Influence of Fiber Content and Concentrate Level on Chewing Activity, Ruminal Digestion, Digesta Passage Rate and Nutrient Digestibility in Dairy Cows in Late Lactation

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ABSTRACT : The influence of fiber content of hay (low-fiber 47% NDF and high-fiber 62% NDF of DM) and concentrate level (high 50% and low 20% of ration DM) on chewing activity, passage rate and nutrient digestibility were tested on four restrict-fed (11.1 to 13.7 kg DM/d) Holstein cows in late lactation. Aspects of ruminal fermentation and digesta particle size distribution were also investigated on two ruminally cannulated (100 mm i.d.) cows of the same group of animals. All digestion parameters studied were more affected by the fiber content of the hay and its ratio to non structural carbohydrates than by the concentrate level. Giving a diet of high-fiber (62% NDF) hay and low concentrate level (20%) increased chewing activity but decreased solid passage rate and total digestibility of nutrients due to a limited availability of fermentable OM in the late cut fiber rich hay. A supplementation of high-fiber hay with 50% concentrate in the diet seems to improve the ruminal digestion of cell contents, whilst a depression of the ruminal fiber digestibility was not completely avoided. Giving a diet of low-fiber (47% NDF) hay and high concentrate level (50%) reduced markedly the chewing and rumination activity, affected negatively the rumen conditions and, consequently, the ruminal digestion of fiber. A reduction of the concentrate level from 50 to 20% in the diet of low-fiber hay improved the rumen conditions as reflected by an increase of the ruminal solid passage rate and of fiber digestibility and in a decrease of the concentration of large particles and of the mean particle size of the rumen digesta and of the faeces. Generally, it can be summarised that, (i) concentrate supplementation is not a strategy to overcome limitations of low quality (fiber-rich) hay, and (ii) increase of the roughage quality is an effective strategy in ruminant nutrition, especially when concentrate availability for ruminants is limited. ***(Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 8 : 1116-1124)***

Key Words : Dairy Cows, Chewing Activity, Rumen Digestion, Passage Rate, Hay/Concentrate-ratio

INTRODUCTION

Roughage quality is an important factor affecting for the intake and utilization of forage and for the reducing the use of concentrates in ruminant feeds. Fiber content is the main determinant of roughage quality. Higher fiber content is usually associated with a higher degree of lignification (Van Soest, 1994; Krause and Pell, 2003) and, consequently, with a low degradation rate and availability of fiber in the rumen, which leads to a prolonged chewing time and to a decrease of passage rate, of total digestibility of fiber and of voluntary roughage intake. These effects of fiber on digestive processes are also confounded by the amount and characteristics of concentrate in the diet. These interactions are mainly related to the ratio between structural (SC) and non fiber carbohydrates (NFC) and the ratio between nitrogen and carbohydrates. It can be expected that a moderate amount of concentrate in the diet can improve the utilization of fiber due to the better supply of fermentable organic matter, energy and nitrogen to rumen bacteria. Concentrate-rich diets have a high amount of NFC which are rapidly degraded in the rumen, produce a high amount of short chain fatty acids (SCFA) within a short time (Allen, 1997) and, consequently, lower the pH. Low pH in the rumen (Mould et al., 1983) and the competition for substrates (Hoover, 1986) inhibit the growth and activity of fibrolytic bacteria. The influence of concentrate on fiber digestion and roughage utilization depends also on composition and ruminal fermentability of concentrate feed in the diet (Tamminga, 1993; Krause et al., 2002; Choi et al., 2003). With increasing the concentrate fermentability in the rumen the depressive effect of concentrate level on the roughage fiber digestion increases. The separate effects of roughage quality and concentrate content of the diet on digestive processes in dairy cows are well known, while the interactions between them are often neglected. The objective of the present study is to investigate the effects of the interactions between the fiber content in the hay (hay quality) and the level of highly degradable concentrate in the diet on whole tract digestion (chewing activity, passage rate, apparent digestibility and faecal particle size) and ruminal digestion (fermentation and digesta particle size) on dairy cows in late lactation.

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MATERIALS AND METHODS

Animals, experimental design and feeding

Four ruminally cannulated (two with large cannulaes 100 mm i.d.) Holstein cows (510 to 560 kg BW) in late lactation phase, were used in a two-way factorial block design to investigate the influence of two hay qualities (H)

Table 1. Feed intake and nutritional characteristics of diets

	Treatments (Hay quality×concentrate level)			
H[1]	Low-fiber hay		High-fiber hay	
C[2]	20%	50%	20%	50%
Total DMI (kg/d)	11.2	13.7	11.1	13.6
Hay DMI (kg/d)	8.7	6.9	8.5	6.9
Concentrate DMI (kg/d)	2.5	6.8	2.6	6.7
Concentrate percentage (%)	22	49.9	23.4	49.2
NE_l[3](kcal/kg DMI)	1,453	1,623	1,243	1,475
Contents (% DM basis)				
NDF	42.5	36.3	53.6	44.0
NDF of hay (NDF_h)	36.7	23.7	47.4	31.4
Cellulose	22.4	16.4	30.0	21.7
Hemicellulose	16.7	17.4	19.8	19.4
CF	21.1	15.2	26.9	19.3
NFC[4]	33.6	39.5	22.9	32.1
CP	12.3	13.8	11.1	12.9
NDF/NFC	1.26	0.92	2.34	1.37
NDF_h/NFC	1.09	0.60	2.07	0.98

[1] Hay quality: High-fiber hay (62% NDF) and low-fiber hay (47% NDF in DM).

[2] Concentrate level: 50% and 20% of DM ration. Concentrate mixture composition (per 100 kg as fed): 53.8 kg barley, 28.2 kg wheat, 14.6 kg soybean meal [44% CP], 1.1 kg plant oil and 2.3 kg mineral premix for dairy cows [14.0% Ca, 6.0% P, 11.0% Na, 3.0% Mg and per 1 kg premix: 1,000,000 IU Vit. A, 100,000 IU Vit. D_3, 1,000 mg Vit. E, 7,500 mg Zn, 4,500 mg Mn, 1,000 mg Cu, 100 mg J, 30 mg Co and 20 mg Se].

[3] Predicted by the Hohenheim gas test.

[4] NFC = Non fiber carbohydrates (NFC = 100-NDF-CP-EE-CA).

The diets with low concentrate amount were supplemented with 100 g mineral premix per animal and day from the same mineral premix.

and concentrate levels (C) on the whole digestion and the ruminal digestion. A low-fiber hay (11.3% CP, 47.1% NDF, 31.1% ADF, 4.0% ADL and 29.2% non fiber carbohydrates (NFC) in DM, 1,314.5 kcal NE_l/kg DM, degradation rate of DM 7.0%/h) and a high-fiber hay (9.6% CP, 61.9% NDF, 41.8% ADF, 4.6% ADL and 14.9% NFC in DM, 1,027.7 kcal NE_l/kg DM, degradation rate of DM 5.7%/h) were offered in combination with a concentrate mixture (16.6% CP, 25.3% NDF and 50.2% NFC in DM, 1,936 kcal NE_l/kg DM, degradation rate of DM 15.0%/h) at two levels in the diet (high level or 50% and low level or 20% of ration DM) in four consecutive treatments in turn. The treatment 'high-fiber hay and 50% concentrate' was tested in three cows because one cow finished lactating at the start of the 4th experimental run.

Hay quality was defined based on the chemical composition, mainly by NDF content. The degradation characteristics of hay and concentrate were determined based on the cumulative *in vitro* gas production (incubation time 72 h) using the Hohenheim Gas Test method. The rumen fluid for incubation was collected from two cows 1 h before the morning feeding. The sample degradation characteristics were defined by the equation $p = a+b(1-e^{-ct})$, where p is gas production at time t, a+b is the potential gas production, c is the rate of gas production and a, b and c are constants in the exponential equation (Khazaal et al., 1993).

The detailed data of the diets and feed intake are presented in Table 1. Cows were housed and fed in individual tie stalls and milked twice daily in their stalls at 06:30 and 16:00 h. The experimental diets were offered restricted (Table 1) twice daily at 08:00 and 20:00 h and animals had free access to water. In all treatments hay was chopped long (mean particle length 27.9 to 37.0 mm). The adaptation period to diets lasted 14 d.

Analysis and measurements

Chewing activity : Chewing activity was recorded on 4 to 6 consecutive days during the 3rd week of the experiment. The cows wore a halter to which was attached a small rubber ballon that was compresed by each jaw movement. The ballon was connected to a thin tube, from which airflow was detected. A computer recorded each jaw movement so that the time spent for eating as well as the number of chews could be determined. Less than two jaw movements within 4 sec were not taken as eating activity, and a pause of chewing was defined as no jaw movement for eating within a 4-sec period. This correction was necessary to remove signals by single jaw movements, which were not caused by eating; they accounted for 2.3% of total signals (Susenbeth et al., 2004).

Passage rate : This parameter was measured during the 3rd week of the experiment. Passage rate of solid mass was determined using NDF from fiber-rich (69.3% NDF of DM) hay (retained particles on a sieve of 1.0 mm mesh) labelled with Yb (Yb-NDF) according to the method described by Mambrini and Peyraud (1997). Liquid passage rate was measured using the complex of LiCo-EDTA·$3H_2O$ as described by Uden et al. (1980). Fifteen grams LiCo-EDTA

per animal was administered as pulse dose via the cannulae into the rumen. Immediately after administration of liquid marker, 146 g Yb-NDF per animal was mixed with 1.000 g concentrate and given as pulse dose before the morning feeding. Faecal samples (150-200 g each) were collected from the rectum at the following times (h after marker dosage): 0, 4, 8, 12, 16, 24, 28, 32, 36, 40, 52, 56, 60, 72, 80, 88, 96, 104, 112, 120, 132, 144 and 168 h. The faecal samples were dried at 60°C and ground to pass a 1 mm sieve. The concentration of Yb and Co was determined by atomic absorption spectrophotometer (Spectra AA, Varian, 220FS) using nitrous oxide flame, against standard with faecal matrix. A sample of 4 to 5 g dried faeces was ashed (550°C for 12 h) and the total ash was determined. About 0.5 g ash was digested with 5 ml 65% nitric acid at 180°C for 3 h, transferred to flask (50 ml volume) with a 0.20% aqueous solution of potassium chloride (KCl). Wavelengths chosen were 398.8 and 240.7 nm for Yb and Co, respectively. Time Delay (TD), Rumen Mean Retention Time (RMRT = 1/Ks), Fast Mean Retention Time (FMRT = Gn/λ) and Total Mean Retention Time (TMRT = TD+ RMRT+FMRT) were calculated by a model of Moore et al. (1992). The excretion curves of markers were fitted using one age-independent model (G1G1) and three age-dependent models (GnG1: n = 2, 3, 4).

Apparent nutrient digestibility : The digestibility was also determined during the 3rd week of the experiment using TiO_2 as an external marker (35 g/animal and day were mixed with 1.000 g concentrate and fed for 10 d twice daily in two equal meals). Faecal samples were collected for 7 d every 8 h from the rectum. The samples were pooled, mixed, dried at 65°C and ground at 1 mm. One g of dried faeces was digested in 15 ml concentrated H_2SO_4 according to Kjeldahl, filled up with water to 100 ml. After 24 h the samples were filtered. Two ml H_2O_2 (30%) were mixed with 5 ml of the filtrate and the absorption was measured in a spectrophotometer at 405 nm. Five ml of filtrate with 2 ml distilled water were used as blank. The Ti concentration was calculated from a linear calibration curve derived from a Ti standard solution. The daily amount of faeces was calculated after the following equation: Faecal output (g DM/d) = (Intake of TiO_2 [g/d]×100)/Faecal TiO_2-concentration [%].

Ruminal fermentation : These parameters together with those of digesta particle size distribution, were measured during the 4th week of the experiment in two cows (of the same group of animals) fitted with 100 mm i.d. rumen cannulae. Ruminal fluid was collected from the ventral rumen by a vacuum pump (as used for the Hohenheim Gas Test). After collection the fluid was filtered through cloth bags (pore size 0.25 mm) and the pH was measured by an electrode (InLab 412, Mettler-Toledo, Greifensee, Switzerland). Approximately 100 ml fluid was centrifuged at 2,010×*g* for 20 min. Two replicates of supernatant, each of 5 ml, were taken for the measurement of free CO_2 and ammonia by a gas sensitive electrode (Type 15 232 3000 for CO_2 and Type 15 230 3000 for ammonia; Ingold Messtechnik AG, Urdorf, Switzerland). For determination of short chain fatty acids (SCFA) concentrations, two replicates each of 5 ml of the same supernatant were frozen at -30°C. The SCFA concentration was determined by gas chromatography (GC System, HP 6890 plus, Germany). Before measurement of SCFA, the supernatant was mixed with standard solution (2.5% iso-caproic acid) and after 2 h was centrifuged at 19,000×*g* for 20 min. The concentration of bicarbonate was calculated based on the measured values of pH and CO_2 concentration in rumen samples using the equation of Henderson-Hasselbalch (Kaufmann and Hagemeister, 1969; Gäbel, 2000): $pH = pK_1 + \log([HCO_3^-]/[CO_2])$, where: pH is the measured pH value; pK_1 = -logK (K is the dissociation constant; pK_1 of the HCO_3/CO_2 -system is 6.1 for a temperature = 37°C), and CO_2 is the measured concentration (mmol/l) by gas sensitive electrodes.

Particle size distribution in rumen digesta and faeces : Rumen solid digesta was collected from the bottom of the rumen (5-10 cm above the rumen floor) using the sampling technique of Tafaj et al. (2001). For the sampling a self-made sampler was used, which had a container (about 460 cm^3) consisted of two acrylic glass cylinders which can be manually rotated. It had an aperture (70×50 mm) which could be opened and closed (to avoid, as much as possible, contamination of sample) by rotating a metal "T" rod (about 60 cm long with a scale as a reference for the defined sampling positions). The collected digesta was manually squeezed through double cloth bags (pore size 0.25 mm), in order to win the solid digesta mass. Sub-samples of about 150 g solid digesta were stored at -20°C for sieve analysis.

Sieve analysis was performed on the wet sieving apparatus (Analytical Sieve Shakers, F. Kurt Retsch GmbH and Co.KG, Haan, Type AS 200 Digit) using sieves (200 mm i.d.) having square holes sides of 4.0, 2.0, 1.0, 0.5, 0.25, 0.125 and 0.063 mm. Conditions for the analyses were similar with those reported by Lechner-Doll and Engelhardt (1989). Duplicate samples of digesta particles (approximately 40 g) were sieved for 12 min with a water spray of 2.9 l per min (sprayed on the top sieve), a vibration amplitude of 2.0 mm and an interval pause of 2 sec every 20 sec. Material retained on the sieves was washed onto pre-weighed filter paper, dried at 80°C for 24 h and weighed. The particle size distribution of the hay and of faeces samples was measured by the same method. For the sieve analysis of the hay one sieve with 6.40 mm pore size was added to the sieving apparatus. Duplicate samples of approximately 50 g faeces and 15 g hay were sieved. Particles were grouped into three classes or fractions: large particles (>1.0 mm, i.e. retained on a sieve of 1.0 mm, 2.0 mm and 4.0 mm mesh and have a

Table 2. Influence of hay quality and concentrate level on the chewing and rumination activities in dairy cows (Lsmeans±SEM)

H[1] / C[2]	Treatments (Hay quality×concentrate level)				Significance
	Low-fiber hay		High-fiber hay		
	20% (n = 18)	50% (n = 15)[4]	20% (n = 21)	50% (n = 14)	(p≤0.05)[3]
Total chewing time (min/d)	663±48.3[a]	681±55.2[a]	841±47.7[b]	803±55.7[b]	H
Rumination time (min/d)	311±25.9[a]	328±29.1[a]	459±24.8[b]	477±29.6[b]	H
Eating time (min/d)	351±38.8	354±44.8	380±38.7	322±44.8	-
Frequency (total chews/min)	58.0±1.3[a]	59.4±1.5[a]	62.9±1.3[ab]	64.2±1.5[b]	H
Rumination chews/min	62.8±2.7	63.8±3.1	67.1±2.7	68.8±3.1	-
Rumination chews/Bolus	44.9±3.4[a]	50.7±3.9[b]	56.0±3.3[b]	65.0±3.9[c]	H, C
Rumination periods:					
Number/d	14.9±1.0	15.1±1.1	15.7±1.0	15.4±1.1	
Duration (min/period)	20.8±2.5[a]	21.8±2.8[a]	30.4±2.4[b]	31.6±2.9[b]	H
Chewing indices refer to total ration:					
Chewing (min/kg DM)	56.6±3.40[a]	48.7±3.86[a]	74.4±3.30[b]	59.2±3.91[a]	H, C
Chewing (min/kg NDF)	134.2±7.42	130.1±8.53	139.8±7.16	132.9±8.50	-
Rumination (min/kg DM)	26.9±2.76[ab]	23.6±3.11[a]	40.8±2.67[b]	35.2±3.17[b]	H
Rumination (min/kg NDF)	63.7±5.93	63.5±6.68	76.4±5.71	79.2±6.79	H
Chewing indices refer to hay:					
Chewing (min/kg hay DM)	71.3±5.1[a]	93.6±5.8[b]	95.3±5.0[b]	115.4±5.9[c]	H, C
Chewing (min/kg hay NDF)	153.2±9.3[a]	193.8±10.5[b]	155.7±9.1[a]	183.3±10.7[b]	C
Rumination (min/kg hay DM)	33.9±3.9[a]	45.5±4.4[b]	52.4±3.8[b]	69.1±4.5[c]	H, C
Rumination (min/kg hay NDF)	72.7±7.3[a]	94.4±8.2[b]	85.5±7.1[ab]	109.9±8.4[b]	C

[1] H = Hay quality: high-fiber (62% NDF) and low-fiber (47% NDF in DM).
[2] C = Concentrate level: high (50%) and low (20% of total DMI). [3] H, C = main factors.
[4] Number of observations in each treatment (3 to 4 animals and 3 to 7 measurement days per animal).
Different superscripts in the line indicate significant differences between treatments (p≤0.05).

low probability of escape from the reticulo-rumen), small particles (0.063 mm<particles<1.00 mm, i.e. retained on sieve of 0.063 mm, 0.125 mm, 0.25 mm and 0.5 mm mesh and have a high probability of escape from the reticulo-rumen) and very fine particles or soluble fraction (particles <0.063 mm, i.e. passed the sieve of 0.063 mm). The mean particle size (MPL) was calculated based on the dried particles retained on different sieves using the equation 1 (without 0 mm = 100%, i.e. the digesta passed the sieve 0.063 mm was not included in the calculation) of Fisher et al. (1988) by the NLIN Procedure of SAS (Release 8.2, 2001).

For a main plot (H·C), repeated digesta samples for ruminal fermentation and particle size distribution were collected at four times intervals (1 h before and 2, 5 and 10 h after the morning feeding) on two days (4 sampling times ×2 days = 8 measurements for each animal and treatment).

Statistical analysis

The data of particle size in faeces, passage and digestibility were analysed by GLM procedure of SAS (SAS, Release 8.2, 2001) using a model considering the fixed effects of factors H, C, animal (A), the interaction H·C and the intake level (I: g DM/kg $BW^{0.75}$) as co-variable. For the chewing activity analysis a model for repeated measures (measurement days as random) was used. The data of ruminal fermentation and of particle size distribution in the rumen digesta were analysed by MIXED procedure of SAS (SAS, Release 8.2, 2001) using a split-plot model for repeated measures according to Littell et al. (1998). In addition to the fixed effects of H, C and A, the corresponding two-way interaction of H and C (H·C), the repeated measures on two days (D) and four sampling times (T) on the same main plot and animal (H·C·A) in the random part of the model were considered. In order to control the experimental error related to the lactation stage, the lactation day was recorded as a covariate in the analysis of covariance (ANCOVA which is based on the assumption of parallelism). The significance of the influence was tested by lack-of-fit term. The lactation day showed no significant influence on parameters studied. Therefore it was not included into further analysis of variance. The differences between the Least squares means (Lsmeans) of different H·C-treatments were tested by the option PDIFF for p≤0.05.

RESULTS

Chewing activity

High fibre content in the hay caused longer chewing and rumination time and longer rumination periods irrespective of concentrate level. Chew frequency and rumination chews per bolus were also higher in diets with high-fibre hay.

Table 3. Influence of hay quality and concentrate level on passage parameters of solid and fluid digesta and on the apparent nutrient digestibility in dairy cows (Lsmeans±SEM)

H[1] C[2]	Treatments (Hay quality×concentrate level)				Significance
	Low-fiber hay		High-fiber hay		
	20% (n = 4)	50% (n = 4)[3]	20% (n = 4)	50% (n = 3)	(p≤0.05)[4]
Solid digesta passage[5]					
TD (h)	10.3±3.09	10.1±3.46	17.1±3.09	9.2±4.74	-
RMRT (h)	24.2±2.16^{a}	40.8±2.42^{c}	28.7±2.16^{ab}	30.1±3.32^{bc}	H·C, C, A, I
FMRT (h)	16.7±2.27^{b}	11.4±2.54^{a}	11.3±2.27^{a}	11.2±3.47^{a}	-
TMRT (h)	51.3±3.29^{a}	62.3±3.69^{b}	57.2±3.29^{ab}	50.5±5.05^{a}	H·C
Fluid digesta passage					
TD (h)	11.9±2.18	11.3±2.44	9.8±2.18	6.6±3.34	-
RMRT (h)	14.5±1.35	15.2±1.51	15.9±1.35	12.5±2.07	A, I
FMRT (h)	1.7±1.44	1.2±1.62	2.2±1.44	3.8±2.21	A, I
TMRT (h)	28.2±2.21	27.7±2.47	27.9±2.21	22.9±3.38	I
Digestibility (%)					
OM	72.0±0.89^{b}	73.4±0.89^{b}	62.9±0.89^{a}	71.8±1.07^{b}	H, C, H·C
CF	63.0±1.81^{b}	49.2±1.81^{a}	54.0±1.81^{a}	51.3±2.17^{a}	C, H·C
NDF	69.8±1.47^{b}	56.2±1.47^{a}	55.1±1.47^{a}	57.4±1.76^{ab}	H·C
NFC	92.0±0.98^{b}	93.5±0.98^{b}	87.1±0.98^{a}	92.0±1.18^{b}	H, C
Hemicellulose	82.0±1.39^{b}	74.9±1.16^{a}	76.4±1.16^{a}	73.0±1.39^{a}	H, C

[1] H = Hay quality: high-fiber (62% NDF) and low-fiber (47% NDF in DM).
[2] C = Concentrate level: high (50%) and low (20% of total DMI).
[3] Number of animals in each treatment.
[4] H·C = Interactions between main factors; A = factor animal considered as random factor in the model of the analysis of variance; I = Intake level (kg DM/d) considered as co-variable in the model of analysis of variance.
[5] Passage parameters: TD = time delay, RMRT = ruminal mean retention time, FMRT = fast mean retention time, and TMRT = total mean retention time.
Different superscripts in the line indicate significant differences between treatments (p≤0.05).

Lower NDF content in the hay (470% vs. 620% NDF in DM) reduced the rumination time by 18 to 24 min/kg hay-DM and 13 to 16 min/kg hay-NDF, when feeding low (20%) and high (50% in ration DM) concentrate level, respectively (Table 2). When the concentrate level was increased from 20% to 50% (DM basis) and feeding level was restricted, cows spent significantly more time for rumination per unit of roughage DM and NDF (Table 2), but absolutely (rumination time/day) the increase was only 5 and 4% in the low-fibre and high-fibre hay diet, respectively.

Passage rate and nutrient digestibility

Increasing the concentrate to hay ratio caused a significant increase of solid retention time in the rumen (RMRT) when low-fibre hay was offered, only (Table 3). The RMRT was decreased by feeding high-fibre hay with 50% but not with 20% concentrate. The increase of the fibre content in the hay decreased the apparent nutrient digestibility at the low concentrate (20%) treatments, only (Table 3). The digestibility of CF and NDF of treatments with low-fibre hay was significantly decreased when concentrate level was increased from 20% to 50% in the diet.

Ruminal fermentation

In the Table 4 the parameters of fermentation of 1 h before, 2 h after the morning feeding and the mean of four sampling times within a day are presented. The results of 5 h and 10 h after the morning feeding are described in the text. The ruminal pH was significantly ($p<0.05$) affected by C, T and H·C·T. The great differences are observed especially at 2 h and 5 h after the morning feeding. Higher concentrate level in the diet deceased pH by 0.3 to 0.6 units compared to low concentrate level regardless the hay quality (Table 4). Prior to the morning feeding pH in all treatments studied was in the physiological optimal range 6.7±0.5 (Van Soest, 1994). The bicarbonate concentration before the morning feeding was affected mainly by H. With increasing concentrate level the bicarbonate concentration at 2 h and 5 h after the morning feeding decreased (Table 4). Giving higher concentrate amount in the diet decreased ($p<0.05$) the acetate to propionate ratio (A:P ratio) and slightly ($p>0.05$) increased the SCFA concentration. Ammonia concentration was affected ($p<0.05$) by H, C and T (Table 4). Lower fibre content in the hay and higher concentrate level in the diet decreased ($p<0.05$) the ammonia concentration in the rumen.

Particle distribution in the rumen and faeces

Higher fibre content in the hay increased the concentration of large (>1.0 mm sieve size) and small (<1.0 mm sieve size) particles (in the rumen slightly) and

Table 4. Influence of hay quality and concentrate level on the ruminal fermentation (Lsmeans)

Parameters	Sampling time[2]	N	Treatments (Hay quality×concentrate level)				SEM[1]	Significant factors [3] (p≤0.05)
			Low-fiber hay		High-fiber hay			
			20%	50%	20%	50%		
pH	-1 h	4[4]	6.87^{ab}	6.96^{b}	6.76^{ab}	6.63^{a}	0.110	
	+2 h	4	6.49^{b}	5.99^{a}	6.55^{b}	6.09^{a}	0.110	C, T, H·C·T
	Mean	16[5]	6.68^{b}	6.34^{a}	6.58^{b}	6.31^{a}	0.057	
SCFA (mmol/l)	-1 h	4	91.8	81.0	92.5	82.3	9.73	
	+2 h	4	108.7	116.3	101.8	120.9	9.73	T
	Mean	16	96.9	99.7	97.7	105.2	4.54	
Acetate/ propionate	-1 h	4	4.75^{b}	3.92^{a}	4.16^{ab}	3.54^{a}	0.310	
	+2 h	4	3.46	2.92	3.56	2.98	0.310	T, C ($p = 0.076$)
	Mean	16	4.20^{b}	3.45^{ab}	3.83^{ab}	3.31^{a}	0.238	
Bicarbonate (mmol/l)	-1 h	4	50.31^{b}	47.93^{b}	38.80^{ab}	26.83^{a}	6.709	
	+2 h	4	25.81^{b}	7.76^{a}	23.94^{b}	7.08^{a}	6.709	H, C, T
	Mean	16	36.37^{b}	22.60^{a}	26.58^{a}	16.89^{a}	3.421	
Ammonia (mmol/l)	-1 h	4	5.65^{b}	3.60^{a}	6.56^{b}	4.31^{ab}	0.830	
	+2 h	4	9.84^{b}	7.86^{a}	12.87^{c}	8.02^{ab}	0.830	H, C, T
	Mean	16	5.36^{b}	3.70^{a}	7.86^{c}	5.39^{b}	0.419	

[1] Least square means (Lsmeans) and standard error of the mean (SEM).

[2] Sampling times within a day: -1 h = one hour before morning feeding; −2 h = two hours after morning feeding; Mean = mean of all measurements carried out on two animals, repeated over two days and four sampling times (1 h before, 2 h, 5 h and 10 h after morning feeding) within the day for each treatment (H·C).

[3] H = hay quality; C = concentrate level; T = sampling time; H·C and H·C·T = interactions between main factors.

[4] Four measurements (2 animals×2 days) were carried out per sampling time for each treatment.

[5] 16 measurements (2 animals×2 days×4 sampling times) were carried out for each treatment (H·C).

Different superscripts in the line indicate significant differences between treatments (p≤0.05).

decreased that of very fine (<0.063 mm sieve size) particles or soluble fraction in the bottom rumen and faeces (Table 5). The enhancement of the concentrate level from 20 to 50% in the diet increased the share of large particles, did not affect the concentration of small particles in the rumen and faeces and decreased the concentration of soluble fraction in the rumen, but not in the faeces. The MPL of rumen digesta and of faeces increased with increasing fibre content in the hay and concentrate level in the diet. The shortest MPL was found on the treatment "low-fibre hay and 20% concentrate" (Table 5).

DISCUSSION

The effects of different treatments on digestive processes are primarily related to the roughage: concentrate ratio, the content of structural and non structural carbohydrates in the diet as well as their degradation rate in the rumen. These effects are already reported by several authors (Welch, 1982; Mould et al., 1983; Hooper, 1986; Beauchemin, 1991; Robinson and McQueen, 1997; Mertens, 1997; Offer and Dixon, 2000; Choi et al., 2003; Moon et al., 2004). Therefore the discussion chapter is focused mainly on the effects of increasing of hay quality on digestion processes, particularly when low concentrate amount was included in the diet.

The diet consisting of low-fiber hay (47.1% NDF) and low concentrate amount (20%) had a better SC: NFC- ratio and a moderate degradation rate, which seems to influence positively the digestion processes, particularly the rumen conditions and ruminal fiber digestion. In this treatment a significant reduction of the time spent for chewing and rumination time per unit of hay DM and NDF occurred compared to the treatment "low-fiber hay+50% concentrate". The shorter time spent for chewing and rumination per unit DM or fiber leads to the assumption that the contribution of the rumen microbial digestion to the fiber breakdown is increased and, consequently less ruminating is needed. The contribution of chewing (eating and ruminating) activity to the particle reduction is about 70 to 80% and that of the microbial degradation is 15 to 25% (McLeod and Minson, 1988; Beauchemin, 1991). It is highly probable that ruminants try to compensate a low microbial fiber breakdown through an increase of the physical breakdown, which extends the chewing time. This should mean that the digestion through the mastication and that one through the ruminal microbial work complementarily in the fiber breakdown. Through the

Table 5. Particle size distribution (retained DM particle in % of DM) and Mean Particle Length (MPL, mm) of rumen digesta (ventral sac) and faeces of cows fed with various hay qualities and concentrate levels in the diet (Lsmeans)

Particle fraction[2]	Compartment[3]	Sampling time[4]	n	Hay quality×concentrate level				SEM[1]	Significant factors[5] (p≤0.05)
				Low-fiber hay		High-fiber hay			
				20%	50%	20%	50%		
Large >1.0 mm	Rumen	-1 h	4[6]	26.5^{a}	36.2^{ab}	30.2^{a}	38.6^{b}	3.01	
		+2 h	4	37.9^{a}	40.9^{ab}	39.0^{ab}	46.2^{b}	3.01	C, T, H[0.09]
		Mean	16[7]	34.0^{a}	39.2^{ab}	38.2^{ab}	42.8^{b}	1.61	
	Faeces	-	4[8]	2.29^{a}	3.95^{b}	3.16^{b}	6.16^{c}	0.33[9]	H, C, H·C[0.09]
Small <1.0 mm	Rumen	-1 h	4	47.4	47.2	48.4	48.4	3.51	
		+2 h	4	34.3	39.3	42.5	39.7	3.51	T
		Mean	16	40.5	43.0	42.8	44.8	1.76	
	Faeces	-	4	39.2^{a}	36.9^{a}	50.5^{b}	47.3^{ab}	3.18	H
Very fine <0.063 mm	Rumen	-1 h	4	26.1^{b}	16.6^{a}	21.4^{ab}	12.9^{a}	2.04	
		+2 h	4	27.8^{b}	19.8^{a}	18.5^{a}	14.2^{a}	2.04	H, C
		Mean	16	25.6^{c}	17.8^{b}	18.9^{b}	12.4^{a}	1.02	
	Faeces	-	4	58.6^{b}	59.1^{b}	46.3^{a}	46.6^{a}	3.17	H
MPL (mm)	Rumen	-1 h	4	1.18	1.67	1.34	1.96	0.426	
		+2 h	4	2.19^{a}	2.19^{a}	2.12^{a}	3.26^{b}	0.426	H, T, C[0.078]
		Mean	16	1.77^{a}	2.09^{ab}	2.12^{ab}	2.59^{b}	0.218	
	Faeces	-	4	0.26^{a}	0.43^{b}	0.36^{ab}	0.46^{b}	0.050	C

[1] Least square means (Lsmeans) and standard error of the mean (SEM).

[2] Large particles >1.0 mm sieve size (the sum of retained DM particle on sieves 1.0, 2.0 and 4.0 mm); small particles >0.063 mm and <1.00 mm sieve size (the sum of retained DM particle on sieves 0.5, 0.25, 0.125 and 0.063 mm); very fine particles >0.063 mm sieve size (DM particle passed the sieve 0.063 mm sieve size).

[3] Compartments: digesta of ventral rumen sac and faeces.

[4] Sampling times within a day: -1 h = one hour before morning feeding; −2 h = two hours after morning feeding; Mean = mean of all measurements carried out on two animals, repeated over two days and four sampling times (1 h before, 2 h, 5 h and 10 h after morning feeding) within the day for each treatment (H·C).

[5] H = hay quality; C = concentrate level; T = sampling time; H·C and H·C·T = interactions between main factors.

[6] On rumen digesta, 4 measurements (2 animals×2 days) were carried out per sampling time for each treatment.

[7] On rumen digesta, 16 measurements (2 animals×2 days×4 sampling times) were carried out for each treatment

[8] Number of animals in each treatment (in the treatment fiber-high hay+50% concentrate 3 animals).

[9] SEM for the treatment "high-fiber hay+50% concentrate" differ from other treatments due to the number of animals. SEM values for this treatment: 0.395, 3.826, 3.810 and 0.060 for large particle, small particle, soluble fraction and MPL, respectively.

Different superscripts in the line indicate significant differences between treatments (p≤0.05).

ingestive and ruminative mastication the feed particles are intensively disintegrated and lacerated, their available surface is increased and therefore the microbes gain a better access to cellulose (Krause and Pell, 2003). On the other hand, the microbial digestion weakens cell walls and increases the fragility of the particles, which in turn makes them more susceptible to mastication and as result improves the rumination efficiency (Kennedy and Doyle, 1993). The assumption about an increased contribution of rumen microbial digestion to the fiber breakdown at the treatment "low-fiber hay+20% concentrate" is also supported by the significant decrease of the solid RMRT and TMRT and the significant increase of the digestibility of CF and NDF and of the acetate to propionate ratio in total SCFA of ruminal fluid. The decrease of MPL and of large particle (>1.0 mm) to small particles (<1.0 mm) ratio in the ruminal digesta and in the faeces can also be interpreted as a result of an increased fiber digestion in the rumen. It can be concluded that giving a diet of low-fiber hay in combination with low concentrate level (20%) provided the better rumen conditions for fiber digestion due to the better nutrient balance, particularly due to a better ratio between structural and non structural carbohydrates in the diet. The results of this treatment confirm the role of the rumen conditions on fiber digestion, particle outflow from reticulorumen and passage rate, and, consequently, on feed intake. Recent studies (Okine et al., 1998; Poppi et al., 2001) concluded that the digesta flow from the reticulorumen is affected more by rumen conditions than by particle kinetics (particle size, density) *per se*, i.e. that the feeding factors act mainly indirectly on the digesta outflow that is influenced by rumen conditions.

In practical terms, the results of the treatment "low-fiber hay+20% concentrate" confirm that the increase of the roughage quality is an effective strategy in dairy cows nutrition, as an alternative to increasing the concentrate proportion to the diet, especially when concentrate availability is limited. This conclusion can be well illustrated when effects of treatments "low-fiber hay+20% concentrate" and "high-fiber hay+50% concentrate" on digestive processes are compared. These treatments can be considered as representative for different feeding strategies. The treatment "low-fiber hay+20% concentrate" can be considered representative for strategies aimed improving of roughage quality through reduction of fiber content. The treatment "high-fiber hay+50% concentrate" can be considered representative for strategies of increasing the concentrate proportion in the dairy cows diets based on low-quality roughages (e.g. Bwire and Wiktorsson, 2003). Concerning the diet characteristics, there are not great quantitative differences in the level of structural and non-structural carbohydrates (NDF: 42.5 vs. 44%; NDF of hay: 36.7 vs. 31.4%; NFC: 33.6 vs. 32.1%) between these two treatments. However, the chewing and ruminating efficiency (chewing and ruminating time spent per unit of hay DM and NDF) was markedly increased when low-fiber hay combined with 20% concentrate is offered compared to the treatment of high-fiber hay and 50% concentrate. The higher pH and the bicarbonate concentration, the lower SCFA concentration in the rumen 2 h after the morning feeding, the lower concentration of large particle fraction (>1.00 mm) and the lower MPL of particle of ruminal digesta and of faeces at the treatment of low-fiber hay and 20% concentrate confirmed better rumen conditions and higher microbial fiber breakdown compared to the treatment of high-fiber hay and 50% concentrate in the diet. It seems that feeding high amount of high-degradable concentrate (50% DM basis) in combination of fibre-rich or fibre-poor hay altered the rumen conditions and consequently did not avoid a depression of the ruminal fiber digestibility. Several authors (Allen, 1997; Choi et al., 2003) reported that the amount of fermentable carbohydrates consumed is decisive for the production of SCFA and pH in the rumen.

The positive effects of low-fibre hay combined with low concentrate amount can be also related to the degree of lignification of hay. There is a positive correlation between fiber content and degree of lignification (Van Soest, 1994). The low-fiber content is presumably associated with a low degree of lignification, which can favour the bacterial colonisation of fiber and the degradation activity of fibrolytic bacteria in the rumen (Krause and Pell, 2003).

The positive effects of diets consisting of low-fiber hay and low concentrate amount on digestive processes, particularly on ruminal digestion, support the conclusion of Van Soest (1994) that concentrate supplementation is not a way to overcome the limitations of low-quality forages, which are best resolved by managing for better-quality forage. Robinson and McQueen (1997) studied different concentrate levels and forage qualities (defined by the NDF fermentability) in mid lactation cows and reported also that high quality forage is crucial for a successful dairy diet.

CONCLUSIONS

The whole tract digestion (chewing, passage, nutrient digestibility), the ruminal fermentation (pH, concentration of SCFA, bicarbonate and ammonia) and the particle size in the rumen and faeces appear to be more affected by the content and degradation rate of the fiber in the hay than by the concentrate level. The low-fiber hay combined with the low concentrate level in the diet provided better rumen conditions for fiber digestion than the treatments with high-fiber hay. Giving high concentrate amount (50%) and low-fiber hay influenced negatively the rumen digestion. Generally, it can be summarised that, (i) the concentrate supplementation is not a strategy to overcome limitations of low quality (fiber-rich) hay, and (ii) that the increase of the roughage quality is an effective strategy in the ruminant nutrition, especially when the concentrate availability for ruminants is limited.

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REFERENCES

Allen, M. S. 1997. Relationship between fermentation acid production in the rumen and the requirement for physically effective fiber. J. Dairy Sci. 80:1447-1462.

Bwire, J. M. N. and H. Wiktorsson. 2003. Effect of supplementary feeding strategies on the performance of stall fed dual-purpose dairy cows fed grass hay-based diets. Asian-Aust. J. Anim. Sci. 16(3):359-367.

Beauchemin, K. A. 1991. Ingestion and mastication of feed by dairy cattle. Vet. Clinics of North America: Food Animal Practice, 7(2):439-463.

Choi, Y. J., S. S. Lee, J. Y. Song, N. J. Choi, H. G. Sung, S. G. Yun and J. K. Ha. 2003. Effects of dietary acidogenicity values on rumen fermentation characteristics and nutrients digestibility. Asian-Aust. J. Anim. Sci. 16(11):1625-1633.

Fisher, D. S., J. C. Burns and K. R. Pond. 1988. Estimation of mean and median particle size of ruminant digesta. J. Dairy Sci. 71:518-524.

Gäbel, G. 2000. Säure-Basen-Haushalt. In Physiologie der Haustiere. (Ed. W. V. Engelhardt and G. Breves), Enke im Hippokrates Verlag GmbH, Stuttgart, Germany, pp. 295-302.

Hoover, W. H. 1986. Chemical factors involved in ruminal fiber digestion. J. Dairy Sci. 69:2755-2767.

Kaufmann, W. and H. Hagemeister. 1969. Das Puffersystem in den Vormägen von Rindern. J. Anim. Physiol. Anim. Nutr. 25:157-168.

Kennedy, P. M. and P. T. Doyle. 1993. Particle-size reduction by ruminants – Effects of cell wall composition and structure. In Forage cell wall structure and digestibility. (Ed. H. G. Jung, D. R. Buxton, R. D. Hatfield and J. Ralph), Madison, Wisconsin, USA, pp. 499-534.

Khazaal, K., M. T. Dentinho, J. M. Ribeiro and E. R. Orskov. 1993. A comparison of gas production during incubation with rumen contents *in vitro* and nylon bag degradability as predictors of the apparent digestibility *in vivo* and the voluntary intake of hays. Anim. Prod. 57:105-112.

Krause, D. O. and A. N. Pell. 2003. Plant cell wall degradation in the rumen: ecology, constraints to digestion and modelling. In Matching herbivore nutrition to ecosystems biodiversity. Mannetje, L.'t, L. Ramirez-Aviles, (Ed. C. A. Sandoval-Castro and J. C. Ku-Vera). Proceedings of the Sixth International Symposium on the Nutrition of Herbivores. Universidad Autonoma de Yucatan, Mexico, pp. 129-150.

Krause, K. M., D. K. Combs and K. A. Beauchemin. 2002. Effects of forage particle size and grain fermentability in midlactation cows. II. Ruminal pH and chewing activity. J. Dairy Sci. 85:1947-1957.

Lechner-Doll, M. and W. von Engelhardt. 1989. Particle size and passage from the forestomach in camels compared to cattle and sheep fed similar diet. J. Anim. Physiol. Anim. Nutr. 61:120-128.

Littell, R. C., P. R. Henry and C. B. Ammerman. 1998. Statistical analysis of repeated measures data using SAS procedures. J. Anim. Sci. 76:1216-1231.

Mambrini, M. and J. L. Peyraud. 1997. Retention time of feed particles and liquids in the stomachs and intestines of dairy cows; direct measurement and calculations based on faecal collection. Reprod. Nutr. Dev. 37:427-442.

McLeod, M. N. and D. J. Minson. 1988. Large particle breakdown by cattle eating ryegrass and alfalfa. J. Anim. Sci. 66:992-999.

Mertens, D. R. 1997. Creating a system for meeting the fiber requirements of dairy cows. J. Dairy Sci. 80:1463-1481.

Moon, Y. H., S. C. Lee and S. S. Lee. 2004. Effects of neutral detergent fiber concentration and particle size of the diet on chewing activities of dairy cows. Asian-Aust. J. Anim. Sci. 17(11):1535-1540.

Moore, J. A., K. R. Pond, M. N. Poore and T. G. Goodwin. 1992. Influence of model and marker on digesta kinetic estimates for sheep. J. Anim. Sci. 70:3528-3540.

Mould, F. L., E. R. Orskov and S. O. Mann. 1983. Associative effects of mixed feeds. I. Effects of type and level of supplementation and the influence of the rumen fluid pH on cellolysis *in vivo* and dry matter digestion on various roughages. Anim. Feed Sci. Technol. 10:15-30.

Offer, N. W. and J. Dixon. 2000. Factors affecting outflow rate from the reticulo-rumen. Nutr. Abstr. Rev., Series B: Livestock Feeds and Feeding, 70:833-844.

Okine, E. K., G. W. Mathison, M. Kaske, J. J. Kenelly and R. J. Christopherson. 1998. Current understanding of the role of the reticulum and reticulo-omasal orifice in the control of digesta passage from the ruminoreticulum of sheep and cattle. Can. J. Anim. Sci. 78:15-21.

Poppi, D. P., W. C. Ellis, J. H. Matis and C. E. Lascano. 2001. Marker concentration patterns of labelled leaf and stem particles in the rumne of cattle grazing bermuda grass (*Cynodon dactylon*) analysed by reference to a raft model. Br. J. Nutr. 85:353-563.

Robinson, P. H. and R. E. McQueen. 1997. Influence of level of concentrate allocation and fermentability of forage fiber on chewing behaviour and production of dairy cows. J. Dairy Sci. 80:681-691.

SAS. 2001. SAS/STAT, Release 8.2. SAS Inst. Inc., Cary, NC.

Susenbeth, A., T. Dickel, K. -H. Südekum, W. Drochner and H. Steingass. 2004. Energy requirements of cattle for standing and for ingestion, estimated by a ruminal emptying technique. J. Anim. Sci. 82:129-136.

Tafaj, M., B. Junck, A. Maulbetsch, H. Steingaß and W. Drochner. 2001. Research Note: A method for studying of local differences in ruminal fermentation in dairy cattle. Arch. Anim. Nutr. 54:341-347.

Tamminga, S. 1993. Influence of feeding management on ruminant fibre digestibility. In Forage cell wall structure and digestibility. (Ed. H. G. Jung, D. R. Buxton, R. D. Hatfield and J. Ralph), Madison, Wisconsin, USA, pp. 571-602.

Uden, P., P. E. Colucci and P. J. Van Soest. 1980. Investigation of chromium, cerium and cobalt as markers in digesta rate of passage studies. J. Sci. Food Agric. 31:625-632.

Van Soest, P. J. 1994. Nutritional ecology of the ruminant. Second edition. Cornell University Press.

Welch, J. G. 1982. Rumination, particle size and passage from the rumen. J. Anim. Sci. 54:885-894.