Effects of particle size and dry matter content of a total mixed ration on intraruminal equilibration and net portal flux of volatile fatty acids in lactating dairy cows

A. C. Storm and N. B. Kristensen¹

Department of Animal Health and Bioscience, Faculty of Agricultural Sciences, Aarhus University, DK-8830 Tjele, Denmark

ABSTRACT

Effects of physical changes in consistency of ruminal contents on intraruminal equilibration and net portal fluxes of volatile fatty acids (VFA) in dairy cows were studied. Four Danish Holstein cows (121 ± 17 d in milk, 591 ± 24 kg of body weight, mean $\pm$ SD) surgically fitted with a ruminal cannula and permanent indwelling catheters in the major splanchnic blood vessels were used. The experimental design was a 4×4 Latin square with a 2×2 factorial design of treatments. Treatments differed in forage (grass hay) particle size (FPS; 3.0 and 30 mm) and feed dry matter (DM) content of the total mixed ration (44.3 and 53.8%). The feed DM did not affect chewing time, ruminal variables, or net portal flux of VFA. However, decreasing the FPS decreased the overall chewing and rumination times by 151 ± 55 and 135 ± 29 min/d, respectively. No effect of the reduced chewing time was observed on ruminal pH or milk fat percentage. Cows maintained average ventral ruminal pH of 6.65 ± 0.02 , medial ruminal pH of 5.95 ± 0.04 , and milk fat of $4.42 \pm 0.12\%$ with chewing time of 28.0 ± 2.1 min/kg of DM when fed short particles. The medial ruminal pool of wet particulate matter was decreased by 10.53 ± 2.29 kg with decreasing FPS, thereby decreasing the medial pool of total VFA, acetate, propionate, butyrate, isobutyrate, and isovalerate by $1,143 \pm 333$, 720 ± 205 , 228 ± 69 , 140 ± 51 , 8.0 ± 2.3 , and 25.2 ± 5.6 mmol, respectively. Ventral pool variables were not affected by treatments. Relatively large intraruminal differences of VFA concentrations and pH between the ventral and medial pools were observed, VFA concentrations being largest and pH being the lowest medially. This indicates that the ruminal mat acts as a barrier retaining VFA. The effects of reduced FPS were limited to the VFA pool sizes of the mat, leaving ruminal pH, ruminal VFA concentrations, and net portal flux of VFA unaffected. Consequently reduced FPS affected the intraruminal equilibration of

VFA between mat and ventral rumen with an estimated turnover rate of isobutyrate increasing from $50 \pm 3\%/h$ with long particles to $61 \pm 3\%/h$ with short particles. The estimated ruminal fluid flow and therefore intraruminal VFA transport between medial and ventral phase was not affected by the FPS. In conclusion, the ruminal mat pool of VFA was proportional to the size of the mat and the only detected effects of decreasing FPS were decreasing the mat size and an increasing turnover of the mat pool of VFA.

Key words: dairy cow, forage particle size, portal absorption, rumen volatile fatty acid kinetics

INTRODUCTION

Volatile fatty acids of forestomach origin are absorbed across the ruminal epithelium, and from compartments distal to the rumen for the VFA being washed out of the rumen (Peters et al., 1990). Three processes can be hypothesized to limit VFA absorption across the ruminal epithelium: 1) intraruminal transport of VFA from the site of production to the site of absorption, 2) transport across the ruminal epithelium, and 3) epithelial blood flow limiting removal of VFA from the serosal side of the reticulorumen epithelium.

Intraruminal equilibration of VFA from the site of production to the site of absorption is facilitated by the contractive cycles of reticulorumen muscle bands mixing the ruminal contents and exposing VFA to the ruminal wall for absorption. Under common feeding situations, ruminal pH and VFA concentrations differ between the medial mat and the ventral rumen sac, showing lowest pH and highest VFA concentrations in the mat region (Smith et al., 1956; Tafaj et al., 2004). This indicates that the mixing of ruminal contents is imperfect and that the intraruminal VFA equilibration is one of the factors contributing to accumulation of VFA in the ruminal mat.

The intraruminal equilibration of VFA is probably limited by the consistency of ruminal contents, which is dependent on DM to fluid ratio, ruminal mat stratification, and fluid viscosity. The feed particle size is known

Received December 16, 2009.

Accepted May 11, 2010.

¹Corresponding author: nbk@agrsci.dk

to affect the mat size by increasing the ruminal DM to fluid ratio (Shaver et al., 1988), but the DM content of the TMR has also been shown to affect the ruminal pH (Hosseinkhani et al., 2007).

The amount of large forage particles in the ration has been proposed as a practical measure to ensure a healthy ruminal environment by reducing the risk of ruminal acidosis (Mertens, 1997). However, a healthy ruminal environment induced by increasing chewing activity, attributed to increased particle size, has been difficult to prove. One of the reasons could be that saliva secretion and thereby bicarbonate buffer capacity does not increase with increased chewing activity (Maekawa et al., 2002). The effect of increasing particle size on ruminal pH may be attributable to change in the consistency of ruminal contents, which reduces the rate of intraruminal equilibration of VFA, consequently delaying the absorption and protecting the epithelium from the peak load of VFA.

Our hypothesis was that large particles in the ruminal mat and high DM content of the ruminal mat after feeding a dry TMR would decrease the rate of ruminal fermentation and VFA equilibration from the ruminal mat to the ventral rumen and result in a flatter profile of net portal flux of VFA with the same total daily flux. The objective of the study was to investigate the effects of physical changes in the ruminal mat on chewing time, ruminal environment, and intraruminal equilibration and net portal flux of VFA in lactating dairy cows.

MATERIALS AND METHODS

Animals and Feeding

The present experiment complied with Danish Ministry of Justice law number 382 (June 10, 1987) act number 726 (September 9, 1993) concerning experiments with animals and the care of experimental animals. Four Danish Holstein cows (second parity, 591 ± 24 kg of BW, 121 ± 17 DIM, mean $\pm$ SD) were used. The cows were surgically fitted with a ruminal cannula and permanent indwelling catheters in the mesenteric or intercostal artery, mesenteric vein, hepatic portal vein, and hepatic vein on -45 ± 9 d relative to calving, with first sampling after surgery at -12 ± 4 d relative to calving. Surgery was done according to Huntington et al. (1989), with slight modifications of the catheters (Kristensen et al., 2007).

Cows were housed in tie stalls and fed 3 times daily (0800, 1600, and 2400 h). Cows were restrictively offered 20 kg of DM/d to avoid feed refusal and were milked at 0600 and 1600 h. Cows were randomly allocated to one of 4 experimental diets in a 4×4 Latin square

design with 14-d periods, with blood and ruminal fluid sampling on d 13 and ruminal evacuations on d 14.

The treatments were arranged as a 2×2 factorial design with 2 levels of forage particle size (**FPS**), short and long, and 2 levels of feed DM (**FDM**), moist and dry, resulting in 4 treatments (short dry, short moist, long dry, and long moist). The diets were based on corn silage, grass hay, and rolled barley and were supplemented as shown in Table 1. The factor FDM was controlled by adding water to the TMR, resulting in 44.3% DM in the moist rations and 53.8% DM in the dry rations. The FPS factor was controlled by varying the theoretical length of cut (**TLC**) of the grass hay in the TMR. Long particles had a TLC of 30 mm (Cormall tractor cutter, 30 mm mesh, Cormall A/S, Sønderborg, Denmark) and short particles had a TLC of 3.0 mm (Cormall cutter, 10 mm mesh, followed by President hammer mill, 3.0 mm mesh, President Milling, Herning, Denmark).

Chromium oxide (Table 1) was used as a digestibility marker. It was mixed and pelleted with the nonforage feed components other than barley before addition to the TMR. The TMR was mixed 2 wk before initiation of the experiment, vacuum packed in plastic bags of 10 to 15 kg, and stored at -20 to -25°C until start of the experiment. The bagged TMR was stored at -6°C during the experiment and defrosted overnight before use.

Experimental Samplings

On d 13 of each period a continuous infusion of *p*-aminohippuric acid (**pAH**; 29.3 ± 0.4 mmol/h) into the mesenteric vein was initiated at 0530 h. From 0730 to 1430 h, 8 sets of ruminal fluid (medial ruminal mat and ventral rumen sac) and blood (artery, hepatic portal vein, and hepatic vein) samples were obtained at hourly intervals from 30 min before to 6.5 h after the morning feeding. Ruminal pH was measured immediately using a combination electrode (PHC2002-8, Hach Lange APS, Brønshøj, Denmark) and a pH meter calibrated at pH 4.005 and 7.000 (PHM 240, Hach Lange APS) and stored at -20°C .

Medial ruminal fluid was sampled by grab sampling the ruminal mat through the cannula. One large handful was obtained 10 to 15 cm beneath the mat surface in the midsection of the rumen. The ruminal mat particle associated fluid was squeezed from the sample through 1 piece of cheese cloth into a 45-mL test tube. The ventral rumen was sampled with a suction strainer (#RT, Bar Diamond Inc., Parma, ID). Subsamples of 8 mL of ruminal fluid were combined with 2 mL of 25% metaphosphoric acid and stored at -20°C .

For blood gas measurements, a 1-mL sample of blood was drawn from each catheter into heparinized syringes.

Table 1. Ingredients and nutrient composition of experimental diets¹

Item	Short		Long	
	Dry	Moist	Dry	Moist
Ingredient, % of DM				
Corn silage ²	35	35	35	35
Grass hay ³	30	30	30	30
Rolled barley	20	20	20	20
Molasses sugar beet	1	1	1	1
Rapeseed cake	11	11	11	11
NaHCO ₃	0.8	0.8	0.8	0.8
Vitamin-mineral premix ⁴	1	1	1	1
Urea	0.7	0.7	0.7	0.7
CaCO ₃	0.2	0.2	0.2	0.2
Chromiumoxide	0.1	0.1	0.1	0.1
Nutrient				
Water added, g/kg of feed	68	225	68	225
DM, g/kg of feed	523	449	553	437
OM, g/kg of DM	946	946	943	938
NDF, g/kg of DM	353	345	338	353
CP, g/kg of DM	155	161	164	152
Crude fat, g/kg of DM	40	37	41	40

¹Diets comprised 2 levels of forage particle size, short and long, and 2 levels of feed DM, moist and dry. All diets were designed to contain a similar nutritional value, differing only in the forage particle size and DM content.

²Corn silage: DM, 36.6%; ash, 3.9 g/100 g of DM; CP, 8.7 g/100 g of DM; NDF, 37.5 g/100 g of DM.

³Grass hay: DM, 92.8%; ash, 6.1 g/100 g of DM; CP, 14.4 g/100 g of DM; NDF, 54.1 g/100 g of DM.

⁴Vitamin-mineral premix (VM 1, Vitfoss, Gråsten, Denmark): Ca, 18 g/100 g of DM; P, 0.4 g/100 g of DM; Mg, 6.5 g/100 g of DM; Na, 8.0 g/100 g of DM; S, 3.0 g/100 g of DM; vitamin A, 693 IU/g; vitamin D₃, 146 IU/g; vitamin E, 4,620 mg/kg; Mn, 3,080 mg/kg; Cu, 693 mg/kg; Co, 19 mg/kg; Zn, 3,460 mg/kg; I, 173 mg/kg; Se, 38.5 mg/kg.

Afterward, 40 mL of blood was simultaneously sampled from the artery, hepatic portal vein, and hepatic vein catheters using 20-mL syringes. Blood was transferred to heparin vacuettes (#455051, Greiner BioOne GmbH, Kremsmuenster, Austria) and centrifuged at $3,000 \times g$ for 20 min at 4°C, and plasma was stored at -20°C. Daily milk yield was registered and milk samples were collected at each milking on the sampling day. Feces were collected morning and evening on the sampling day and the day before. Continuous registration of jaw movements was recorded by a data logger mounted on a head halter (Rutter et al., 1997) initiated on d 12 at 1600 h and continued for 24 h.

Along with the blood sampling, continuous monitoring of reticular motility was conducted with an air filled balloon-sinker complex, placed in the ventral reticulum. The complex was connected with a plastic pipe to a pneumatic pressure transducer (#24PCEFA6G, Honeywell Sensing and Control, Golden Valley, MN) through the ruminal cannula. The pressure transducer was cable-connected to a data logger PC setup (ML870 PowerLab 8/30, ML224 Quart Bridge, Chart 5, ADInstruments Pty Ltd., Bella Vista New South Wales, Australia).

On d 14 of each period all cows were ruminally evacuated approximately 0.5 h before and approximately 1 h after feeding. The content was strained through a

heavy-duty plastic sieve ($\sim 2 \times 3$ cm) with square holes by applying static weight until little ruminal fluid was running from the wet particulate matter (**WPM**). The free ruminal fluid was thereby separated from WPM, and DM contents of both fractions were determined.

Analytical Procedures

Feed, feces, ruminal fluid, and solid DM were determined after drying at 60°C for 48 h in a forced-air oven. Feed and feces OM were determined as DM minus crude ash, where crude ash was determined after combustion at 525°C for 6 h. Feed crude fat was determined as petroleum ether extract following acid hydrolysis (Stoldt, 1952). Feed and feces CP were determined by the Dumas method according to Hansen (1989), NDF was determined as described by Mertens (2002), and chromium was determined according to Schürch et al. (1950).

Ruminal fluid samples were analyzed for glucose (D-glucose oxidase) and L-lactate (L-lactate oxidase) using the YSI analyzer (YSI 7100, YSI Inc., Yellow Springs, OH). Ruminal VFA (acetate, propionate, butyrate, isobutyrate, isovalerate, valerate, and caproate) were analyzed by gas chromatography (Kristensen et al., 1996). Ruminal fluid NH₃ was analyzed using a Cobas Mira autoanalyzer (Triolab A/S, Brøndby, Denmark)

and a kit based on glutamate dehydrogenase (AM 1015, Randox Laboratories Ltd., Crumlin, UK).

The 1-mL syringes of blood from the 3 catheters were immediately taken for blood gas and oximetry analysis (ABL 520, Radiometer A/S, Copenhagen, Denmark). The packed blood cell volume (hematocrit) was measured by whole blood centrifugation in capillary tubes at $13,000 \times g$ for 6 min at ambient temperature.

Plasma concentrations of glucose, L-lactate, and NH_3 were analyzed as for ruminal fluid described above. Plasma pAH was analyzed by the method described by Kristensen et al. (2009) using a continuous flow analyzer (Autoanalyzer 3, method US-216-72 rev.1, Seal Analytical Ltd., Burgess Hill, UK). Plasma concentrations of VFA (acetate, propionate, butyrate, isobutyrate, isovalerate, valerate, and caproate) were analyzed by gas chromatography (Kristensen, 2000). Plasma BHBA was determined in plasma using a Cobas Mira autoanalyzer and a BHBA dehydrogenase kit (Ranbut, Randox Laboratories Ltd.). Milk was analyzed for fat, protein, and lactose (monohydrate) by infrared spectrometry using a MilkoScan 4000 (Eurofins Steins A/S, Holstebro, Denmark).

Jaw recordings were analyzed using peak recognition algorithm software with a noise threshold capable of identifying periods of resting, eating, and ruminating (Graze, version 0.8, Institute of Grassland and Environmental Research, North Wyke, UK; Rutter, 2000). The frequency of primary reticuloruminal contractions (**FPRC**) was analyzed by manual counting of reticular double and triple contractions from the computer chart. The number of contractions was counted in a 15-min window from 5 min before to 10 min after initiation of blood sampling.

Calculations and Statistical Procedures

The total tract digestibilities of OM, NDF, and CP were calculated as the quantitative difference in feed and feces divided by the content in feed. The fecal nutrient excretion was determined from the Cr_2O_3 concentration in feces and Cr_2O_3 intake, under the assumption of 100% fecal recovery of Cr_2O_3 . Energy corrected milk was calculated using the procedure described by Sjaunja et al. (1991).

To calculate the ruminal pool sizes, data from the prefeeding evacuation, approximately 0.5 h before feeding, were combined with VFA data from the first and eighth samples (0.5 h before feeding and 1.5 h before the next feeding, respectively). Data from the postfeeding evacuation, approximately 1.0 h after feeding, were combined with VFA data from the second and third samples (0.5 and 1.5 h after feeding, respectively). The ruminal pools of VFA were calculated as follows: me-

dial pool VFA (mmol) = medial VFA concentration $\times$ WPM volume $\times$ (1 - WPM DM); ventral pool VFA (mmol) = ventral VFA concentration $\times$ free ruminal fluid volume $\times$ (1 - free ruminal fluid DM).

Blood and plasma fluxes were calculated according to Kristensen et al. (2007). In short, the net portal flux is the concentration difference between the portal and arterial blood times the portal blood flow. Net hepatic flux is the efferent hepatic vein flux minus the afferent fluxes in portal and hepatic arterial supplies to the liver. A positive flux describes a net release whereas a negative flux describes a net uptake. The net portal flux of acetate was corrected for the portal drained visceral (**PDV**) use of arterial acetate (0.33) to estimate the corrected flux of acetate (Kristensen and Harmon, 2004b). Corrected flux of propionate was calculated using the mean portal recovery correction factors (0.88) from 2 studies with steers (Kristensen and Harmon, 2004a,b). Corrected flux of butyrate was calculated using the recovery correction factors (0.33) corresponding to the butyrate recovery with 12 mmol/kg of butyrate in ruminal fluid (Kristensen and Harmon, 2004a). The corrected flux of isobutyrate was set equal to the net portal flux of isobutyrate because of close to 100% recovery of ruminally infused isobutyrate (Kristensen and Harmon, 2004a). The corrected net portal fluxes of acetate, propionate, butyrate, and isobutyrate are considered to be equal to the production of these metabolites in the fermentation and are therefore used as the rates of fermentation in the following calculations of fractional absorption rate, turnover of the medial rumen, and ruminal clearance (Kristensen, 2005).

The corrected fractional absorption rates of the ventral ruminal pool (**CFAR**) of acetate, propionate, butyrate, and isobutyrate were calculated as $\text{CFAR VFA (\%/h)} = \text{corrected flux VFA/ventral pool VFA}$. The turnover rates of the medial pools for acetate, propionate, butyrate, and isobutyrate were calculated as $\text{medial pool turnover VFA (\%/h)} = \text{corrected flux VFA/medial pool VFA}$. Medial to ventral ruminal clearances and ventral ruminal to blood clearances of acetate, propionate, butyrate, and isobutyrate were calculated as $\text{medial clearance VFA (L/h)} = \text{corrected flux VFA/medial VFA concentration}$ and $\text{ventral clearance VFA (L/h)} = \text{corrected flux VFA/ventral VFA concentration}$, respectively. The flow of ruminal fluid through the medial mat was calculated as $\text{ruminal fluid flow (L/h)} = \text{corrected flux isobutyrate}/(\text{medial isobutyrate concentration} - \text{ventral isobutyrate concentration})$.

Data on the medial ruminal mat to ventral ruminal fluid difference variables were statistically analyzed by the T-TEST procedure; all other data were analyzed using the MIXED procedure of SAS (SAS Institute, 2003) with cow $\times$ treatment as random factor. Data

Table 2. Feed intake, digestibility, and milk yield variables in dairy cows fed 2 levels of TMR DM and 2 levels of forage particle size

Item	Short		Long		SEM (n = 4)	P-value ¹		
	Dry	Moist	Dry	Moist		FPS	FDM	FPS × FDM
DMI, kg/d	20.9	21.2	21.7	20.3	0.2	0.97	0.02	<0.01
DMI/DM fed	1.00	1.00	0.98	0.99	0.01	0.13	0.83	0.85
Digestibility, ² % of DM								
OM	66.5	66.1	66.2	69.3	1.0	0.20	0.22	0.12
NDF	40.6	40.4	40.9	49.0	2.9	0.17	0.22	0.16
CP	63.1	64.8	61.5	63.1	0.9	0.11	0.12	0.94
Yield								
Milk, kg/d	30.6	29.8	31.4	29.1	0.9	0.95	0.11	0.41
ECM, ³ kg/d	29.8	30.4	32.2	27.8	0.8	0.88	0.04	0.01
Fat, g/100 g of milk	4.4	4.4	4.7	4.4	0.2	0.63	0.65	0.53
Protein, g/100 g of milk	3.0	3.1	3.0	2.9	0.2	0.58	0.89	0.58
Lactose, g/100 g of milk	4.9	4.9	4.9	4.8	0.1	0.56	0.20	0.25

¹FPS = forage particle size; FDM = feed DM.

²Total tract digestibility.

³Calculated according to Sjaunja et al. (1991).

on milk composition, ECM, chewing activities, BW, and digestibility were analyzed using a model including fixed effects of FPS, FDM, and period, including FPS and FDM interactions with compound symmetry covariate structure. Data on DMI and milk yield were analyzed using data from the last 6 d before the collection day. The model included fixed effects of FPS, FDM, time (days in period), and period, including FPS, FDM, and time interactions. Days in period were considered as a repeated measure with autoregressive covariate structure because of the homogenous distribution of data points. Data on ruminal and blood variables were analyzed using a model including fixed effects of FPS, FDM, time (sampling time), and period, including FPS, FDM, and time interactions. Sampling time was considered as a repeated measure. For ruminal and blood variables other than ruminal pool variables, an autoregressive covariate structure was used because of the homogenous distribution of data points. For ruminal pool variables, the spatial Gaussian covariate structure was used because the distance between data points were heterogeneous. Significance and tendency were considered at $P < 0.05$ and $0.05 \leq P < 0.10$, respectively. Data are presented as least squares means $\pm$ residual standard error of the mean except for overall means, which is presented as mean $\pm$ standard error of the mean.

RESULTS

Feed Composition, Intake, and Milk Yield

The dietary treatments were visually very different and DM differed 9.5 percentage units from moist to dry rations (44.3 to 53.8% DM, respectively; Table 1). Small amounts of sugar beet pulp pellets were found

in treatments. They presumably originated from residues in the feed mixer from the prior feed mix and affected, among other things, the DM adjustment. The difference in nutrient composition between treatments (Table 1) because of the DM adjustment corresponds to 0.4, 2.1, 3.5, and 4.4% (SD/mean, $n = 4$) for OM, NDF, CP, and fat, respectively. Dry matter intake was intended to be restricted to 20 kg/d. However, mixing inaccuracies and sugar beet pulp residues in the TMR resulted in excess feeding of 0.85, 1.16, 1.69, and 0.32 ± 0.18 kg of DM/d for short dry, short moist, long dry, and long moist treatments, respectively (Table 2). Dry matter intake relative to DM fed was close to unity and was not affected by dietary treatments, nor were milk yield variables relative to DMI affected. The excess feed intake did however increase the ECM yield ($P = 0.01$). The total tract digestibility of OM and NDF were not affected ($P = 0.11$ to 0.22) by dietary treatments.

Chewing Time Variables

The overall chewing time (min/d, min/kg of DM, and min/kg of NDF) was affected by FPS ($P = 0.02$). Cows chewed 151 ± 55 min/d longer with long particle rations compared with short particle rations (Table 3). This was primarily an effect of rumination time (min/d, min/kg of DM, and min/kg of NDF; $P < 0.01$), which increased by 135 ± 39 min/d (374 to 508 ± 20 min/d). Eating time was on average 220 ± 13 min/d and was not affected by FPS ($P = 0.60$). The FDM did not affect ($P = 0.45$ to 0.90) any chewing, rumination, or eating time related attributes.

Ruminal Variables

The ruminal variables FPRC, pH, L-lactate, NH_3 , all measured VFA, and medial glucose were affected by

Table 3. Chewing activity variables in dairy cows fed 2 levels of TMR DM and 2 levels of forage particle size

Item	Short		Long		SEM (n = 4)	<i>P</i> -value ¹		
	Dry	Moist	Dry	Moist		FPS	FDM	FPS × FDM
Eating time								
Min/d	207	216	246	209	29	0.60	0.64	0.45
Min/kg of DM	10.1	10.2	11.5	11.1	1.5	0.49	0.90	0.82
Min/kg of NDF	28.5	29.7	34.1	31.1	4.4	0.45	0.84	0.65
Rumination time								
Min/d	393	355	499	518	28	<0.01	0.74	0.35
Min/kg of DM	19.0	16.8	23.5	26.8	1.6	<0.01	0.75	0.11
Min/kg of NDF	54.0	48.6	69.5	75.9	4.5	<0.01	0.91	0.23
Chewing time ²								
Min/d	600	571	745	727	55	0.02	0.67	0.93
Min/kg of DM	29.1	27.0	35.0	37.7	3.0	0.02	0.92	0.44
Min/kg of NDF	83	78	104	107	9	0.02	0.96	0.67

¹FPS = forage particle size; FDM = feed DM.²Chewing = eating + rumination.

time ($P < 0.01$; Table 4). The overall effect of time except for ruminal pH was a postprandial increase with zenith 1.5 h after feeding followed by a decline to near prefeeding levels. Medial pH decreased from 6.6 to 5.6 ± 0.05 from 0.5 h before to 4.5 h after feeding (Figure 1). Ventral pH decreased from 6.9 to 6.5 ± 0.06 from 0.5 h before to 3.5 h after feeding. Ventral pH was affected by a FDM × time interaction ($P = 0.01$) reflecting a greater pH with moist diets, from 2.5 to 4.5 after feeding (data not shown).

The medial mat concentration of glucose, L-lactate, NH₃, total VFA, and the molar proportion of propionate, butyrate, valerate, and caproate were not affected by treatments ($P = 0.18$ to 0.96). The molar proportion of acetate in the medial mat increased ($P = 0.02$) and the molar proportion of isobutyrate tended ($P = 0.09$) to increase with the moist diets. The molar proportion of medial isovalerate increased ($P = 0.05$) with long particles.

The glucose concentration in rumen fluid collected from the ventral sac tended ($P = 0.06$) to increase after feeding. The ventral concentrations of glucose, total VFA, and the molar proportion of propionate were not affected by treatments ($P = 0.60$ to 0.74). The molar proportion of ventral isobutyrate was greater ($P = 0.01$) with moist diets compared with dry diets. The ventral molar proportion of isovalerate tended to increase ($P = 0.07$) with long diets. The ventral concentrations of L-lactate and NH₃ were affected by an FDM × time interaction ($P = 0.02$ and 0.04, respectively), being greatest at 0.5 h after feeding with moist rations.

Intraruminal Differences

A relatively large intraruminal difference was observed between the medial mat and the free fluid of the ventral rumen for all ruminal concentrations and

pH ($P < 0.01$) except for glucose and NH₃ (Table 4). The intraruminal differences of these variables were all affected by time ($P < 0.01$), with an increase after feeding. The pH of the mat fluid was on average 0.66 ± 0.03 pH units lower than the pH of the ventral fluid. The mat fluid concentrations were greater than the ventral fluids concentrations for total VFA, acetate, propionate, butyrate, valerate, isovalerate, caproate, and isobutyrate, the differences being 44.5 ± 2.3 , 27.3 ± 1.4 , 8.8 ± 0.5 , 6.2 ± 0.4 , 0.9 ± 0.1 , 0.6 ± 0.04 , 0.4 ± 0.04 , and 0.3 ± 0.02 mmol/L, respectively. Ruminal L-lactate was the only variable with the greatest concentration difference in the ventral fluid. The intraruminal differences for L-lactate and butyrate were affected by FDM × time interaction ($P = 0.04$), reflecting a greater concentration difference for L-lactate and lower concentration difference of butyrate 0.5 h after feeding moist diets.

Ruminal Pools of Contents and VFA

The FDM did not affect ($P = 0.39$ to 0.91) any of the measured ruminal content pools (Table 5). The medial mat weight increased ($P < 0.01$; 10.5 ± 2.3 kg) 21% with long particles. The FPS had no effect on the pool ($P = 0.48$) of free ruminal fluid. The total ruminal pools of fluid, DM, and total content increased ($P = 0.01$, 0.02, and 0.01, respectively) with long forage diets (6.9 ± 1.9 , 1.7 ± 0.5 , and 8.6 ± 2.2 kg, respectively). All ruminal content pools increased ($P < 0.01$ to 0.02) after feeding. The ruminal DM to fluid ratio increased ($P = 0.04$) from 0.16 ± 0.004 before feeding to 0.17 ± 0.004 after feeding.

The medial pool of total VFA, acetate, propionate, butyrate, isobutyrate, and isovalerate increased ($P < 0.01$ to 0.02) with long diets ($1,143 \pm 333$, 720 ± 205 , 228 ± 69 , 140 ± 51 , 8.0 ± 2.3 , and 25 ± 6 mmol,

Table 4. Ruminal variables in dairy cows fed 2 levels of TMR DM and 2 levels of forage particle size

Item	Short		Long		SEM (n = 4)	<i>P</i> -value ¹				
	Dry	Moist	Dry	Moist		FPS	FDM	FPS × FDM	Time	<i>t</i> -test
FPRC, ² min ⁻¹	1.5	1.5	1.5	1.4	0.1	0.34	0.50	0.52	<0.01	
Medial rumen ³										
pH	5.9	6.0	6.0	6.0	0.1	0.42	0.33	0.10	<0.01	
Glucose, mmol/L	0.065	0.064	0.088	0.074	0.023	0.50	0.74	0.79	<0.01	
L-Lactate, mmol/L	0.30	0.32	0.28	0.39	0.06	0.68	0.31	0.46	<0.01	
NH ₃ , mmol/L	10.2	10.7	9.4	8.8	1.1	0.24	0.96	0.62	<0.01	
Total VFA, mmol/L	144	137	139	138	5	0.68	0.38	0.48	<0.01	
Acetate, mol/100 mol	62.7	63.8	62.8	63.8	0.4	0.91	0.02	0.89	<0.01	
Propionate, mol/100 mol	18.9	18.7	19.0	18.6	0.2	0.88	0.22	0.62	<0.01	
Butyrate, mol/100 mol	14.2	13.1	13.5	13.3	0.4	0.61	0.18	0.29	<0.01	
Isobutyrate, mol/100 mol	0.64	0.66	0.65	0.68	0.01	0.33	0.09	0.88	<0.01	
Valerate, mol/100 mol	1.7	1.7	1.8	1.6	0.1	0.65	0.20	0.11	<0.01	
Isovalerate, mol/100 mol	1.1	1.2	1.4	1.3	0.1	0.05	0.73	0.26	<0.01	
Caproate, mol/100 mol	0.85	0.86	0.96	0.72	0.08	0.85	0.18	0.14	<0.01	
Ventral rumen ⁴										
pH*	6.6	6.7	6.6	6.6	0.1	0.66	0.58	0.71	<0.01	
Glucose, mmol/L	0.062	0.217	0.331	0.077	0.142	0.66	0.74	0.19	0.06	
L-Lactate,* mmol/L	0.72	0.83	0.85	0.63	0.30	0.91	0.86	0.61	<0.01	
NH ₃ ,* mmol/L	10.5	9.8	8.6	9.0	1.5	0.40	0.91	0.72	<0.01	
Total VFA, mmol/L	95	96	94	94	3	0.62	0.73	0.95	<0.01	
Acetate,** mol/100 mol	63.6	64.1	63.7	64.3	0.5	0.82	0.34	0.90	<0.01	
Propionate, mol/100 mol	18.4	18.4	18.7	18.4	0.4	0.60	0.74	0.62	<0.01	
Butyrate,** mol/100 mol	13.9	13.2	13.2	13.1	0.5	0.37	0.40	0.51	<0.01	
Isobutyrate, mol/100 mol	0.64	0.69	0.64	0.72	0.02	0.56	0.01	0.51	<0.01	
Valerate,** mol/100 mol	1.5	1.5	1.5	1.5	0.1	0.94	0.71	0.26	<0.01	
Isovalerate, mol/100 mol	1.1	1.2	1.4	1.4	0.1	0.07	0.58	0.51	<0.01	
Caproate,** mol/100 mol	0.81	0.85	0.90	0.72	0.09	0.83	0.48	0.25	<0.01	
Medial to ventral difference ⁵										
pH	-0.73	-0.67	-0.59	-0.65	0.09	0.42	0.99	0.57	<0.01	<0.01
Glucose, mmol/L	0.004	-0.15	-0.24	-0.003	0.14	0.73	0.77	0.18	0.13	0.06
L-Lactate,* mmol/L	-0.42	-0.51	-0.57	-0.24	0.29	0.84	0.69	0.50	<0.01	<0.01
NH ₃ , mmol/L	-0.30	0.89	0.77	-0.21	0.79	0.99	0.88	0.18	0.16	0.47
Total VFA, mmol/L	49.6	40.0	45.1	43.3	7.0	0.94	0.44	0.59	<0.01	<0.01
Acetate, mmol/L	29.9	25.1	27.1	27.1	4.3	0.93	0.59	0.59	<0.01	<0.01
Propionate, mmol/L	9.8	7.8	9.1	8.3	1.3	0.95	0.32	0.65	<0.01	<0.01
Butyrate,* mmol/L	7.4	5.1	6.3	5.9	1.1	0.90	0.23	0.39	<0.01	<0.01
Isobutyrate, mmol/L	0.32	0.24	0.30	0.27	0.05	0.95	0.31	0.68	<0.01	<0.01
Valerate, mmol/L	1.1	0.9	1.0	0.8	0.2	0.72	0.24	0.90	<0.01	<0.01
Isovalerate, mmol/L	0.60	0.46	0.73	0.54	0.11	0.38	0.17	0.81	<0.01	<0.01
Caproate, mmol/L	0.50	0.41	0.53	0.33	0.11	0.79	0.20	0.64	<0.01	<0.01

¹FPS = forage particle size; FDM = feed DM; time = sample time (n = 8); *t*-test of difference. *t*-Test was used to determine whether the medial to ventral concentration difference of VFA was different from zero.

²FPRC = frequency of primary reticulorumen contractions.

³Medial ruminal mat-associated fluid grab sampled 10 to 15 cm below the mat surface.

⁴Ventral ruminal free fluid sampled from the ventral rumen sac by suction strainer.

⁵Difference = medial rumen – ventral rumen.

*FDM × time, *P* < 0.05; **FPS × FDM × time, *P* < 0.05.

respectively). The medial pool of valerate tended (*P* = 0.08) to increase with long particles. The medial pool of caproate and all ventral pools of VFA were unaffected (*P* = 0.18 to 0.80) by FPS. However, all ruminal pools of VFA increased (*P* < 0.01) after feeding. As for the pools of contents, no effect was observed of FDM on any of the VFA pools.

Arterial Variables

Arterial pH and concentrations of oxygen, pAH, acetate, propionate, butyrate, valerate, isovalerate,

glucose, L-lactate, and BHBA were not affected (*P* = 0.12 to 0.96) by treatments. Numerically small effects of treatments were observed for arterial concentrations of isobutyrate and caproate (Table 6).

The hematocrit was affected by FPS × time interaction (*P* = 0.02), pointing to a marginally higher hematocrit 0.5 h after feeding with short particles. All measured arterial variables except isovalerate and caproate were affected by time (*P* < 0.01 to 0.04). The overall effects of time were a postprandial increase followed by a decline to near prefeeding levels except for arterial oxygen, where the pattern was opposite.

Figure 1. Ruminal pH as a function of time relative to feeding: medial pH, short (Δ) and long ($\square$); ventral pH, short ($\blacktriangle$) and long ($\blacksquare$). The arrow indicates the time of feeding. Each data point is the mean of 8 observations $\pm$ SE.

Blood Flow

Portal and hepatic vein blood plasma flows increased ($P = 0.04$ to 0.01) after feeding ($1,185$ to $1,496 \pm 70$ and $1,366$ to $1,694 \pm 66$ L/h for portal and hepatic plasma flows, respectively; Table 7). The hepatic arterial blood plasma flow was not affected ($P = 0.25$ to 0.95) by treatments or time.

Net Portal Fluxes

Net portal blood flux of oxygen, carbon dioxide, and net portal plasma flux of glucose, L-lactate, and BHBA were not affected by treatment or time ($P = 0.12$ to 0.99 ; Table 7). The net uptake of oxygen in the PDV was on average $1,606 \pm 43$ mmol/h. The corresponding net release of carbon dioxide from the PDV was $1,941 \pm 278$ mmol/h. There was PDV release of glucose from 0.5 to 1.5 h after feeding and net uptake was observed outside this time period.

The net portal blood fluxes of all VFA increased ($P < 0.01$) after feeding. The net portal blood flux of total VFA was on average $3,104 \pm 88$ mmol/h and increased from $2,438$ to $3,802 \pm 252$ mmol/h from 0.5 h before to 3.5 h after feeding. There were no effects of treatments on net portal fluxes of VFA ($P = 0.14$ to 0.99).

Net Hepatic Fluxes

Net hepatic blood flux of carbon dioxide, acetate, and the net hepatic plasma flux of glucose were not affected by treatments or time ($P = 0.23$ to 0.99 ; Table 7). The net hepatic blood fluxes of oxygen, propionate, butyrate, isobutyrate, valerate, isovalerate, caproate, and net hepatic plasma fluxes of L-lactate and BHBA increased after feeding ($P < 0.01$ to 0.02). The net hepatic flux of glucose was on average 645 ± 19 mmol/h and was not affected by time ($P = 0.31$). The net hepatic uptake of L-lactate, propionate, isobutyrate, and valerate relative to the net hepatic production of glucose was 25.4 ± 2.2 , 52.3 ± 2.5 , 1.8 ± 0.07 , and $0.18 \pm 0.06\%$, respectively, leaving on a net basis $23.5 \pm 2.4\%$ of glucogenic precursors unaccounted for.

Medial Ruminal Turnover and Fractional Absorption Rates of VFA

The turnover of the medial ruminal isobutyrate pool calculated from the net portal flux of isobutyrate and the medial ruminal isobutyrate pool increased 11.2 ± 3.6 percentage units/h ($P = 0.01$) with decreasing total ruminal pool (Table 8). The same trends were observed for medial turnover of acetate and butyrate

Table 5. Pools of ruminal contents and VFA in dairy cows fed 2 levels of TMR DM and 2 levels of forage particle size

Item	Short		Long		SEM (n = 4)	P-value ¹			
	Dry	Moist	Dry	Moist		FPS	FDM	FPS × FDM	Time
Ruminal content pools, ² kg									
WPM ³	49.7	50.8	60.9	60.8	2.5	<0.01	0.84	0.81	<0.01
Free fluid ⁴	16.3	18.0	14.8	15.7	2.0	0.30	0.48	0.81	0.02
Fluid ⁵	56.8	59.6	64.8	65.4	2.5	0.01	0.39	0.57	<0.01
DM	9.3	9.2	10.9	11.0	0.5	0.02	0.91	0.87	<0.01
Total content	66.0	68.8	75.6	76.4	2.9	<0.01	0.45	0.66	<0.01
Medial VFA pools, ⁶ mmol									
Total VFA	5,499	5,481	6,646	6,620	333	<0.01	0.95	0.99	<0.01
Acetate	3,460	3,510	4,172	4,238	205	<0.01	0.79	0.97	<0.01
Propionate	1,039	1,024	1,285	1,232	69	<0.01	0.64	0.79	<0.01
Butyrate	771	716	893	873	51	0.02	0.48	0.74	<0.01
Isobutyrate	37	37	44	46	2	<0.01	0.67	0.66	<0.01
Valerate	87	86	104	99	7	0.08	0.72	0.74	<0.01
Isovalerate	64	66	93	87	6	<0.01	0.69	0.48	<0.01
Caproate	43	43	55	46	5	0.18	0.44	0.41	<0.01
Ventral VFA pools, ⁶ mmol									
Total VFA	1,495	1,657	1,386	1,506	244	0.60	0.58	0.93	<0.01
Acetate	963	1,069	891	976	154	0.60	0.55	0.95	<0.01
Propionate	278	305	260	280	47	0.66	0.64	0.94	<0.01
Butyrate	199	218	180	190	35	0.51	0.69	0.90	<0.01
Isobutyrate	9.3	11.4	8.8	10.2	1.7	0.62	0.34	0.84	<0.01
Valerate	18.6	21.9	17.8	19.8	3.1	0.64	0.42	0.84	<0.01
Isovalerate	16.5	20.0	18.4	19.6	2.8	0.80	0.43	0.70	<0.01
Caproate	9.8	11.5	10.0	10.1	1.5	0.71	0.56	0.59	<0.01

¹FPS = forage particle size; FDM = feed DM; time = sample time.

²Rumen evacuations pre- and postfeeding (n = 2).

³Rumen wet particulate matter (WPM), ruminal mat material plus associated fluid.

⁴Rumen free fluid = total ruminal content – WPM.

⁵Rumen fluid = total ruminal content – ruminal DM.

⁶Average rumen pool size (mmol) pre- and postfeeding.

($P = 0.07$). An FDM × time interaction affected the values of propionate and isobutyrate ($P = 0.01$ and 0.02 , respectively), both higher before feeding with dry diets. The FDM did not affect ($P = 0.29$ to 0.53) the medial ruminal pool turnover.

Even though the turnover rate of the medial pool of isobutyrate increased with short particles, no effects of treatments were observed for the CFAR from the ventral pool of isobutyrate, acetate, propionate, or butyrate. Nor were there observed time effects for acetate, propionate, butyrate, and isobutyrate corrected ventral rumen fluid fractional absorption rates.

Clearance of Ruminal VFA and Intraruminal Fluid Flow

The intraruminal flow of fluid through the medial mat was 108 ± 23 L/h and not affected by dietary treatments or time ($P = 0.34$ and 0.86 , respectively; data not shown). The clearance of ruminal acetate from the medial to ventral ruminal pool was on average 32.8 ± 1.1 L/h and was not affected by dietary treatments or time (Table 8). The medial to ventral pool clearance

of propionate, butyrate, and isobutyrate was on average 28.8 ± 1.0 , 30.2 ± 1.3 , and 24.7 ± 0.8 L/h, respectively. Medial butyrate clearance increased ($P < 0.01$) from 25.6 ± 2.4 to 36.4 ± 2.4 L/h after feeding. The medial clearance of propionate and isobutyrate were affected by an FDM × time interaction ($P = 0.02$ and 0.04 , respectively), reflecting greater clearance with dry diets before feeding.

Ventral pool to blood clearances of acetate, propionate, butyrate, and isobutyrate were not affected ($P = 0.23$ to 0.93) by treatments and were on average 46.6 ± 2.1 , 42.9 ± 2.3 , 45.2 ± 2.4 , and 36.8 ± 1.7 L/h, respectively. The ventral pool clearance of butyrate increased ($P < 0.01$) from 32.9 ± 4.3 to 57.5 ± 4.3 L/h after feeding.

DISCUSSION

Feed Intake, Digestibility, and Milk Variables

Increasing the forage particle size of the feed can induce sorting behavior in dairy cows fed TMR based on grass silage (Leonardi and Armentano, 2003) or corn si-

Table 6. Arterial blood and plasma variables in dairy cows fed 2 levels of TMR DM and 2 levels of forage particle size

Item	Short		Long		SEM (n = 4)	<i>P</i> -value ¹			
	Dry	Moist	Dry	Moist		FPS	FDM	FPS × FDM	Time
Whole blood									
pH	7.44	7.44	7.44	7.43	0.01	0.61	0.92	0.77	<0.01
Hematocrit,* %	25.0	25.0	25.0	24.7	0.8	0.87	0.89	0.88	<0.01
Oxygen, mmol/L	4.76	4.70	4.76	4.72	0.14	0.96	0.74	0.96	0.01
Carbon dioxide, mmol/L	25.6	26.5	26.0	25.6	1.1	0.80	0.85	0.52	<0.01
p-Aminohippuric acid, mmol/L	0.08	0.08	0.08	0.09	0.01	0.72	0.76	0.88	0.01
Acetate, mmol/L	1.77	1.72	1.65	1.61	0.12	0.33	0.72	0.97	<0.01
Propionate, mmol/L	0.063	0.068	0.067	0.071	0.005	0.45	0.30	0.89	<0.01
Butyrate, mmol/L	0.040	0.048	0.052	0.049	0.004	0.12	0.45	0.19	<0.01
Isobutyrate, mmol/L	0.003	0.004	0.004	0.006	0.0007	0.06	0.04	0.52	<0.01
Valerate, mmol/L	0.004	0.008	0.010	0.005	0.002	0.47	0.77	0.09	<0.01
Isovalerate, mmol/L	0.003	0.004	0.005	0.008	0.003	0.23	0.43	0.83	0.20
Caproate,** mmol/L	0.001	0.001	0.002	0.003	0.0007	0.10	0.37	0.43	0.31
Blood plasma, mmol/L									
Glucose	3.77	3.72	3.63	3.52	0.18	0.35	0.66	0.87	0.04
L-Lactate	0.54	0.45	0.39	0.52	0.09	0.66	0.83	0.27	<0.01
BHBA	1.18	1.20	1.01	0.92	0.26	0.40	0.90	0.82	<0.01

¹FPS = forage particle size; FDM = feed DM; time = sample time (n = 8).

*FPS × time, *P* < 0.05; **FDM × time, *P* < 0.05.

lage (Beauchemin and Yang, 2005). Sorting was avoided in the present study by offering the diets restrictively.

The isonutritional value of the offered diets was compromised by the DM adjustments and the sugar beet pulp pellet residues in the TMR. This resulted in excess feeding affecting the ECM yield. However, the inaccuracy of the experimental setup affected only the comparison between dry and moist diets, not the comparison between short and long particle diets where cows were fed 21.0 ± 0.1 kg/d with both treatments.

Extensive feed particle size reduction is believed to decrease the rumen mean retention time of particulate matter as well as fiber digestibility. On the other hand, Kononoff and Heinrichs (2003) observed an increased rate of ruminal fermentation from increasing the specific particle surface area by reducing alfalfa haylage particle size from 22.3 to 4.8 mm TLC. These 2 parameters are in theory inversely proportional when observing the digestibility of the TMR. In favor of this theory, decreasing dietary corn silage particle size did not affect the digestibility of OM and tended to decrease the NDF digestibility in dairy cows (Yang and Beauchemin, 2005). The same was observed with decreasing alfalfa hay particle size in a corn silage-based TMR (Yansari et al., 2004), indicating that the increase in rate of particle passage did not exceed the increase in fermentation rate. This is in line with the lack of treatment effect in the present study for the total tract digestibility of OM and NDF. The ruminal fiber digestion can be negatively affected by low ruminal pH. Cellulolytic activity in vitro has been shown to be reduced at pH values below 6 and impaired at pH values below

5.5 (Hoover, 1986; Le Liboux and Peyraud, 1999). Most of the fibrous material in the rumen is situated in the medial ruminal mat and not in the ventral rumen, most commonly sampled. It is remarkable that the major fiber pool in the rumen of the cows were bathed in fluid with pH that stayed below 6 for large parts of the sampling window even though the cows would be considered to have a healthy rumen environment based on the ventral ruminal fluid. However, the literature on medial ruminal environment and its effect on fiber degradation is sparse and precludes strong conclusions on the importance of medial pH on ruminal fermentation.

Chewing Time and Ruminal pH

In agreement with previous studies, the cows responded to increased forage particle size by increasing the overall chewing time (Beauchemin et al., 1997; Krause et al., 2002). The effect was solely on rumination time because the eating time was unaffected by treatments in accordance with Beauchemin et al. (1994a).

Chewing time has been proposed as a practical measure to ensure sufficient effective fiber for lactating dairy cows to sustain rumen health and avoid milk fat depression. The correlation of low ruminal pH and milk fat depression is strong, but other factors, such as unsaturated fatty acids, contribute to the problem. Sudweeks et al. (1981) proposed a minimum chewing time of 31 min/kg of DM to ensure a healthy ruminal environment and milk fat percentage of 3.5%. However, Woodford and Murphy (1988b) reported that a chewing time of 28.2 min/kg of DM was sufficient to main-

Table 7. Blood plasma flow in the portal vein, hepatic vein, and hepatic artery and net portal flux and net hepatic flux of oxygen, carbon dioxide, and metabolites in dairy cows fed 2 levels of TMR DM and 2 levels of forage particle size

Item	Short		Long		SEM (n = 4)	P-value ¹			
	Dry	Moist	Dry	Moist		FPS	FDM	FPS × FDM	Time
Blood plasma flow, L/h									
Portal	1,433	1,262	1,322	1,335	50	0.71	0.15	0.10	0.04
Hepatic vein	1,499	1,488	1,475	1,482	69	0.83	0.98	0.90	0.01
Hepatic artery	66	226	153	147	63	0.95	0.25	0.22	0.71
Net portal blood flux, mmol/h									
Oxygen	-1,774	-1,545	-1,646	-1,451	131	0.40	0.14	0.87	0.32
Carbon dioxide	2,760	1,821	1,651	1,521	460	0.32	0.26	0.42	0.79
Acetate	2,274	2,019	2,143	2,153	210	0.99	0.57	0.54	<0.01
Propionate	709	630	675	650	66	0.91	0.45	0.70	<0.01
Butyrate	208	197	213	198	24	0.92	0.60	0.92	<0.01
Isobutyrate	23.4	22.2	22.4	23.3	1.7	0.99	0.91	0.54	<0.01
Valerate	13.8	11.7	12.3	12.6	1.1	0.82	0.44	0.31	<0.01
Isovalerate	41.4	36.0	37.9	33.9	4.3	0.53	0.30	0.86	<0.01
Carproate	46.1	36.1	37.2	32.5	6.0	0.32	0.24	0.66	<0.01
Net portal plasma flux, mmol/h									
Glucose	-34.9	-16.1	-11.9	-46.7	15.9	0.82	0.63	0.12	0.83
L-Lactate	169	142	145	128	17	0.29	0.22	0.76	0.40
BHBA	209	178	162	209	22	0.73	0.72	0.12	0.26
Net hepatic blood flux, mmol/h									
Oxygen	-1,757	-1,948	-1,751	-1,726	155	0.48	0.69	0.45	<0.01
Carbon dioxide	1,317	1,585	1,387	1,150	687	0.90	0.96	0.71	0.23
Acetate	8	294	182	122	99	0.99	0.28	0.11	0.53
Propionate	-656	-574	-621	-599	62	0.94	0.43	0.64	<0.01
Butyrate	-166	-150	-166	-156	21	0.87	0.55	0.90	<0.01
Isobutyrate	-21.8	-20.1	-20.8	-21.6	1.5	0.90	0.75	0.42	<0.01
Valerate	-11.0	-7.7	-8.4	-9.1	1.4	0.63	0.36	0.17	<0.01
Isovalerate	-39.0	-34.4	-37.0	-32.6	4.0	0.63	0.26	0.96	<0.01
Carproate	-46.0	-36.7	-37.4	-32.9	5.8	0.31	0.26	0.69	<0.01
Net hepatic plasma flux, mmol/h									
Glucose	701	630	620	631	52	0.46	0.57	0.45	0.31
L-Lactate	-340	-297	-247	-275	49	0.27	0.89	0.49	0.01
BHBA	273	298	290	234	44	0.60	0.76	0.38	0.02

¹FPS = forage particle size; FDM = feed DM; time = sample time (n = 8).

tain 3.1% of milk fat. Zebeli et al. (2006) evaluated recent studies and found that cows required 36.5, 30.0, and 23.3 min/kg of DM of chewing time to maintain 3.6, 3.4, and 3.2% milk fat, respectively. The cows in the present study chewed 28 min/kg of DM on short particle rations and produced milk containing $4.42 \pm 0.12\%$ milk fat with no effect of FPS or FDM. Thus, the study brings no evidence to the simple correlation between chewing time and milk fat depression and concurs with the statement of Krause et al. (2002) that milk fat depression is not a simple effect of chewing time. It could be speculated that chewing time will correlate with the ability of the diet to sustain the ruminal mat and stratification.

Chewing time has been considered one of the most important factors controlling ruminal environment in high producing dairy cows because of the proposed correlation to saliva secretion and the buffering capacity with the HCO_3^- -rich saliva (Bailey and Balch, 1961). However, the correlation between chewing time and ruminal pH when feeding TMR to dairy cows is not

clear. Most studies feeding isonutritional rations differing in particle size show no effect of changes in chewing time on ruminal pH (Woodford and Murphy, 1988b; Beauchemin and Yang, 2005; Yang and Beauchemin, 2006; Zebeli et al., 2008). Further, the correlation between saliva secretion and chewing time has been difficult to prove because studies have shown a compensatory effect of decreased chewing time enhancing the saliva secretion in the resting periods. This indicates that the saliva secretion reaches the same overall level (L/d) regardless of the forage to concentrate ratio (Maekawa et al., 2002) or type of forage (Cassida and Stokes, 1986).

The DM content of the feed has also been proposed to induce saliva secretion by increasing the need of feed lubrication to facilitate the swallowing process (Carter and Grovum, 1990). Beauchemin et al. (2008) showed, however, that the level of lubrication was related more to the need of particle reduction than to DM because concentrate boli held 40 to 50% DM and forage boli held 10 to 20% DM. The lubrication of feed in the

Table 8. Medial pool turnover, corrected¹ fractional absorption rate, and corrected¹ ruminal clearance of VFA in dairy cows fed 2 levels of TMR DM and 2 levels of forage particle size

Item	Short		Long		SEM (n = 4)	<i>P</i> -value ²			
	Dry	Moist	Dry	Moist		FPS	FDM	FPS × FDM	Time
Medial pool turnover, ³ %/h									
Acetate	85.9	73.7	67.5	64.3	6.9	0.07	0.29	0.53	0.28
Propionate*	72.5	66.5	60.1	57.1	6.7	0.14	0.52	0.83	0.26
Butyrate	72.8	71.2	63.6	57.8	5.6	0.07	0.53	0.72	0.06
Isobutyrate*	63.0	58.6	50.3	48.8	3.8	0.01	0.47	0.72	0.63
CFAR ventral ruminal pool, ⁴ %/h									
Acetate	373	261	333	310	58	0.94	0.27	0.46	0.13
Propionate	360	245	309	274	70	0.88	0.31	0.58	0.22
Butyrate	333	251	332	287	39	0.58	0.17	0.74	0.35
Isobutyrate	293	205	268	239	42	0.94	0.20	0.50	0.85
Medial to ventral clearance, ⁵ L/h									
Acetate	35.0	31.1	33.6	31.6	3.1	0.89	0.37	0.76	0.34
Propionate*	29.6	28.0	29.7	28.0	2.8	0.98	0.57	0.99	0.62
Butyrate	29.7	30.2	32.4	28.5	3.2	0.88	0.61	0.51	<0.01
Isobutyrate*	25.6	24.8	24.9	24.0	1.8	0.66	0.70	0.95	0.33
Ventral to blood clearance, ⁶ L/h									
Acetate	52.7	43.6	46.1	44.2	5.3	0.58	0.32	0.51	0.76
Propionate	50.0	40.1	42.0	39.4	7.0	0.55	0.39	0.62	0.84
Butyrate	47.9	42.9	47.4	42.6	4.5	0.93	0.31	0.99	<0.01
Isobutyrate	42.5	34.1	35.8	34.2	4.2	0.40	0.23	0.41	0.02

¹Corrected portal flux of acetate, propionate, butyrate, and isobutyrate (Kristensen and Harmon, 2004a,b).

²FPS = forage particle size; FDM = feed DM; time = sample time (n = 4).

³Medial pool turnover = corrected VFA flux/medial ruminal VFA pool.

⁴Corrected fractional absorption rate (CFAR) = corrected VFA flux/ventral ruminal VFA pool.

⁵Medial clearance = corrected VFA flux/medial ruminal concentration of VFA.

⁶Ventral clearance = corrected VFA flux/ventral ruminal concentration of VFA.

*FDM × time, *P* < 0.05.

present study was not measured, but there was no effect of FDM on eating time, indirectly indicating that the TMR DM did not affect the saliva secretion during eating.

Even though chewing time increased with long particles, this had no effect on ruminal pH, nor did FDM affect ruminal pH. Thus, the present study does not provide evidence for the saliva secretion hypothesis, nor do our observations point toward any direct relationship between chewing time and ruminal environment.

Ruminal Fermentation

The heterogeneous environment of the rumen is commonly divided in a particulate mat medially and free ruminal fluid ventrally, with substantial intraruminal differences in VFA concentrations (Lane et al., 1968). Nevertheless, many studies use ventral ruminal samples to describe the overall ruminal concentration of various metabolites and pH. Fewer studies use multisite sampling to estimate the average ruminal concentrations (Beauchemin, 1991; Beauchemin et al., 1994b) and even fewer present values from the different sampling sites (Smith et al., 1956; Zebeli et al., 2008). The observed relative increase in ruminal concentrations of acetate, propionate, and butyrate from medial mat to ventral

ruminal fluid was 29, 32, and 31%, in accordance with Smith et al. (1956) but larger than reported by Zebeli et al. (2008). The ruminal production of VFA is largest in the medial mat compared with the free fluid of the ventral rumen, also confirmed by Tafaj et al. (2004). The VFA produced in the mat flows to the rest of the rumen associated with the fluid and are continuously removed from the reticulorumen through epithelia absorption and liquid passage to distal compartments (Peters et al., 1990). Thus, lower ventral VFA concentrations can be explained through 2 processes: 1) ruminal fluid dilution with drinking water and saliva or 2) VFA removal (absorption and passage). However, fluid dilution with drinking water and saliva seems less likely because the mat to ventral concentration difference was relatively constant throughout the collection period. Absorption and passage of VFA from the ventral free fluid seems a more likely explanation. From an overall point of view, VFA in the medial mat has to pass 2 barriers to be absorbed: 1) the transport from the medial mat to the ventral fluid and 2) the absorption from the ventral fluid to the blood. It seems that the ruminal mat acts as a barrier retaining the fluid, consequently decreasing the intraruminal VFA equilibration. The chemical composition of the mat material affects the mat to ventral differences because the pH and VFA concentration

difference increased after feeding but decreased to near prefeeding levels at last sample. Zebeli et al. (2008) studied concentrate to forage ratio combined with different forages particle sizes. These authors observed a significant increase in ruminal acetate and butyrate because of a higher concentrate level. This resulted in a numerical increase in the intraruminal difference of acetate and butyrate with no effect of forage particle size. Tafaj et al. (2004) observed the difference to be related to the level of forage NDF in the ration rather than the forage itself, showing the same VFA concentration in the top layer of the mat and lower in the ventral rumen when feeding low levels of forage NDF. Therefore, a faster medial ruminal VFA equilibration can be hypothesized if the ruminal stratification diminishes from feeding low fiber diets resulting in more homogenous ruminal content with a high level of easily fermentable feed components. This would increase the intraruminal fluid flow, consequently increasing ventral VFA concentrations to near mat level, and thereby increase the risk of ruminal acidosis, as has been observed when high concentrate, low fiber diets are fed to both dairy cows and growing bulls (T.C. Jarltoft, A. C. Storm, M. Vestergaard, M. Larsen, and N. B. Kristensen, Faculty of Agricultural Sciences, Aarhus University, Tjele, Denmark; unpublished data). The previous reports of the effect of forage NDF level on ruminal pH might relate more to the mat ability to retain VFA than to the NDF effect on chewing time and buffer capacity (Tafaj et al., 2004).

Pools of Ruminal Contents and VFA

The ruminal mat is believed to consist of large feed particles suspended in ruminal fluid. The buoyancy is caused by entrapped fermentation gas within the void space of forage particles. As the feed particles are reduced in size, mediated by mastication and microbial fiber degradation, the capability to entrap gas diminishes. The specific gravity of the feed particles thereby increases, resulting in a release of small particles from the mat for further passages out of the reticulorumen and thereby effectively retaining large particles in the rumen for further breakdown (Sutherland, 1988; Allen, 1996).

The observed increase in the total pool of ruminal content and fluid with increasing particles size was in agreement with Yang et al. (2002). However, in other studies no effects on the total pool of ruminal content, fluid, and DM were observed with decreasing forage particle size (Woodford and Murphy, 1988a; Kononoff and Heinrichs, 2003) or forage to concentrate ratio (Maekawa et al., 2002; Penner et al., 2009). The variation between studies may reflect the extent of forage

particle size reduction because the present study and the study by Yang et al. (2002) used finer particles than Woodford and Murphy (1988a) and Kononoff and Heinrichs (2003).

The thickness of the ruminal mat was not directly measured, but the size of the mat was estimated from the WPM. With a significant increase in WPM with long particles and without an effect on the free ruminal fluid, it is clear that the increased particle size affected the amount of DM plus associated fluid and thereby increased the size of the mat. The total pool of WPM increased 21% from short to long particle rations. Zebeli et al. (2007) reported a 5.6% relative increase in the total pool of ruminal content when increasing the forage particle size from 6 to 30 mm. The relative increase was even larger (14.6%) when forage DMI was doubled at the expense of concentrate. The total pool of free ruminal fluid was not affected in that study, meaning that there was an increase in the pool of WPM. The DMI also increased the pool of WPM by 80% in an experiment with steers, when DMI was increased from 1 to 2 times maintenance (Kovács et al., 1997). This indicates that forage intake possibly affects the mat construction more than forage particle size. However, the extensive effect of particle size on the pool of ruminal content, DM, fluid, and WPM in the present study compared with other studies is probably attributable to the extensive particle size reduction from the hammer milling processing of the hay compared with simple chopping.

The heterogeneity of the ruminal contents underlines the need of a 2-compartment model when describing the total ruminal pool of VFA in the intact rumen. The total pool of ruminal VFA, acetate, propionate, isobutyrate, and isovalerate increased with long particles (12, 12, 13, 13, and 29%, respectively). This increase was solely related to the medial VFA pool increase because the ventral pool of VFA was unaffected by dietary treatments. This lack of increase in the ventral VFA pool indicates a medial accumulation of VFA.

Arterial Variables

There were no observed treatment effects on arterial variables other than isobutyrate (FDM $P = 0.04$, FPS $P = 0.06$). This increase in arterial isobutyrate because of low feed DM was consistent with the increase in ventral ruminal concentrations and molar proportions. The overall levels of arterial variables are in agreement with the control treatment in Kristensen and Raun (2007).

Absorption and Intraruminal VFA Kinetics

Previous studies on reticulorumen absorption of VFA have been restricted to the absorption across the epithe-

lia and how the chemical conditions of the ruminal fluid as VFA concentrations, osmolarity, and pH affect the rate of ruminal disappearance (Dijkstra et al., 1993) or portal appearance (Kristensen and Harmon, 2004a,b). Given that the intraruminal carbon flux through the VFA pool is larger than the net production of VFA for absorption, the net VFA production is not obtained from typical isotopic dilution studies. The relatively high time resolution of portal appearance rates of VFA combined with relatively robust recovery estimates for several of the VFA absorbed from the forestomachs (Kristensen, 2005) makes flux-based VFA production estimates the most obvious tool for modeling VFA kinetics.

Rate of reticuloruminal VFA absorption relies on the rate at which VFA are supplied from the ruminal mat to the reticulorumen epithelia. Because of the large volume of the rumen, the cow cannot rely on passive diffusion to facilitate the intraruminal equilibration. The mixing of ruminal contents through ruminal motility is therefore crucial for effective VFA absorption. Controlling the reticulorumen motility may be a route for the cow to control VFA absorption. In acute ruminal acidosis, the frequency of primary reticuloruminal contractions is reduced or even absent, reducing the mixing and passage of ruminal content to the omasum (Crichlow, 1988). Through this action, the cow may affect the rate at which ruminal VFA flows from the medial to the ventral pool and consequently downregulate the absorption of VFA from the reticulorumen. The FPRC was not affected by dietary treatments in the present study; however, because of the reduced ruminal mat together with the shorter particles, the relative movement of ruminal fluid by the contractions might increase, affecting the intraruminal equilibration of VFA. With the setup used we cannot measure the

amplitude of the contractions and therefore cannot account for any frequency $\times$ amplitude (work) effect. However, the flow from the medial to the ventral pool (108 ± 23 L/h) of ruminal fluid was not affected by the size of the medial pool. Thus, intraruminal fluid flow and thereby intraruminal VFA flow seems not to be dependent on the size of the ruminal mat.

The overall values of net portal flux of VFA were in agreement with previous studies using dairy cows receiving corn silage-based rations with the same DMI as in the present study (Kristensen and Raun, 2007). Changing the physical characteristic of the ruminal mat was expected to affect the rate of fermentation and intraruminal turnover, consequently affecting the net portal flux of VFA; however, this was not observed. The best estimate of corrected absorption was evaluated to be isobutyrate because of the limited PDV metabolism and high portal blood recovery of ruminal infused isobutyrate (Kristensen and Harmon, 2004b). Isobutyrate was therefore evaluated to be the best VFA marker in relation to intraruminal turnover and corrected flux using the ruminal VFA model (Figure 2). The model is constructed under the assumption that VFA is primarily absorbed from the ventral ruminal pool and that VFA equilibrates from the medial mat to the ventral pool with the same rate that VFA is absorbed. The experimental design enables us to measure the real time dynamic of VFA between ruminal pools and the VFA exchange between ventral ruminal pool and portal blood. The ruminal CFAR links the corrected flux of specific metabolites to the ruminal pool of interest, thereby describing the pool turnover rate. The CFAR of the total pool of VFA has previously been studied in vivo under intact (Krehbiel et al., 1995; Resende Júnior et al., 2006; Penner et al., 2009) and

Figure 2. Model of intraruminal VFA kinetics. The straight arrows show the routes of VFA transport into, between, and out of the 2 ruminal pools. The curved arrows illustrate the flow of ruminal fluid between the 2 ruminal pools.

washed (Dijkstra et al., 1993; Kristensen and Harmon, 2004a,b) rumen conditions. The present study used ventral ruminal pool and corrected flux to estimate the CFAR and can therefore not directly be related to these studies. No effect ($P = 17$ to 0.94) was revealed when calculating the CFAR of acetate, propionate, butyrate, and isobutyrate (319 ± 21 , 297 ± 22 , 298 ± 18 , and $251 \pm 14\%/h$, respectively) because the ventral ruminal pool and corrected flux of these metabolites were not affected by dietary treatments. However, the effect of FPS on the medial pool of isobutyrate was clear when calculating the intraruminal turnover. The turnover of isobutyrate increased ($P = 0.01$) as the medial pool decreased with short particles; the same trend was observed for acetate and butyrate ($P = 0.07$). This shows that the effect of dietary particle size is related to the turnover of the medial pool and that the size of the mat affects the retention of isobutyrate. It can be hypothesized that this effect is the same for other VFA because the estimated fluid flow through the mat was not affected.

Medial to ventral ruminal clearance and ventral rumen to portal blood clearance of acetate, propionate, butyrate, and isobutyrate were not affected by dietary treatments because no effects were detected on the ruminal concentrations or corrected flux of these metabolites. The clearance describes the VFA dynamic of the rumen in relation to the mixing of ruminal contents and exposure of the VFA to the epithelia for absorption. The medial ruminal clearance of isobutyrate indicates that 24.7 ± 0.8 L/h of medial ruminal fluid was cleared of this metabolite whereas 36.8 ± 1.7 L/h of ventral ruminal fluid was cleared. In relation to the ruminal pool sizes, this indicates the incomplete equilibration of the mat with the ventral ruminal fluid.

CONCLUSIONS

Total mixed ration DM between 45 and 55% did not affect chewing time, ruminal function, or net or corrected portal flux of VFA in lactating dairy cows. Forage particle size reduction decreased the rumination time and the size of the ruminal mat but did not affect milk fat concentration, ventral and mat fluid concentrations of VFA, or ruminal pH. Forage particle size reduction affected the ruminal mat construction by decreasing the medial pool of wet particulate matter and thereby the VFA accumulation in the mat and affected the intraruminal equilibration by increasing the medial turnover of isobutyrate.

ACKNOWLEDGMENTS

We gratefully acknowledge Birgit H. Løth, Anne Krustup, colleagues, and barn staff for their skillful

technical assistance (Faculty of Agricultural Sciences, Aarhus University, Tjele, Denmark). Funding for the present study was provided by the Directorate for Food, Fisheries and Agri Business (#3304-VMP-05-005; Copenhagen, Denmark), the Danish Cattle Federation (#Stivelsesprojektet; Aarhus, Denmark), and the Faculty of Agricultural Sciences, Aarhus University.

REFERENCES

- Allen, M. S. 1996. Physical constraints on voluntary intake of forages by ruminants. *J. Anim. Sci.* 74:3063–3075.
- Bailey, C. B., and C. C. Balch. 1961. Saliva secretion and its relation to feeding in cattle. *Br. J. Nutr.* 15:383–402.
- Beauchemin, K. A. 1991. Effects of dietary neutral detergent fiber concentration and alfalfa hay quality on chewing, rumen function, and milk production of dairy cows. *J. Dairy Sci.* 74:3140–3151.
- Beauchemin, K. A., L. Eriksen, P. Norgaard, and L. M. Rode. 2008. Short communication: Salivary secretion during meals in lactating dairy cattle. *J. Dairy Sci.* 91:2077–2081.
- Beauchemin, K. A., B. I. Farr, L. M. Rode, and G. B. Schaalje. 1994a. Effects of alfalfa silage chop length and supplementary long hay on chewing and milk production of dairy cows. *J. Dairy Sci.* 77:1326–1339.
- Beauchemin, K. A., B. I. Farr, L. M. Rode, and G. B. Schaalje. 1994b. Optimal neutral detergent fiber concentration of barley-based diets for lactating dairy cows. *J. Dairy Sci.* 77:1013–1029.
- Beauchemin, K. A., L. M. Rode, and M. V. Eliason. 1997. Chewing activities and milk production of dairy cows fed alfalfa as hay, silage, or dried cubes of hay or silage. *J. Dairy Sci.* 80:324–333.
- Beauchemin, K. A., and W. Z. Yang. 2005. Effects of physically effective fiber on intake, chewing activity, and ruminal acidosis for dairy cows fed diets based on corn silage. *J. Dairy Sci.* 88:2117–2129.
- Carter, R. R., and W. L. Grovum. 1990. A review of the physiological significance of hypertonic body fluids on feed intake and ruminal function: Salivation, motility and microbes. *J. Anim. Sci.* 68:2811–2832.
- Cassida, K. A., and M. R. Stokes. 1986. Eating and resting salivation in early lactation dairy cows. *J. Dairy Sci.* 69:1282–1292.
- Crichlow, E. C. 1988. Ruminal lactic-acidosis-forestomach epithelial receptor activation by undissociated volatile fatty acids and rumen fluids collected during loss of reticulorumen motility. *Res. Vet. Sci.* 45:364–368.
- Dijkstra, J., H. Boer, J. Vanbruchem, M. Bruining, and S. Tamminga. 1993. Absorption of volatile fatty acids from the rumen of lactating dairy cows as influenced by volatile fatty acid concentration, pH and rumen liquid volume. *Br. J. Nutr.* 69:385–396.
- Hansen, B. 1989. Determination of nitrogen as elementary N, an alternative to Kjeldahl. *Acta Agric. Scand.* 39:113–118.
- Hoover, W. H. 1986. Chemical factors involved in ruminal fiber digestion. *J. Dairy Sci.* 69:2755–2766.
- Hosseinkhani, A., R. Valizadeh, A. Naserian, M. D. Mesgharan, and F. E. Shahroodi. 2007. Effect of alfalfa hay particle size and water addition to barely base diets on dairy cows performance in early lactation. *J. Anim. Vet. Adv.* 6:1482–1488.
- Huntington, G. B., C. K. Reynolds, and B. H. Stroud. 1989. Techniques for measuring blood flow in splanchnic tissues of cattle. *J. Dairy Sci.* 72:1583–1595.
- Kononoff, P. J., and A. J. Heinrichs. 2003. The effect of reducing alfalfa haylage particle size on cows in early lactation. *J. Dairy Sci.* 86:1445–1457.
- Kovács, P. L., K.-H. Sudekum, and M. Stangassinger. 1997. Rumen contents and ruminal and faecal particle size distribution in steers fed a mixed diet at three amounts of intake. *Anim. Feed Sci. Technol.* 64:143–154.
- Krause, K. M., D. K. Combs, and K. A. Beauchemin. 2002. Effects of forage particle size and grain fermentability in midlactation cows.

- II. Ruminant pH and chewing activity. *J. Dairy Sci.* 85:1947–1957.
- Krehbiel, C. R., K. R. A. Britton, D. L. Harmon, T. J. Wester, and R. A. Stock. 1995. The effects of ruminal acidosis on volatile fatty acid absorption and plasma activities of pancreatic enzymes in lambs. *J. Anim. Sci.* 73:3111–3121.
- Kristensen, N. B. 2000. Quantification of whole blood short-chain fatty acids by gas chromatographic determination of plasma 2-chloroethyl derivatives and correction for dilution space in erythrocytes. *Acta Agric. Scand. A* 50:231–236.
- Kristensen, N. B. 2005. Splanchnic metabolism of volatile fatty acids in the dairy cow. *Anim. Sci.* 80:3–10.
- Kristensen, N. B., A. Danfaer, V. Tetens, and N. Agergaard. 1996. Portal recovery of intraruminally infused short-chain fatty acids in sheep. *Acta Agric. Scand. A* 46:26–38.
- Kristensen, N. B., and D. L. Harmon. 2004a. Effect of increasing ruminal butyrate absorption on splanchnic metabolism of volatile fatty acids absorbed from the washed reticulorumen of steers. *J. Anim. Sci.* 82:3549–3559.
- Kristensen, N. B., and D. L. Harmon. 2004b. Splanchnic metabolism of volatile fatty acids absorbed from the washed reticulorumen of steers. *J. Anim. Sci.* 82:2033–2042.
- Kristensen, N. B., and B. M. L. Raun. 2007. Ruminal and intermediary metabolism of propylene glycol in lactating Holstein cows. *J. Dairy Sci.* 90:4707–4717.
- Kristensen, N. B., B. A. Røjen, B. M. L. Raun, A. C. Storm, L. Puggaard, and M. Larsen. 2009. Hepatic acetylation of the blood flow marker *p*-aminohippuric acid affect measurement of hepatic blood flow in cattle. Pages 558–559 in XIth International Symposium on Ruminant Physiology, Clermont-Ferrand, France. Y. Chilliard, F. Glasser, Y. Faulconnier, F. Bocquier, I. Veissier, and M. Doreau, ed. Wageningen Academic Publishers, Wageningen, the Netherlands.
- Kristensen, N. B., A. Storm, B. M. L. Raun, B. A. Røjen, and D. L. Harmon. 2007. Metabolism of silage alcohols in lactating dairy cows. *J. Dairy Sci.* 90:1364–1377.
- Lane, G. T., C. H. Noller, V. F. Colenbrander, K. R. Cummings, and R. B. Harrington. 1968. Apparatus for obtaining ruminoreticular samples and the effect of sampling location on pH and volatile fatty acids. *J. Dairy Sci.* 51:114–116.
- Le Liboux, S., and J. L. Peyraud. 1999. 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.* 76:297–319.
- Leonardi, C., and L. E. Armentano. 2003. Effect of quantity, quality, and length of alfalfa hay on selective consumption by dairy cows. *J. Dairy Sci.* 86:557–564.
- Maekawa, M., K. A. Beauchemin, and D. A. Christensen. 2002. Effect of concentrate level and feeding management on chewing activities, saliva production, and ruminal pH of lactating dairy cows. *J. Dairy Sci.* 85:1165–1175.
- Mertens, D. R. 1997. Creating a system for meeting the fiber requirements of dairy cows. *J. Dairy Sci.* 80:1463–1481.
- Mertens, D. R. 2002. Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing in beakers or crucibles: Collaborative study. *J. AOAC Int.* 85:1217–1240.
- Penner, G. B., M. Taniguchi, L. L. Guan, K. A. Beauchemin, and M. Oba. 2009. Effect of dietary forage to concentrate ratio on volatile fatty acid absorption and the expression of genes related to volatile fatty acid absorption and metabolism in ruminal tissue. *J. Dairy Sci.* 92:2767–2781.
- Peters, J. P., R. Y. W. Shen, J. A. Robinson, and S. T. Chester. 1990. Disappearance and passage of propionic acid from the rumen of the beef steer. *J. Anim. Sci.* 68:3337–3349.
- Resende Júnior, J. C., M. N. Pereira, H. Boer, and S. Tamminga. 2006. Comparison of techniques to determine the clearance of ruminal volatile fatty acids. *J. Dairy Sci.* 89:3096–3106.
- Rutter, S. M. 2000. Graze: A program to analyze recordings of the jaw movements of ruminants. *Behav. Res. Methods Instrum. Comput.* 32:86–92.
- Rutter, S. M., R. A. Champion, and P. D. Penning. 1997. An automatic system to record foraging behaviour in free-ranging ruminants. *Appl. Anim. Behav. Sci.* 54:185–195.
- SAS Institute. 2003. The SAS System for Windows. Version 9.1. SAS Institute, Cary, NC.
- Schürch, A. F., L. E. Lloyd, and E. W. Crampton. 1950. The use of chromic oxide as an index for determining the digestibility of a diet. *J. Nutr.* 41:629–636.
- Shaver, R. D., A. J. Nyles, L. D. Satter, and N. A. Jorgensen. 1988. Influence of feed intake, forage physical form, and forage fiber content on particle size of masticated forage, ruminal digesta, and feces of dairy cows. *J. Dairy Sci.* 71:1566–1572.
- Sjaunja, L. O., L. Bævre, L. Junkkarinen, J. Pedersen, and J. Setälä. 1991. A Nordic proposal for an energy corrected milk (ECM) formula. Pages 156–157 in Proc. 27th Session of International Committee for Recording Productivity of Milk Animals (ICRPMA). Eur. Assoc. Anim. Prod. Publ. No. 50. Eur. Assoc. Anim. Prod., Wageningen, the Netherlands.
- Smith, P. H., H. C. Sweeney, J. R. Rooney, K. W. King, and W. E. C. Moore. 1956. Stratifications and kinetic changes in the ingesta of the bovine rumen. *J. Dairy Sci.* 39:598–609.
- Stoldt, W. 1952. Vorschlag zur vereinheitlichung der fettbestimmung in lebensmitteln. *Fette Seifen* 54:206–207.
- Sudweeks, E. M., L. O. Ely, D. R. Mertens, and L. R. Sisk. 1981. Assessing minimum amounts and form of roughages in ruminant diets: Roughage value index system. *J. Anim. Sci.* 53:1406–1411.
- Sutherland, T. M. 1988. Particle separation in the forestomachs of sheep. Pages 43–73 in *Aspects of Digestive Physiology in Ruminants*. A. Dobson and M. J. Dobson, ed. Cornell University Press, Ithaca, NY.
- Tafaj, M., B. Junck, A. Maulbetsch, H. Steingass, H. P. Piepho, and W. Drochner. 2004. Digesta characteristics of dorsal, middle and ventral rumen of cows fed with different hay qualities and concentrate levels. *Arch. Anim. Nutr.* 58:325–342.
- Woodford, S. T., and M. R. Murphy. 1988a. Dietary alteration of particle breakdown and passage from the rumen in lactating dairy cattle. *J. Dairy Sci.* 71:687–696.
- Woodford, S. T., and M. R. Murphy. 1988b. Effect of forage physical form on chewing activity, dry matter intake, and rumen function of dairy cows in early lactation. *J. Dairy Sci.* 71:674–686.
- Yang, W. Z., and K. A. Beauchemin. 2005. Effects of physically effective fiber on digestion and milk production by dairy cows fed diets based on corn silage. *J. Dairy Sci.* 88:1090–1098.
- Yang, W. Z., and K. A. Beauchemin. 2006. Effects of physically effective fiber on chewing activity and ruminal pH of dairy cows fed diets based on barley silage. *J. Dairy Sci.* 89:217–228.
- Yang, W. Z., K. A. Beauchemin, and L. M. Rode. 2002. Effects of particle size of alfalfa-based dairy cow diets on site and extent of digestion. *J. Dairy Sci.* 85:1958–1968.
- Teimouri Yansari, A., R. Valizadeh, A. Naserian, D. A. Christensen, P. Yu, and F. E. Shahroodi. 2004. Effects of alfalfa particle size and specific gravity on chewing activity, digestibility, and performance of Holstein dairy cows. *J. Dairy Sci.* 87:3912–3924.
- Zebeli, Q., M. Tafaj, H. Steingass, B. Metzler, and W. Drochner. 2006. Effects of physically effective fiber on digestive processes and milk fat content in early lactating dairy cows fed total mixed rations. *J. Dairy Sci.* 89:651–668.
- Zebeli, Q., M. Tafaj, I. Weber, J. Dijkstra, H. Steingass, and W. Drochner. 2007. Effects of varying dietary forage particle size in two concentrate levels on chewing activity, ruminal mat characteristics, and passage in dairy cows. *J. Dairy Sci.* 90:1929–1942.
- Zebeli, Q., M. Tafaj, I. Weber, H. Steingass, and W. Drochner. 2008. Effects of dietary forage particle size and concentrate level on fermentation profile, in vitro degradation characteristics and concentration of liquid- or solid-associated bacterial mass in the rumen of dairy cows. *Anim. Feed Sci. Technol.* 140:307–325.