fresh grass fed ad libitum and supplemented with 6.0 kg hay either offered once daily at 18:00 h (H1) or in three equal portions at 07:00, 13:00 and 17:00 h (H3). The hay supplements were offered for 3 h in treatment H1 and for 1 h each in treatment H3, respectively. Each cow passed consecutively three experimental periods, which consisted of a 14 d adaptation period and of 7 d of intensive data and sample collection each. During the adaptation periods, the cows were tethered in individual stalls on rubber mats and were turned outside for 2 h, three times per week. For each data collection period, the cows were kept in metabolism crates equipped with slatted floors. The experiment was conducted as outlined in the Swiss guidelines for animal welfare.

2.2. Feeds, animals, and climate conditions

The experimental grass was harvested daily between 07:00 and 08:00 h after 36 to 42 days of regrowth from a ley. For the first experimental period, the second cut and for the second and third periods the third cut obtained from three adjoining swards were used. The botanical composition of the grass was determined weekly. The hay was of a second cut and harvested in the previous year from a ley. At the time of cutting, the botanical composition was determined.

Cows had no access to the grass in the hay feeding periods. The cows were offered 300 g per day of a mineral mixture containing per kg: 118 g Ca, 45.5 g P, 21.6 g Mg, 89.7 g Na, 1.24 g Zn, 475 mg Cu, 70 mg Se, 25 mg I, 5 mg Co. In order to facilitate the production process and to ensure complete consumption, the mixture also contained 50 $g \cdot kg^{-1}$ fat, 511 $g \cdot kg^{-1}$ barley and 7.2 $g \cdot kg^{-1}$ wheat middlings. This mixture was provided daily after the morning milking.

Experimental cows were Brown Swiss and Holstein (1:1) and were multiparous. At the start of the study, the cows were on average 89 ± 15 (mean ± SD) days in milk, had a mean body weight of 647 ± 55 kg and produced 32.9 ± 3.7 kg of milk per day. The cows had been surgically fitted with flexible ruminal cannulas of 10 cm diameter (Bar Diamond, Parma, ID, USA).

Ambient temperature and air humidity were recorded indoors by an automatic combined temperature and humidity sensor data logger (No. 2001, Escort Messtechnik AG, Aesch, Switzerland) and outdoors with a stationary meteorological station (Meteo-Schweiz, Station Grangeneuve, Switzerland) being less than 1 km away from the field where the grass was harvested. The data measured between 06:00 and 18:00 h and 18:00 and 06:00 h were summarised. The indoor mean values (± SD) were 26.2 ± 2.7 °C and 51.6 ± 11.0% air humidity and the corresponding outdoor values were 24.9 ± 6.5 °C and 50.7 ± 22.0%. Nocturnal data (i.e., between 18:00 and 06:00 h) were 26.2 ± 2.6 °C and 52.4 ± 9.9% indoors and 21.6 ± 5.6 °C and 61.0 ± 20.2% outdoors, respectively.

2.3. Sampling and data collection

In the intensive sample and data collection period, milk yield was determined at each of the two milkings per day and individual milk samples were obtained. One part of the sample was preserved with Broad-Spectrum® microtabs (Gerber Instruments AG, Effretikon, Switzerland) and stored at 5 °C until further analysis. The other part was stored at 5 °C until the end of each collection period. These samples were pooled per cow afterwards and frozen at –20 °C.

Daily at 06:30 h, individual herbage intake was determined by removing and

registering the refusals of grass and hay. Hay and grass samples were collected daily at the same time. Forage samples were first stored at 5 °C and then pooled across the collection periods. Excreta were quantitatively collected. A homogenised sample was taken once a day between 07:00 h and 08:00 h and frozen at –20 °C. At that time, urine samples were also collected per cow and day in a pitcher and frozen at –20 °C, but only in the second and third collection period. Blood samples were taken at 08:00 h on days 1 and 7 of the collection period from the jugular vein, collected in vacuum tubes and cooled on ice. Plasma was produced using heparinised vacuettes® (Greiner bio-one, Solingen, Germany) and centrifuging the samples (Universal 16, Hettich, Tuttlingen, Germany) at 1500 × *g* for 15 min. Afterwards, the samples were stored at –20 °C.

During each data collection period, ruminal pH was measured continuously every day for 22 h using a self-constructed device described in detail by Graf et al. [16]. The central instrument of the device was an indwelling pH electrode (Solitrode-combined LL pH electrode, PP-shaft, Metrohm plug-in head G, No. 6.0220.100, Metrohm, Herisau, Switzerland) placed in the rumen through the rumen cannula. A wire connected the electrode consecutively with a pH adapter (EP-ADP-PH, Escort Messtechnik AG, Aesch, Switzerland) and a data logger (EX-2E-DDD, Escort) which recorded the ruminal pH every minute. There was another tube attached to the wire tube, which ended at the site of the pH electrode and allowed the collection of rumen fluid samples through the cannula without removing the device installed in the cannula. A weight was attached to the end of the indwelling pH electrode around the two tubes in order to keep the electrode permanently in the liquid phase in the ventral sac of the rumen. The electrodes were removed once daily, between 10:00 and 12:00 h, for calibration and cleaning. During this time, no pH records were obtained. For evaluation of the continuously obtained pH data, the day was separated into a diurnal (07:00 to 19:00 h) and a nocturnal (19:00 to 07:00 h) period. For these periods, minimum, maximum and mean values were calculated as well as the time when pH was below 5.8 as the threshold level for a subclinical ruminal acidosis [25]. The complete system was extensively tested before use and has been successfully applied in a previous experiment [16]. On days three to five, rumen fluid was sampled from the ventral sac of the rumen directly through the cannula. Samples were taken at 08:00, 14:00 and 18:00 h, and were preserved with 5% $HgCl_2$ before being stored at –20 °C. Untreated rumen fluid subsamples were cooled on ice for immediate determination of pH and bicarbonate concentration, the latter only in the morning samples. After thawing, rumen fluid samples were analysed, separately for each daytime and cow, for concentrations of volatile fatty acids (VFA) and ammonia.

The IGER Behaviour Recorders (Institute of Grassland and Environment Research, North Wyke, UK) as developed by Rutter et al. [36] were used in each collection period to follow individual eating and ruminating behaviour by registering jaw movements. Every 24 h, the batteries and memory cards of the recorders were exchanged by recharged batteries and empty memory cards. The data from the used memory cards were downloaded to a computer. Records lasting for more than 23 h were used for analysis and extrapolated to 24 h. The data of the jaw movement recordings were read and analysed automatically by using the Microsoft® Windows TM program 'Graze' [35]. From the jaw movement pattern, the program discriminated time spent eating, ruminating and time without jaw movement. In the present study, jaw movements lasting for less than 2 min and being away more than 1 min from the next movement were defined as idle time additional to the time where no movements were recorded. The chewing activities were divided into two periods comprising 07:00 to 19:00 h (daytime) and 19:00 to 07:00 h (nocturnal data). The device has been extensively

tested for its accuracy [22] and was found appropriate [14, 16] for the determination of chewing activity.

2.4. Laboratory analyses

In order to determine dry matter (DM) and ash contents, forages and excreta samples were lyophilised, milled through a 1.0 mm screen, heated at 105 °C for 3 h and at 550 °C for 4 h. In the ash residue of the forage samples, minerals were precipitated with nitric acid and then analysed for Ca, P, Mg, Na and K by optical emission spectrometry (ICP-OES Optima 2000 DV, Perkin Elmer, Shelton, CT, USA) as described by Boss and Fredeen [6]. The contents of neutral detergent fibre (NDF), acid detergent fibre (ADF) and, acid detergent lignin (ADL) only in forages, were analysed according to standard protocols [28, 42]. The sugar content was analysed as described by Shannon [39] with an Autoanalyser (Type II, Bran + Luebbe, Nordstet, Germany). The dietary contents of net energy lactation (NEL) and absorbable protein at the duodenum (PDI) were calculated according to RAP [33]. The digestibilities of NDF and ADF and the intake of indigestible NDF were calculated from the intake and the contents of the excreta.

In urine, pH was determined by a pH electrode (No. 6.0202.10, Metrohm, Herisau, Switzerland) attached to a pH meter (EA 940, Orion, Cambridge MA, USA) as described by Graf et al. [16]. The fractionated net acid-base excretion was analysed by titration and the base-acid ratio was calculated using the formula recommended by Bender et al. [3].

The frozen rumen fluid samples were defrosted and enzymatically analysed for their ammonia concentration by the test kit and method of bioMérieux SA, Lyon, France (No. 61236). The analysis of VFA was performed according to Alén et al. [1] on a gas chromatograph (HP 5890, Hewlett Packard, Waldbronn, Germany). Ruminal pH of the drawn, but not previously frozen, samples was determined with a pH electrode (No. 6.0220.100, Metrohm, Herisau, Switzerland) and a pH meter (No. 692, Metrohm, Herisau, Switzerland). For the determination of the ruminal bicarbonate concentration, cooled samples were first centrifuged (Universal 16, Hettich, Tuttlingen, Germany) at 2000 × *g* for 20 min at 4 °C (recommended by Tafaj et al. [41] and Tafaj, oral comm.). The analysis of the concentrations and the calculation of the results were carried out according to the manual of Mettler Toledo (Greifensee, Switzerland) and Graf et al. [16].

In the preserved and refrigerated milk samples, the contents of fat, protein and lactose were analysed by infrared spectrometry (Combis-Foss, Gerber Instruments AG, Effretikon, Switzerland). The frozen milk samples were defrosted and enzymatically analysed for their urea N content using a test kit (No. 61974, UV 250, bioMérieux, Lyon, France).

In the blood plasma the concentrations of metabolites were determined enzymatically using the following commercial test kits: glucose (No. 1447513, Roche, Basle, Switzerland), total protein (No. 1553836, Roche), albumin (No. 1553836, bioMérieux), urea (No. 61974, UV 250, bioMérieux), creatinine (Jaffé, Roche diagnostics, Basel, Switzerland), alanine aminotransferase (No. 63312, bioMérieux), aspartate aminotransferase (No. 63212, bioMérieux), creatinine kinase (No. 763870, Roche diagnostics) and glutamyl dehydrogenase (No. 1929992, Roche diagnostics).

2.5. Statistical analysis

Data were analysed by the general linear model (GLM) with the Statistical Analysis System [37] with treatment and period as effects. Data that had been determined on several days per period were averaged over this period before statistical analysis. Multiple comparisons among treatment means were performed by the Tukey procedure. Those rumen fluid variables that were

Table I. Chemical composition as well as calculated energy and protein supply of the experimental feeds [means of three (pasture grass) and of two (hay) determinations].

Forage treatment	Grass		Hay	
	X	SD	X	SD
Analysed nutrient and mineral composition ($g \cdot kg^{-1}$ dry matter (DM))				
DM ($g \cdot kg^{-1}$)	251	36	906	12
Organic matter	854	9	863	5
Crude protein	153	9	115	6
Total fatty acids	41.2	7.32	21.6	7.95
Neutral detergent fibre	343	14	464	10
Acid detergent fibre	226	40	285	0
Acid detergent lignin	54.2	5.0	35.0	1.6
Sugar	80.7	5.25	79.9	3.0
Ca	10.7	1.10	5.17	0.93
P	2.60	0.24	3.70	0.05
Mg	2.31	0.29	1.53	0.17
Na	0.28	0.04	0.18	0.01
K	26.2	2.6	36.2	0.5
Calculated energy and protein supply (kg^{-1} DM)[1]				
NEL (MJ)	6.16	0.13	5.07	0.07
PDI (g)	103.1	2.0	84.3	0.4

[1] NEL: net energy for lactation; PDI: absorbable protein at the duodenum from microbial origin and dietary origin; calculated according to RAP (1999).

determined three times per day were analysed by using the repeated measurement statement of SAS. In the tables, mean values and either standard deviations (SD) or standard errors of means (SEM) are presented.

3. RESULTS

3.1. Forage composition

The average botanical composition of the grass was characterised by high proportions of legumes (53 ± 7%; *Trifolium pratense*, *Trifolium repens*) and fewer grasses (38 ± 10%; dominated by *Lolium perenne, Agrostis repens, Phleum pratense*) and herbs (11 ± 18%; *Taraxacum officinalis*). The hay was composed of 56% grasses (dominated by *Lolium perenne*) and 40% legumes. The grass was lower in fibre content (NDF and ADF, on average –24%) than the hay but more lignified (ADL, +35%; Tab. I). Furthermore, there were other compositional differences between the two forages, especially in crude protein and mineral contents. The grass presented contents of net energy lactation (NEL) and absorbable protein at the duodenum (PDI) being higher by about 20% than that of the hay. However, the ratio of PDI to NEL was the same for grass and hay with 16.7 and 16.6, respectively. The summer when the experiment took place had been unusually hot and dry. This explains the high DM content of the grass and its large variation in botanical composition. Since the treatments were balanced across experimental periods, this effect was excluded by considering the period as an effect in the analysis of variance.

Table II. Treatment effects on feed intake, fibre digestibilities and eating pattern[1].

Item	Treatment[2]			SEM	Treatment effect (P)
	G	H1	H3		
Intake, per cow per day					
Grass (kg DM)	17.6[a]	13.0[b]	12.7[b]	0.80	< 0.001
Hay (kg DM)	–	5.1[b]	5.3[a]	0.10	< 0.05
Total DM[3] (kg)	17.9	18.4	18.3	0.79	0.87
Digestibility					
NDF (%)	60.5	59.0	64.7	7.93	0.87
ADF (%)	58.3	57.3	62.7	8.26	0.89
Eating pattern					
Daytime[4]					
Eating, min	384[a]	350[ab]	330[b]	12.4	< 0.05
Rumination, min	184	184	198	6.8	0.27
Time idle, min	151[b]	187[ab]	191[a]	10.9	< 0.05
Nocturnal[5]					
Eating, min	133	111	128	8.7	0.19
Rumination, min	291	321	299	10.0	0.12
Time idle, min	297	287	295	16.2	0.91
Average of daytime and nocturnal data					
Eating					
min·d^{-1}	518	460	458	17.4	0.05
min·kg^{-1} DM consumed	29.2	25.3	25.1	1.16	0.05
min·kg^{-1} NDF consumed	86.9[a]	67.9[b]	67.3[b]	3.24	< 0.01
Rumination					
min·d^{-1}	475	505	497	11.6	0.19
min·kg^{-1} DM consumed	26.8	27.7	27.3	1.07	0.87
min·kg^{-1} NDF consumed	79.8	74.3	73.1	3.26	0.32
Time idle					
min·d^{-1}	448	474	486	25.0	0.56

[1] Means within the same row with unequal superscripts are significantly different ($P < 0.05$).
[2] G = grass-only; H1 = grass plus once hay; H3 = grass plus three times hay.
[3] Including mineral supplement.
[4] 07:00 through 19:00 h.
[5] 19:00 through 07:00 h.

3.2. Feed intake, fibre digestibilities and eating pattern

Cows receiving hay reduced their grass intake ($P < 0.05$) to an extent where the same total forage DM intake as in the grass-only fed cows was reached (Tab. II). The cows offered the hay once per day consumed somewhat less hay (–4%, $P < 0.05$) than the cows offered the hay in three equal portions, but this was compensated for by correspondingly higher grass DM intakes.

Table III. Treatment effects on milk performance, blood plasma metabolites and enzyme activities[1].

Item	Treatment			SEM	Treatment effect (*P*)
	G	H1	H3		
Milk yield and composition					
Yield (kg·d^{-1})	24.6	24.7	24.2	0.33	0.84
Fat (%)	3.79	3.51	3.75	0.150	0.39
Protein (%)	2.91	2.91	2.95	0.076	0.92
Lactose (%)	4.90	4.73	4.89	0.105	0.47
Urea N (mg·100g^{-1})	20.3	17.9	17.5	0.79	0.05
Metabolites					
Glucose (mmol·L^{-1})	3.25	3.27	3.29	0.126	0.89
Total protein (g·L^{-1})	83.5	82.7	86.5	4.95	0.47
Albumin (g·L^{-1})	40.8	40.5	41.3	1.27	0.62
Urea N (mmol·L^{-1})	7.87	6.46	6.71	0.928	0.07
Creatinine (µmol·L^{-1})	84.3	87.9	85.2	7.20	0.72
Enzyme activities (U·L^{-1})					
Alanine aminotransferase	29.6	28.4	29.1	5.59	0.94
Aspartate aminotransferase	89.4	89.2	93.0	13.85	0.89
Creatine kinase	164.6	143.4	175.5	42.06	0.49
Glutamyl dehydrogenase	25.1	25.9	23.7	4.70	0.76

[1] Explanations see footnote of Table II.

There were no treatment differences observed for daily intakes of crude protein (2.61 kg), sugar (1.45 kg), NEL (106 MJ) and PDI (176 kg; data not shown in tables).

The digestibilities of NDF and ADF as well as the intakes of NDF and ADF, which were on average 6.58 kg·d^{-1} NDF and 4.73 kg·d^{-1}, respectively (data not shown in table), were not affected by the treatments.

During the day, grass-only fed cows spent more time eating ($P < 0.05$) compared to cows receiving hay three times per day (Tab. II). Since rumination time was not different among treatments, idling time was significantly shorter in the grass-only group than in the group receiving three times hay ($P < 0.05$). During the night, chewing activities were not influenced by the treatments. Hay supplementation tended to reduce ($P = 0.05$) the time spent eating per 24 h by 11%, independent of the frequency of the hay supply, and the same was observed for the time spent to consume 1 kg of DM. The time spent eating per kg NDF intake was higher by 22% in the grass-only group ($P < 0.01$). Rumination per 24 h as well as per kg DM and NDF consumed did not differ among the treatments. This also held true for the time idle per 24 h.

3.3. Milk performance and metabolic status

Hay supplementations had no significant influence on milk yield and milk composition (Tab. III). This includes milk fat content which is most frequently used to retrospectively estimate the structural fibre content of the diet. Milk urea N showed a trend ($P = 0.05$) towards lower values in the hay-supplemented cows.

The concentration of urea N in the blood plasma (Tab. III) tended to be higher in the

Table IV. Treatment effects on rumen fluid pH and other variables related to ruminal pH in rumen fluid and urine[1].

Item	Treatment			SEM	Treatment effect (*P*)
	G	H1	H3		
Rumen fluid pH (permanent on-line measurement)					
Daytime[2]					
Mean	6.46	6.44	6.46	0.048	0.91
Minimum	5.87	5.91	5.97	0.075	0.67
Maximum	6.89	6.79	6.85	0.054	0.48
pH < 5.8 (min·day^{-1})	39	31	18	9.7	0.34
Nocturnal[3]					
Mean	6.46	6.27	6.41	0.068	0.16
Minimum	5.79	5.72	5.83	0.079	0.59
Maximum	6.93	6.73	6.88	0.067	0.13
pH < 5.8 (min·day^{-1})	49	125	42	39.9	0.30
pH in drawn samples					
Rumen fluid[4]	6.65	6.62	6.63	0.057	0.92
Urine[5]	8.48	8.52	8.49	0.039	0.59
pH-related rumen fluid variables					
CO_2 (mmol·L^{-1})	10.6	12.4	12.2	1.12	0.60
Bicarbonate (mmol·L^{-1})	35.0	32.7	33.5	1.00	0.43
Acid-base variables[5]					
Urine net acid-base excretion (mmol·L^{-1})	244	283	276	31.5	0.36
Urine base-acid ratio	8.41	9.07	9.07	0.646	0.43

[1] Explanations see footnote of Table II.
[2] 07:00 through 19:00 h, with a break between 10:00 and 12:00 h.
[3] 19:00 through 07:00 h.
[4] Average of samples drawn at 08:00, 14:00 and 18:00 h.
[5] Reduced dataset (determined only in periods 2 and 3; $n = 4$).

grass-only group compared to the supplemented groups ($P = 0.07$). The other analysed metabolites and enzymes, which are indicative of metabolic status and liver stress, were not significantly different among treatment groups again confirming that energy and nutrient supply did not differ much between grass-alone fed and hay supplemented cows.

3.4. Ruminal pH fluctuation, fermentation pattern and urine acid-base equilibrium

Neither daytime nor nocturnal values of ruminal pH (mean, minimum, maximum and min with pH < 5.8) were significantly different among groups, and also rumen fluid pH in drawn samples did not differ (Tab. IV).

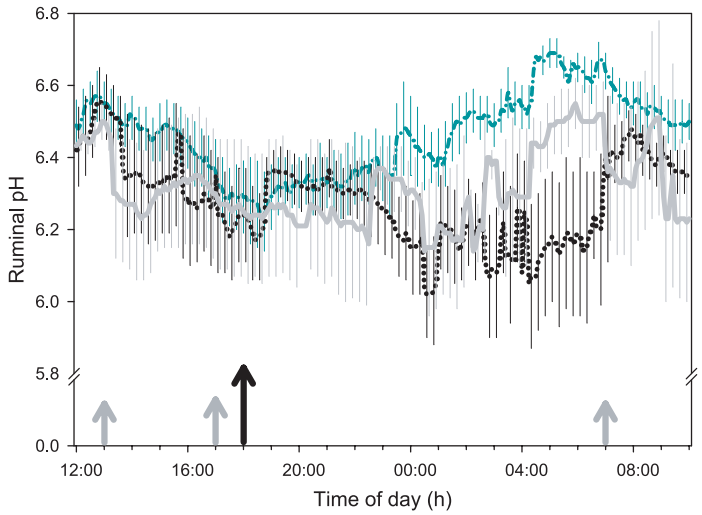

Figure 1. Diurnal fluctuation of ruminal pH (means over 5 min from continuous measurements) of cows fed grass only (dashed-dotted, grey), or grass supplemented with hay either once (dotted, black) or three times per day (solid, grey) (n = 6, treatment means and SE as error bars). Arrows indicate times of hay feeding (↑, one portion; ↑, three portions).

The averages of both measurement techniques correlated with 0.31 ($P < 0.001$). There was a weak trend towards lower nocturnal pH for cows supplemented once with hay (Fig. 1). As a consequence, also the time when pH was below 5.8 was numerically twice as long as with the other treatments.

No type of hay supplementation significantly affected ruminal carbon dioxide and bicarbonate concentration. Furthermore, no treatment effects were found in urine pH, base-acid excretion and ratio (Tab. IV).

Ruminal ammonia concentrations were affected independently by treatment ($P < 0.001$) and sampling time ($P < 0.01$; Tab. V). Concentrations were the highest in the morning and in hay supplemented cows. Treatment effects on total VFA concentration and VFA profile mostly remained insignificant, but there were sampling time differences. No significant interaction between treatment and time occurred. The few significant effects of hay supplementation comprised changes in butyrate ($P < 0.01$) and valerate ($P < 0.05$) proportions which were both the lowest when hay was supplemented in three portions. This was numerically compensated for by acetate ($P = 0.05$). Total VFA concentration and that of many individual VFA was higher at 14:00 h than at 08:00 and 18:00 h. In the VFA profile, the main shift took place between 14:00 and 18:00 h where proportions of all VFA increased at a cost of acetate.

4. DISCUSSION

4.1. Effects of the forage type

In the present study, the grass was composed of legumes and herbs to a relatively high proportion while the hay was dominated by grasses. At the same stage of maturity, *Lolium perenne* was reported to have a higher content of NDF and ADF and a lower content of ADL than *Trifolium pratense* [17] which might be one explanation for the differences in fibre contents of the

Table V. Treatment effects on ruminal fluid variables determined at different daytimes[1].

Item	Treatment (T)			Time (h)			SEM	Effect (*P*)		
	G	H1	H3	08:00	14:00	18:00		T	Time	T × Time
Ammonia (mmol·L^{-1})	6.49^{b}	8.01^{a}	7.32^{ab}	9.01^{x}	6.69^{y}	6.11^{y}	0.343	< 0.05	< 0.001	0.19
Volatile fatty acids (VFA) (mmol·L^{-1})	123	126	125	123^{xy}	136^{x}	116^{y}	4.3	0.87	< 0.01	0.65
Acetate (molar %)	70.7	70.7	71.7	72.3^{x}	72.0^{x}	68.8^{y}	0.31	0.05	< 0.001	0.31
Propionate (molar %)	17.7	18.0	17.7	16.8^{z}	17.8^{y}	18.8^{x}	0.22	0.63	< 0.001	0.63
Butyrate (molar %)	9.00^{a}	8.40^{ab}	7.88^{b}	8.00^{y}	7.86^{y}	9.41^{x}	0.191	< 0.01	< 0.001	0.87
Iso-butyrate (molar %)	1.48	1.60	1.57	1.53^{xy}	1.44^{y}	1.68^{x}	0.04	0.17	< 0.01	0.17
Valerate (molar %)	0.789^{ab}	0.815^{a}	0.701^{b}	0.704^{xy}	0.713^{y}	0.887^{x}	0.0280	< 0.05	< 0.001	0.27
Iso-valerate (molar %)	0.303	0.510	0.430	0.581^{x}	0.215^{y}	0.448^{x}	0.0628	0.08	< 0.001	0.25
Acetate-to-propionate ratio	4.03	3.96	4.06	4.32^{x}	4.05^{y}	3.67^{z}	0.065	0.55	< 0.001	0.46

[1] Explanations see footnote of Table II.
Means within the same row with unequal superscripts (a, b in treatment means; x, y, z in time point means) are significantly different ($P < 0.05$).

grass and hay. Moreover, due to a greater leaf loss during the hay-making process, hay mostly has a higher fibre content compared to fresh grass of the same quality. However, despite the different botanical composition of the two experimental roughage types, DM and NDF intake as well as fibre digestibility were not affected by the treatments. One possible explanation may be that legumes exhibited a higher undegraded fraction and faster degradation rate of slowly degraded NDF fractions, overall resulting in similar ruminally degraded NDF for legumes and grasses [17]. Dry matter intake was in the same range as that observed by Berry et al. [4] with grazing cows, although the NDF digestibility of the grass was slightly higher as in the present study [4].

The properties of fibrousness of a feed can be influenced by various factors including fibre content, particle size, particle shape, fragility, moisture, and type of preservation [26]. When calculating the physical structure value based on the roughage part in dairy cow diets, which is necessary to avoid decreased milk fat content, decreased milk yield and off feed [11], the present grass diet (2.2 per kg DM) did not differ considerably from the diets supplemented with hay (2.4 per kg DM). This could be the reason why ruminating was not affected by hay supplementation. During ruminating, saliva excretion is higher than during eating and idling [2], which is why it is an important factor to evaluate whether fibre can exhibit its physical effectiveness. Accordingly, differences in particle size of barley silage [40] as well as corn and alfalfa silage [23] were noted to clearly affect rumination time, while different mixtures of barley silage and concentrate remained without an effect [25]. The ruminating time found in the present investigation was shorter than that reported in other studies [25, 40] but longer than that observed by Rook and Yarrow [34] as well as Krause and Combs [23]. Overall, the lower structural efficiency supposed for grass compared to hay by Hoffmann [18] was not confirmed in the present study from the effects on ruminating time. On the contrary, cows fed grass only spent more time eating, especially during the day, in order to achieve the same DM intake. This was in line with the differences noted in eating time of full-time grazing cows compared to cows grazing during the day and fed hay in

the evening [16]. On average the time spent eating per day was similar to the times compiled in a review of grazing experiments [34] but twice as high as that observed in cows fed silage-concentrate diets [25, 40]. Under grazing conditions, feed availability [15] and the need of walk to get access to feed [9] influence eating time. However, longer eating times per day and per kg DM intake for fresh grass compared to preserved grassland products were also observed when the feed was offered in the barn [11]. Grass, compared to hay, has a higher water content and might be more bulky which could cause that cows eat more evenly over a longer time. Since the shorter eating time was not compensated for by a longer rumination time in the hay supplemented diets, feeding grass alone may have a more balanced effect on rumen fermentation.

The permanent measurement, as opposed to taking several spot samples, offers an appropriate method to record the diurnal fluctuation of ruminal pH. The pH per se and even more the time when the pH is below 5.8 are important and direct indicators for describing the effectiveness of the fibre in the diets. In line with a previous study [16], hay supplemented once per day decreased the ruminal pH compared to full-time grazed cows, but in the previous study the minimum had occurred during the day when pH had returned to the original level in the present study. Even supplementation of straw, with assumed very pronounced structural properties, in grazing cows did not positively affect the diurnal variation of rumen fluid pH [43]. The mean ruminal pH measured in the present study in cows fed only grass was in the same range as the values determined in studies where cows received only pasture grass [5, 8, 16].

Ruminal input of bases (e.g. ammonia from degraded protein) and buffers (e.g. bicarbonate) counteract against a depression in ruminal pH by neutralising ruminal VFA [30]. Half the bicarbonate entering the rumen comes from saliva during chewing and the other half enters the rumen in exchange of absorbed VFA [30]. In the present study, bicarbonate concentrations were measured only once per day because the method is very time consuming. Therefore, it was not possible to determine the daily fluctuation of bicarbonate concentrations in the rumen. Samples were drawn 1 h after feeding when the ruminal pH was high. Concentrations analysed were in line with those (34.7 $mmol \cdot L^{-1}$) found by Tafaj et al. [41] 3 h after feeding hay with particle sizes ranging between 9.2 and 28.7 mm but lower compared with those of Graf et al. [16] where samples were taken early in the morning before feeding. Since it is known that saliva excretion is higher during rumination than during eating [2] and cattle tend to ruminate at night and to eat during the day ([15] present results) these results make sense. The concentration of total VFA was the highest in the afternoon when ruminal pH started to decrease. Concomitantly, ammonia concentration was lower compared to the morning sample. Although the daily intake of crude protein did not differ among treatments, supplementation of hay only once per day increased ammonia values. Therefore, it seems that one-time hay feeding compared to grass-only causes an uneven ammonia release pattern. On the contrary, blood and milk urea values reflected the higher crude protein content of the grass which was also observed in a previous study [16]. The majority of the blood metabolites and enzymes were in the normal range [19, 32]. Two exceptions were total protein and albumin, which were slightly beyond the upper threshold values of 80 and 36 $g \cdot L^{-1}$, respectively, indicating a certain longer-term excess in protein intake [44].

The analyses of the acid-base variables and urine pH provide other approaches to investigate the physical effectiveness of fibre [3]. While the urine pH was around the upper level of the standard range (7.8–8.4), the acid-base variables exceeded the normal range and thus indicated a certain metabolic alkalosis [3]. There is experimental evidence in goats that a metabolic alkalosis

may develop as a consequence of a lactic acidosis [7]; however, keeping in mind the relatively short periods of pH < 5.8, this explanation does not seem very likely and other unknown reasons might have been responsible.

4.2. Effects of the feeding frequency of hay

In most of the studies dealing with feeding frequency, total mixed rations [24, 38] or numbers of concentrate portions supplemented additionally to roughage [12, 13, 20, 21] were investigated in their effects on rumen fermentation and milk performance. Much less is known about the influence of feeding frequency of roughage as investigated in the present study. Increasing feeding frequency from two to four and six times per day, respectively, was found to increase the total DMI of a corn-based diet [38] and of a dehydrated alfalfa-based diet [24], respectively, which supports the observed trend to a higher hay intake with feeding three times instead of one time. However, total DMI remained unaffected. Keeping the cows side by side might have reduced treatment differences since Phillips and Rind [31] found that the procedure of feeding the neighbouring cow could initiate feed intake of the feed still available in the trough. On the contrary, it seems more likely that the use of a forage-only diet induced cows to stress their maximum forage intake capacity in order to cover their energy requirements. Differences in times spent eating, ruminating or idling were negligible between the two hay feeding regimes which might be explained by the permanent availability of feed in the form of grass. Cows receiving hay once per day also had the opportunity to eat in the remaining time. Varying the feeding frequency of offering a complete diet is more likely to cause changes in chewing behaviour. However, eating and ruminating time per day were also less influenced by a more frequent supply of a total mixed ration [24] while ruminating time per kg DMI was reduced [24].

Shabi et al. [38] and Le Liboux and Peyraud [24] found that a higher feeding frequency reduces post-feeding variations in ruminal ammonia concentration and ruminal pH. Nevertheless, the results about the mean ruminal pH [24, 38] and the ruminal concentrations of VFA [12, 24] were contradictory. In the present study, total VFA production remained unaffected by hay feeding frequency while butyrate and valerate proportions decreased significantly with feeding hay three times, which was compensated for by a numerical increase of acetate concentration. The ruminal ratio of acetate to propionate proportion is closely correlated with the milk fat content [20]. However, in the present study the effect of the feeding frequency on the VFA profile was not sufficiently large to alter milk fat content and to be able to firmly state an improved property of fibrousness with this feeding regime. The observations of Kaufmann [20] who found a higher ratio of acetate to propionate with an increased feeding frequency and therefore a higher milk fat content could not therefore be confirmed.

5. CONCLUSION

The hypothesis of the present investigation that an increase in the frequency of hay supplementation to grass would prevent fluctuations to low ruminal pH in contrast to hay supplemented once per day was not confirmed. Even though hay supplemented only at one time per day once again seemed to be inferior in the property of fibrousness in a grass-based diet, three times hay feeding turned out to be ineffective in improving chewing pattern, ruminal pH level, fibre digestion and milk fat content. This could have been due to sufficient structural properties of the grass used, but there were still times when the pH was below 5.8. Other effects of the grass, such as the prolonged eating time, may have been the actual reason for the relative inefficiency of the hay, which is generally assumed to have more

favourable properties of fibrousness than young grass. It remains to be investigated whether the structural properties of the grass are still basically sufficient to ensure favourable ruminal fermentation conditions when grass-fed cows of higher milk yield are simultaneously offered significant amounts of concentrate.

ACKNOWLEDGEMENTS

We owe special thanks to J.-F. Bise for constructing the device for on-line ruminal pH measurement. We are also grateful to R. Lavoyer for assistance in sampling and with laboratory analyses, K. Zbinden for assistance in the experimental procedures and R. Daccord for the translation of the abstract into French.

REFERENCES

[1] Alén R., Jännäri P., Sjöström E., Gas liquid chromatographic determination of volatile fatty acids C1-C6, and lactic acid as their benzyl esters on a fused silica capillary column, Finn. Chem. Lett. (1985) 190–192.

[2] Bailey C.B., Saliva secretion and its relation to feeding in cattle. 3. Rate of secretion of mixed saliva in cow during eating, with an estimate of magnitude of total daily secretion of mixed saliva, Brit. J. Nutr. 15 (1961) 443–451.

[3] Bender S., Gelfert C.C., Staufenbiel R., Use of urine samples for monitoring acid-base-equilibrium in herd health management of dairy cattle herds, Tierärztl. Prax. 31 (2003) 132–142.

[4] Berry N.R., Sutter F., Bruckmaier R.M., Blum J.W., Kreuzer M., Limitations of high Alpine grazing conditions for early-lactation cows: effects of energy and protein supplementation, Anim. Sci. 73 (2001) 149–162.

[5] Berzaghi P., Herbein J.H., Polan C.E., Intake, site, and extent of nutrient digestion of lactating cows grazing pasture, J. Dairy Sci. 79 (1996) 1581–1589.

[6] Boss C.B., Fredeen K.J., Concepts, instrumentation and techniques in inductively coupled plasma optical emission spectrometry, Perkin-Elmer Corporation, Norwalk, CT, USA, 1999.

[7] Cao G.R., English P.B., Filippich L.J., Inglis S., Experimentally induced lactic acidosis in the goat, Aust. Vet. J. 64 (1987) 367–370.

[8] Carruthers V.R., Neil P.G., Dalley D.E., Effect of altering the non-structural: structural carbohydrate ratio in a pasture diet on milk production and ruminal metabolites in cows in early and late lactation, Anim. Sci. 64 (1997) 393–402.

[9] Cazcarra R.F., Petit M., D'hour P., The effect of sward heigt on grazing behaviour and herbage intake of three sizes of Charolais cattle grazing cocksfoot (Dactylis glomerata) swards, Anim. Sci. 61 (1995) 511–518.

[10] Clark D.A., Kanneganti V.R., Grazing management systems for dairy cattle, in: Cherney J.H., Cherney D.J.R. (Eds.), Grass for Dairy Cattle, CABI Publishing, New York, USA, 1998, pp. 311–334.

[11] De Brabander D.L., De Boever J.L., Vanacker J.M., Boucque C.V., Botterman S.M., Evaluation of physical structure in dairy cattle nutrition, in: Garnsworthy P.C., Wiseman J. (Eds.), Recent Advances in Animal Nutrition, University Press, Nottingham, UK, 1999, pp. 111–145.

[12] Fan Y.K., Lin Y.L., Chen K.J., Chiou P.W.S., Effect of concentrate feeding frequency versus total mixed ration on lactational performance and ruminal characteristics of Holstein cows, AAJAS 15 (2002) 658–664.

[13] French N., Kennelly J.J., Effects of feeding Frequency on ruminal parameters, plasma insulin, milk yield, and milk composition in holstein cows, J. Dairy Sci. 73 (1990) 1857–1863.

[14] Gibb M.J., Huckle C.A., Nuthall R., Effects of level of concentrate supplementation on grazing behaviour and performance by lactating dairy cows grazing continuously stocked grass swards, Anim. Sci. 74 (2002) 319–335.

[15] Gibb M.J., Huckle C.A., Nuthall R., Rook A.J., Effect of sward surface height on intake and grazing behaviour by lactating Holstein Friesian cows, Grass Forage Sci. 52 (1997) 309–321.

[16] Graf C.M., Kreuzer M., Dohme F., Effects of supplemental hay and corn silage versus full-time grazing on ruminal pH and chewing activity of dairy cows, J. Dairy Sci. 88 (2005) 711–725.

[17] Hoffman P.C., Sievert S.J., Shaver R.D., Welch D.A., Combs D.K., In situ dry matter, protein, and fiber degradation of perennial forages, J. Dairy Sci. 76 (1993) 2632–2643.

[18] Hoffmann M., Tierfütterung, Deutscher Landwirtschaftsverlag, Berlin, Germany, 1990.

[19] Kaneko J.J., Harvey J.W., Bruss M.I., Clinical biochemistry of domestic animals, Academic Press, San Diego, CA, USA, 1997.

[20] Kaufmann W., Influence of the composition of the ration and the feeding frequency on pH-regulation in the rumen and on feed intake in ruminants, Livest. Prod. Sci. 3 (1976) 103–114.

[21] Klusmeyer T.H., Cameron M.R., McCoy G.C., Clark J.H., Effects of feed processing and frequency of feeding on ruminal fermentation, milk production, and milk composition, J. Dairy Sci. 73 (1990) 3538–3543.

[22] Kononoff P.J., Lehman H.A., Heinrichs A.J., Technical Note – A comparison of methods used to measure eating and ruminating activity in confined dairy cattle, J. Dairy Sci. 85 (2002) 1801–1803.

[23] Krause K.M., Combs D.K., Effects of forage particle size, forage source, and grain fermentability on performance and ruminal pH in midlactation cows, J. Dairy Sci. 86 (2003) 1382–1397.

[24] Le Liboux S., Peyraud J.L., Effect of forage particle size and feeding frequency on fermentation patterns and sites and extent of digestion in dairy cows fed mixed diets, Anim. Feed Sci. Technol. 76 (1999) 297–319.

[25] Maekawa M., Beauchemin K.A., Christensen D.A., Effect of concentrate level and feeding management on chewing activities, saliva production, and ruminal pH of lactating dairy cows, J. Dairy Sci. 85 (2002) 1165–1175.

[26] Mertens D.R., Creating a system for meeting the fiber requirements of dairy cows, J. Dairy Sci. 80 (1997) 1463–1481.

[27] Moore K.C., Economics of grass for dairy cattle, in: Cherney J.H., Cherney D.J.R. (Eds.), Grass for Dairy Cattle, CABI Publishing, New York, USA, 1998, pp. 373–391.

[28] Naumann K., Bassler R., Methodenbuch. Band III. Die chemische Untersuchung von Futtermitteln, VDLUFA-Verlag, Darmstadt, 1997.

[29] Nocek J.E., Bovine acidosis: Implications on laminitis, J. Dairy Sci. 80 (1997) 1005–1028.

[30] Owens F.N., Secrist D.S., Hill W.J., Gill D.R., Acidosis in cattle: A review, J. Anim. Sci. 76 (1998) 275–286.

[31] Phillips C.J.C., Rind M.I., The effects of frequency of feeding a total mixed ration on the production and behavior of dairy cows, J. Dairy Sci. 84 (2001) 1979–1987.

[32] Radostits O.M., Gay C.C., Blood D.C., Hinchcliff K.W., Veterinary medicine: a textbook of the diseases of cattle, sheep, pigs, goats and horses, W. B. Saunders company, London, UK, 2000.

[33] RAP (Station fédérale de recherche en production animale), Apports alimentaires recommandés et tables de la valeur nutritive des aliments pour les ruminants, Centrale des moyens d'enseignement agricole, Zollikofen, 1999.

[34] Rook A.J., Yarrow N.H., Incorporating grazing behaviour measurements in models to predict herbage intake by grazing dairy cows, Grass Forage Sci. 57 (2002) 19–24.

[35] Rutter S.M., Graze: A program to analyze recordings of the jaw movements of ruminants, Behav. Res. Meth. Ins. C. 32 (2000) 86–92.

[36] Rutter S.M., Champion R.A., Penning P.D., An automatic system to record foraging behaviour in free-ranging ruminants, Appl. Anim. Behav. Sci. 54 (1997) 185–195.

[37] SAS Institute Inc., SAS Procedures Guide, Cary, NC, USA, 2002.

[38] Shabi Z., Bruckental I., Zamwell S., Tagari H., Arieli A., Effects of extrusion of grain and feeding frequency on rumen fermentation, nutrient digestibility, and milk yield and composition in dairy cows, J. Dairy Sci. 82 (1999) 1252–1260.

[39] Shannon D.W.F., A semi-automated method for determination of available carbohydrate content of poultry feeds, The Analyst 97 (1972) 209–212.

[40] Soita H.W., Christensen D.A., Mckinnon J.J., Influence of particle size on the effectiveness of the fiber in barley silage, J. Dairy Sci. 83 (2000) 2295–2300.

[41] Tafaj M., Steingass H., Susenbeth A., Lang G.U., Drochner W., Influence of hay particle size at different concentrate and feeding levels on digestive processes and feed intake in ruminants, Arch. Anim. Nutr. 52 (1999) 167–184.

[42] Van Soest P.J., Robertson J.B., Lewis B.A., Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition, J. Dairy Sci. 74 (1991) 3583–3597.

[43] Wales W.J., Doyle P.T., Effect of grain and straw supplementation on marginal milk- production responses and rumen fermentation of cows grazing highly digestible subterranean clover pasture, Aust. J. Exp. Agr. 43 (2003) 467–474.

[44] Ward W.R., Murray A.R., White A.R., Rees E.M., The use of blood biochemistry for determing the nutritional status of dairy cows, in: Garnsworthy P.C., Cole D.J.A. (Eds.), Nottingham University Press, Nottingham, UK, 1995, pp. 29–51.