Evaluation of the Response of Ruminal Fermentation and Activities of Nonstarch Polysaccharide-Degrading Enzymes to Particle Length of Corn Silage in Dairy Cows¹

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ABSTRACT

The main objective of this study was to evaluate effects of particle length (PL) of corn silage (CS) on distribution of dietary particle fractions, contents of physically effective neutral detergent fiber (peNDF), cows' intake patterns and sorting activity, fermentation profile, and activities of nonstarch polysaccharide-degrading enzymes as well as degradation in the rumen and total tract in lactating dairy cows. Four ruminally cannulated Holstein cows, weighing 624 ± 50 kg and 60 ± 8 d in milk, were fed ad libitum 3 total mixed rations [about 16% crude protein, 34% neutral detergent fiber (NDF), and 7 MJ of net energy of lactation/kg of dry matter (DM)] containing on DM basis 50% concentrate, 10% grass hay, and 40% CS with 3 different theoretical PL at harvesting (14, 8.1, and 5.5 mm for long, medium, and short, respectively). Results showed that the amount of DM retained on sieves with 19- and 8-mm screens of Penn State Particle Separator decreased linearly with decreasing PL of CS. The latter was reflected in a significant decrease in the content of dietary peNDF including both the DM (peNDF_{>8}) and the NDF (peNDF_{>8-NDF}) retained on 19- and 8-mm screens. In contrast, the fraction of particles retained between the 1.18- and 8-mm screens was increased, such that no differences among the diets were observed regarding the content of peNDF that includes DM of particles >1.18 mm (peNDF_{>1.18}). The intake of particles retained between the 1.18- and 8-mm screens increased linearly, whereas the intake of peNDF_{>1.18} increased quadratically with decreasing PL of CS. Sorting consumption was reduced by feeding the short CS, which was reflected in a reduced proportion of propionate and increased acetate-to-propionate ratio and butyrate pro-

portion in the rumen. In contrast, no effects of PL of CS were observed on the concentration of total volatile fatty acids and pH in the rumen. In general, decreasing the PL of CS significantly increased the activities of nonstarch polysaccharide-degrading enzymes. However, greater ruminal and total tract degradation of fiber and nonfiber carbohydrates were observed only by medium CS. Results of the present study suggest that in addition to fractions of long particles (i.e., >8 mm), the particle fraction retained between 1.18- and 8-mm should also be considered to better predict rumen conditions and digestion. In conclusion, a moderate reduction of PL of CS has beneficial effects on nutrient digestion, and may maximize feed efficiency and energy supply in high-yielding dairy cows.

Key words: particle length, enzyme activity, rumen fermentation, dairy cow

INTRODUCTION

Corn silage (CS) is an ideal forage source for dairy cows because of its high starch and energy concentration, high-yielding and high-ensiling properties, and relatively high content in potentially digestible fiber (Johnson et al., 2002). Because of the positive effects of dietary particle length (PL) on digesta stratification and chewing activity (Yang and Beauchemin, 2006a; Zebeli et al., 2007a), an adequately chopped CS may be advantageous in maintaining proper rumen conditions and reducing the risk of ruminal disorders. However, a moderate decrease of PL increases the available surface area for attachment of ruminal bacteria (Van Soest, 1994), and this may promote ruminal digestion, having positive effects on feed utilization efficiency in dairy cows (Yang and Beauchemin, 2006b). In addition, decreasing forage PL improves the uniformity of TMR resulting in less sorting consumption of the feed (Kononoff et al., 2003a), which may be beneficial in terms of reducing the risk of ruminal disorders (Krause and Oetzel, 2006). Thus, optimization of forage PL is an important aspect in dairy cattle nutrition, particularly in TMR feeding.

Received October 30, 2007.

Accepted February 15, 2008.

¹The results of this study were presented in part at the Joint Annual Meeting of ADSA, AMPA, PSA, ASAS held in San Antonio, Texas, July 8–12, 2007.

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Although the effects of PL of CS on digestive processes in high-yielding dairy cows have been extensively investigated (e.g., Bal et al., 2000; Yang and Beauchemin, 2005, 2006a), results obtained from these studies have not been conclusive. For example, some studies found that PL of CS had no effect on chewing activity (Kononoff et al., 2003a) or ruminal pH (Bal et al., 2000; Beauchemin and Yang, 2005; Yang and Beauchemin, 2006a), and other reports suggested that decreasing PL of CS can compromise chewing activity (Beauchemin and Yang, 2005; Yang and Beauchemin, 2006a) or fiber digestion (Bal et al., 2000; Yang and Beauchemin, 2005). However, most of the studies conducted to investigate effects of PL of CS on digestive parameters in dairy cows used CS as a single forage source with a high concentrate level (>45%), and hence the content of dietary fiber in these studies was maintained at a relatively low level (23 to 32% NDF on DM basis). In a recent meta-analysis, Tafaj et al. (2007) concluded that digestive effects of PL of CS, contrary to effects of PL of grass silage, should be considered in relationship with dietary NDF and particularly forage NDF proportion in the diet. We hypothesized that decreasing PL of CS may still exert positive effects on fermentation and fibrolytic activity in the rumen of high-producing dairy cows fed diets that contain also a small portion of fiber-rich hay (i.e., 33 to 35% dietary NDF on DM basis), regardless of a high dietary concentrate level (50% on DM basis).

The main objective of this study was to evaluate effects of PL of CS on feed intake patterns, sorting consumption, fermentation profile, and activities of non-starch polysaccharide (NSP)-degrading enzymes in the rumen of lactating Holstein cows. Another objective was to examine if certain measures of particle fractions, determined by Penn State Particle Separator (PSPS), are useful in predicting the effect that PL of CS had on digestive parameters measured.

MATERIALS AND METHODS

Corn Silage

Whole-plant corn (variety Benicia SM FAO 280, Pioneer Hi-Bred, Buxtehude, Germany) was harvested at the late-dough stage of kernel maturity at about 37% DM from a single field using a self-propelled forage harvester (Model 675, CLAAS Jaguar, Harsewinkel, Germany) set to obtain forage with 3 different PL; namely 14, 8.1, and 5.5 mm for long, medium, and short PL, respectively (Table 1). A kernel processor was set at a 2-mm roll clearance. The harvested forage (13 t of fresh forage per variant) was immediately ensiled in silo-bags (Ø 2.5 m, Ag-Bag, Budissa Agroservice GmbH, Malschwitz, Germany) using a silage bag presser

Table 1. Chemical composition, particle size distribution, and fermentation parameters of corn silage differing in theoretical particle length (PL) at harvesting

Parameter	PL of corn silage		
	Long	Medium	Short
Chemical composition, % of DM unless stated			
DM, %	42.0	40.6	42.2
OM	95.5	95.4	95.7
CP	7.7	7.6	7.8
NDF	53.1	51.0	50.0
ADF	27.4	27.1	26.5
NFC ¹	32.2	34.3	35.8
NE _L , ² MJ/kg of DM	6.26	6.26	6.33
DM- <i>k_d</i> , ³ %/h	8.2	7.8	8.1
DM retained on sieves, ⁴ % of total			
19 mm	4.9	3.1	1.5
8 mm	59.3	48.9	29.7
1.18 mm	31.5	43.0	61.9
Pan	4.3	5.0	6.9
Fermentation parameters			
pH	4.0	4.0	4.1
Lactic acid, % of DM	4.0	3.7	4.7
Acetic acid, % of DM	0.8	0.5	1.2
Propionic acid, % of DM	<0.01	<0.01	<0.01
Butyric acid, % of DM	<0.01	<0.01	<0.01

¹NFC = 100 – (% NDF + % CP + % ether extract + % crude ash).

²Estimated by Hohenheim Gas test (Menke and Steingass, 1988).

³Fractional rate of 72-h gas production, used as an index for fractional DM degradation rate of corn silage (Zebeli et al., 2008a).

⁴Measured by Penn State Particle Separator (Kononoff et al., 2003b).

(Model 401, Eberhardt, Ulm, Germany) and stored for at least 4 mo before being fed.

Animals, Diets, and Experimental Design

Four multiparous, ruminally cannulated (100 mm diameter, Bar Diamond, Parma, ID), lactating Holstein cows averaging (mean ± SD) 624 ± 50 kg of BW, 60 ± 8 DIM, and producing 38 ± 3 kg milk/d at the start of the experiment were used. Cows were housed in a free-stall barn at the research station of the University of Hohenheim, Germany. The stall lying area consisted of separate boxes bedded with rubber-filled cow mattresses, and the other free-stall floor area was covered with soft slatted rubber mats. Cows were milked twice daily at 0600 and 1500 h in the milking parlor located adjacent to the free-stall barn, and BW were recorded daily directly after morning milking. All experimental procedures were approved by the Animal Protection Committee of the Federal State Baden-Württemberg, Germany.

Cows were fed 1 of 3 diets as TMR containing 40% CS, 10% coarsely chopped grass hay (PL = 30 mm), and 50% concentrate (Table 2). The inclusion of 10% fiber-rich grass hay in TMR aimed at obtaining a “marginal” NDF content for a diet containing 50% concentrates.

Table 2. Components, chemical composition,¹ particle size distribution, and content of physically effective NDF of 3 TMR differing in theoretical particle length (PL) of corn silage

Parameter	PL of corn silage			Pooled SEM	Effects ²	
	Long	Medium	Short		Linear	Quadratic
Components, % of DM						
Corn silage, long cut	40	—	—	—	—	—
Corn silage, medium cut	—	40	—	—	—	—
Corn silage, short cut	—	—	40	—	—	—
Grass hay	10	10	10	—	—	—
Concentrate ³	50	50	50	—	—	—
Chemical composition, % of DM unless stated						
DM, %	45.8	44.5	45.9	0.17	NS	NS
OM	93.3	93.6	93.3	0.10	NS	NS
CP	15.4	15.5	14.9	0.23	NS	NS
NDF	33.9	35.2	33.9	0.77	NS	0.10
ADF	18.8	19.3	18.8	0.47	NS	NS
NFC ⁴	41.4	39.7	41.3	0.60	NS	0.06
NE _L , ⁵ MJ/kg of DM	6.94	7.03	7.08	0.16	NS	NS
DM retained on sieves, ⁶ % of total						
19 mm	3.6	2.7	1.9	0.37	<0.01	NS
8 mm	38.2	32.4	23.1	0.63	<0.01	0.04
1.18 mm	42.0	50.0	57.8	0.47	<0.01	NS
Pan	16.2	15.0	17.3	0.43	NS	0.05
Physically effective NDF, ⁷ % of DM						
peNDF _{>8}	14.0	12.3	8.5	0.38	<0.01	0.02
peNDF _{>8-NDF}	21.7	16.4	12.6	0.43	<0.01	NS
peNDF _{>1.18}	28.1	30.0	28.1	0.60	NS	NS

¹Chemical composition of concentrate and hay (% of DM) was respectively 25.9, 5.9 for CP; 15.9, 67.8 for NDF; 7.0, 40.4 for ADF; and 48.9, 18.7 for NFC.

²Linear or quadratic effect of PL; significant at $P \leq 0.05$; tendency at $0.05 < P \leq 0.10$; NS = $P > 0.10$.

³Concentrate contained (% of DM) 30% wheat, 14% corn, 15% barley, 32% soybean meal, 6% sunflower kernels, and 3% vitamin-mineral mixture (wheat, corn, and barley were ground to pass a 3-mm screen); chemical composition was corrected for feed refusals.

⁴NFC = $100 - (\% \text{ NDF} + \% \text{ CP} + \% \text{ ether extract} + \% \text{ crude ash})$.

⁵Estimated by Hohenheim gas test method (Menke and Steingass, 1988).

⁶Physically effective NDF measured as the NDF content of the diet multiplied by amount of DM retained on 8-mm and 1.18-mm sieves (Kononoff et al., 2003b) of the Penn State Particle Separator; all measurements were corrected for feed refusals.

⁷peNDF_{>8}, peNDF_{>1.18} = physically effective NDF measured as the NDF content of the diet multiplied by amount of DM retained on 8- and 1.18-mm sieves of the Penn State Particle Separator, respectively; peNDF_{>8-NDF} = NDF content retained on 8-mm screen; all measurements were corrected for feed refusals.

Diets were chemically similar, but differed only in the PL of CS (i.e., long, medium, and short). Each experimental period lasted 23 d with the first 11 d used for adaptation to the diet. To protect silage from aerobic deterioration, only 1 silo bag per 23-d period was opened, and 3 treatments were sequentially and randomly allocated to the cows in 4 experimental periods.

Diets were formulated to meet the nutrient requirements for a 650-kg cow producing 35 kg/d of milk with 3.5% fat (GfE, 2001). The TMR were prepared daily using a self-propelled and self-trailed feed mixer-wagon (model III, Seko-Sammurai, Cuartorolo, Italy) and subsequently offered to the cows at 0730 h for ad libitum intake. The TMR amounts offered to the cows were corrected after each day to allow approximately 7 to 10% feed refusals.

Particle Size Distribution and Physically Effective Fiber of Diets

Samples of TMR, CS, and feed refusals were collected on 8 occasions during each period and used for particle size distribution analysis and determination of physically effective NDF (**peNDF**) contents. The new compact, manually operated PSPS with 3 sieves and a solid bottom pan (model C24682N, Nasco, Fort Atkinson, WI) was used as described by Kononoff et al. (2003b). The material remaining on each sieve and pan was removed, weighed, and oven-dried at 60°C to determine the distribution of feed DM retained on each sieve and on the pan. The peNDF was determined either as the sum of DM proportion retained on sieves of 19- and 8-mm of PSPS multiplied by the NDF content of the diet

(**peNDF**_{>8}), or as the sum of particles retained on sieves of 19-, 8-, and 1.18-mm multiplied by the NDF content of the diet (**peNDF**_{>1.18}) multiplied by the NDF content of the diets (Kononoff et al., 2003b). For particles retained on the 19- and 8-mm sieves (i.e., >8 mm), the NDF content was also determined, and this proportion of **peNDF** contained in particles >8 mm was defined as **peNDF**_{>8-NDF}.

Feed Intake and Sorting Index

Feed intake was recorded for each cow after the adaptation period for 6 d of each period. The TMR was offered in intake control feeders (WestfaliaSurge GmbH, Bönen, Germany). Each feeder was equipped with an access gate that was programmed to allow a specific cow to access a feed bin, and 2 infrared sensors that recorded the presence of the cow in the feeder. A computer connected to the feeder recorded time at the start and end of each visit of the cows continuously over 24 h. Start and stop times were recorded to the nearest 1 s and bin weights to the nearest 0.05 kg of feed. Therefore, the time spent eating was calculated based on the time the cows were present in the feeders and at least 0.05 kg of feed was consumed. The feed refusals were collected daily at 0700 h.

To evaluate the selective feed consumption for particular fractions of particles retained on the screens of PSPS, a sorting index was calculated based on the differences in particle size distribution between the offered TMR and feed refusals as described by Silveira et al. (2007). A sorting index of 1 indicated no sorting, whereas a sorting index of <1 indicated sorting against, and >1 indicated sorting in favor of particles retained on a particular screen of PSPS.

Chemical Analyses and In Vitro Degradation Kinetics

Samples of CS were taken on 4 occasions from each silo, isolated in plastic bags, immediately frozen at -20°C, and analyzed for fermentation parameters using the method of von Lengerken and Zimmermann (1991). The degradation characteristics of CS were determined based on cumulative in vitro gas production (incubation time of 72 h) using the Hohenheim Gas method (Menke and Steingass, 1988). Rumen fluid for incubation was collected from 2 cows 1 h before morning feeding. Degradation kinetics were defined by fitting the exponential equation: $p = a + b(1 - e^{-ct})$, where p is gas production at time t , $a + b$ is the potential gas production, and c is the fractional rate of gas production used as an index for fractional DM degradation rate (Zebeli et al., 2008a).

Samples of CS, TMR, and orts were collected daily during each collection period and used for DM determi-

nation, and 4 composite samples were stored for proximate analysis. Dried, composite samples were ground to pass a 1-mm screen using a lab mill (SM 100, Retsch, Haan, Germany). Dry matter content and the chemical composition of feeds and orts were determined according to official analytical methods of the Union of German Agricultural Research Stations (VDLUFA; DM: section 3.1; OM: section 8.1; ether extract: section 5.1.1; CP: section 4.1.1; Naumann and Basler, 1997). The methods described by Van Soest et al. (1991) were used in analyses of NDF and ADF including the use of amylase and sodium sulfite in the NDF procedure.

Parameters of Ruminal Fermentation

Fermentation profile was investigated in 2 ruminal phases (i.e., particle-associated rumen liquid, **PARL**; and free rumen liquid, **FRL**) to test separate responses to PL within the rumen. A sample of approximately 250 mL of FRL was collected from the ventral rumen sac using a vacuum pump (VT 4.2, Becker, Wuppertal, Germany) and filtered through 4 layers of cheesecloth, as used in the Hohenheim Gas technique (Menke and Steingass, 1988). Collection of **PARL** was as described previously (Zebeli et al., 2008a). The sampling of digesta was carried out at 0700, 1000, 1500, and 1800 h on d 18 of each experimental period.

The pH value was measured immediately after sampling using a pH electrode (InLab 412, Mettler-Toledo, Greifensee, Switzerland). Approximately 100 mL of fluid was centrifuged at $2,010 \times g$ for 20 min. Two replicates (5 mL each) of supernatant were frozen at -30°C for determination of VFA concentrations as described by Zebeli et al. (2008a).

Enzyme Activities

The activities of selected NSP-degrading enzymes were measured in supernatants obtained from **PARL** or **FRL** using an agar diffusion assay. Digesta samples were collected on d 21 of each period at 2 sampling times (i.e., 0700 and 1100 h). Sampling of digesta was the same as described above, and the procedure for obtaining the supernatants from **PARL** and **FRL** for agar diffusion assay was described by Ölschläger (2007). Briefly, 4 g of homogenate of digesta was mixed with 4 mL of protease inhibitor (Sigma-Aldrich, Schnelldorf, Germany) and centrifuged (Megafuge 2.0, Heraeus, Hanau, Germany) for 10 min at $1,800 \times g$ at 4°C. The supernatants were collected and recentrifuged (Biofuge fresco, Heraeus) for 20 min in $13,000 \times g$ at 4°C. The obtained supernatants were collected using a 10-mL sterile syringe (Becton Dickinson, Franklin Lakes, NJ) and sterile-filtered through a cellulose-acetate-fil-

ter (FP 030/3, 0.2 μm , Rotrand, Schleicher and Schuell, Dassel-Rellinhausen, Germany).

To determine the activities of NSP-degrading enzymes, digesta supernatants were assayed in specific agar substrates including 1.3–1.4- β -glucan (medium viscosity barley), carboxymethyl-cellulose (CMC)-4M, galactan (potato), arabinoxylan (medium viscosity wheat), polygalacturonic acid (orange), and 1.4- β -mannan (medium viscosity guar). With exception of polygalacturonic acid that was purchased from Sigma-Aldrich (Schnelldorf, Germany), all other specific substrates were purchased from Megazyme International Ltd. (Wicklow, Ireland). The resulting enzymatic degradation of specific agar substrates was used as an indication of the activities of NSP-degrading enzymes, respectively, of 1.3–1.4- β -glucanase, carboxymethylcellulase (CMCase), galactanase, xylanase, polygalacturonase, and mannanase.

The agar diffusion assay used in this study to measure the enzyme activities was the same as described by Vahjen et al. (1997) with minor modifications. Briefly, after dissolving each substrate in 20 mM Bis/Tris at pH 6.3 (Sigma-Aldrich), the solution was boiled for 10 min in a microwave oven (600 W). Subsequently, Gelrite (Roth, Karlsruhe, Germany) was added as solidifier and boiled again until all particles were dissolved. After this, the hot solution was cooled to 60°C and uniformly poured into 25- × 25-cm polycarbonate Petri dishes (Nunc, Wiesbaden-Schierstein, Germany) and allowed to solidify at room temperature to obtain the specific agar substrates. The measurement of enzymatic degradation of substrates in agar plates in form of lysis zones was also described previously (Vahjen et al., 1997). The procedure for determination of enzymatic degradation of substrates in agar plates and quantification of lysis zones through a computer-assisted evaluation system (Raytest, Straubenhardt, Germany) are described in details by Vahjen et al. (1997).

In Sacco and Total-Tract Apparent Digestibility

To investigate the effect of PL of CS on DM and NDF degradation in the rumen, *in situ* bags made of Dacron polyester cloth (5 × 10 cm) with pore size of $52 \pm 5 \mu\text{m}$ (mean $\pm$ SD) were used. In each period, 3 replicates of TMR (~5 g of sample each) identical to that fed to the animals (i.e., not dried or ground samples) were weighed into bags and soaked in warm water for 10 min to simulate the addition of saliva before insertion into the rumen for 24 h. Bags were dried at 60°C for 48 h and ruminal DM and NDF degradability were measured by difference.

Apparent digestibility of nutrients in the total digestive tract was determined using TiO_2 as an external

marker (40 g per animal per day was mixed with 500 g of concentrate and fed for 10 d twice daily in 2 equal meals, shortly after milking). Fecal samples (~200 g, wet weight) were collected during the last 7 d of marker feeding, every 8 h from rectum, and were pooled and stored at 4°C. After mixing, subsamples were dried at 65°C and ground in a Retsch mill to pass a 1-mm screen and stored for chemical analysis. Apparent nutrient digestibility in the total tract was calculated as described by Zebeli et al. (2007a).

Statistical Analyses

All data were analyzed using PROC MIXED of SAS (SAS Institute, 2003). The model included the random cow effect and the fixed effect of PL. In addition, period was considered as a random effect, and lactation day of the cows was used as a covariate in the analysis of covariance (Mielenz et al., 2006) to account for the error term related to differences among periods.

Feed intake and eating patterns were analyzed by considering the measurements in 6 d and 24 h, respectively, as longitudinal repeated measures using a first-order autoregressive covariance structure, which assumes that the covariance of measurements at the same animal i decays with time t [$\text{cov}(e_{it}, e_{it'}) = \sigma^2 \rho^{|t-t'|}$, where $0 \leq \rho \leq 1$; Littell et al., 1998]. For the analysis of ruminal fermentation and enzyme activities, the measurements of the same animal at different sampling sites and times were considered as time repeated measures and were analyzed using a compound symmetry and unstructured covariance structure (Littell et al., 1998). Data of particle size distribution of diets and orts were averaged 4 times per period and analyzed by considering the fixed effect of PL and the random effect of replication within treatment. To test a linear or quadratic effect of PL on response parameters, the orthogonal contrasts were performed using the CONTRAST statement of SAS (SAS Institute, 2003). The LSM and the respective pooled SEM were computed. The degrees-of-freedom were adjusted with the Satterthwaite method. Significance was declared at $P \leq 0.05$ and a tendency was considered if $0.05 < P \leq 0.10$.

RESULTS AND DISCUSSION

The inclusion of 10% fiber-rich, coarsely chopped grass hay in TMR might have modified the influence of PL of CS on distribution of particle size fractions and the contents of peNDF in the diet as well as on the digestive parameters measured. Although the level and PL of hay was maintained at a constant level in the diet throughout the study, interactions between hay and PL of CS on different proportions of particles re-

tained on the 3 sieves of PSPS and the contents of peNDF cannot be completely excluded. Therefore, effects of PL of CS obtained in this study can be considered as relevant for current feeding conditions (i.e., TMR containing 50% concentrate and a mixture of 10% coarsely chopped hay with 40% CS differing in PL at harvesting; 14, 8.1, or 5.5 mm).

The results in Table 1 showed that CS did not differ in chemical composition, concentrations of VFA and lactate, as well as pH and in vitro degradation characteristics, excluding an effect of PL on the quality of CS.

Particle Size Distribution and Contents of Physically Effective Fiber

Proportions of particles retained on different screens of PSPS and the contents of peNDF in TMR are shown in Table 2. As expected, proportion of DM retained on 19- and 8-mm screens of PSPS linearly decreased ($P < 0.01$) as the PL of CS decreased, and this was reflected in a significant decrease in $\text{peNDF}_{>8}$ and $\text{peNDF}_{>8-\text{NDF}}$. These results are in agreement with other studies that used the new PSPS to measure particle size distribution of CS-based TMR (Kononoff et al., 2003a; Yang and Beauchemin, 2006a). Decreasing PL of CS at harvesting from 28.6 (long) to 15.9 (medium) and then to 4.8 (short) mm, Yang and Beauchemin (2006a) reported significant decreases of dietary $\text{peNDF}_{>8}$ or $\text{peNDF}_{>8-\text{NDF}}$ from 17.6 to 14.8 and then to 10% or from 22.2 to 18.8 and then to 11.9%, respectively. The lower contents of $\text{peNDF}_{>8}$ compared with $\text{peNDF}_{>8-\text{NDF}}$ in the diets can be attributed to the fact that the method of $\text{peNDF}_{>8}$ underestimates the NDF content of the fraction of particles >8 mm by assuming uniform NDF content of all particle fractions. However, given that both these methods gave similar rankings of the dietary peNDF contents, and that the measurement of $\text{peNDF}_{>8}$ is a more practical and less costly procedure than the measurement of $\text{peNDF}_{>8-\text{NDF}}$, the first method would be more applicable on the farm.

In contrast, the $\text{peNDF}_{>1.18}$ contents of the diets were not different among treatments ($P > 0.10$; Table 2). This is in agreement with Yang and Beauchemin (2006a), who also did not find any differences in the content of dietary $\text{peNDF}_{>1.18}$ (28.3, 26.6, and 26.5% for long, medium, and short, respectively). In both studies, the differences in $\text{peNDF}_{>1.18}$ content among treatments were leveled out because of a significant increase (i.e., from 42.0 to 50.0 and then to 57.8% in present study and from 33.8 to 38.6 and then to 52.8% in the study of Yang and Beauchemin, 2006a) of the fraction of particles retained between the 1.18- and 8-mm sieves with decreasing PL of CS.

Eating and Sorting Patterns

Results of feed intake and sorting index are given in Table 3, whereas data of eating patterns and time are shown in Figure 1. Cows linearly decreased sorting in favor of the particle fraction retained between 1.18- and 8-mm and fine particles (i.e., particles passing through the 1.18-mm screen; $P < 0.01$) as well as against $\text{peNDF}_{>1.18}$ content of TMR ($P = 0.04$) as PL of CS was decreased. Decreased sorting activity in favor of fine particles with decreasing PL of CS in TMR was also reported by Kononoff et al. (2003a). In contrast, sorting against long particles (i.e., >19 or 8 mm) and against the contents of $\text{peNDF}_{>8}$ or $\text{peNDF}_{>8-\text{NDF}}$ of TMR was not affected by treatment in this study (Table 3). In addition, decreasing PL of CS linearly increased DMI of particles retained between the 1.18- and 8-mm sieves, which led to a quadratic increase of $\text{peNDF}_{>1.18}$ intake ($P < 0.01$; Table 3). In contrast, intakes of DM retained on 19- and 8-mm screens of PSPS and $\text{peNDF}_{>8}$ or $\text{peNDF}_{>8-\text{NDF}}$ were significantly decreased with decreasing PL of CS.

In general, eating patterns were not different among treatments (Figure 1). However, decreasing PL of CS increased feed intake and time spent eating at 0100 h ($P < 0.05$). In contrast, cows fed medium and long PL of CS increased feed intake and eating time immediately after feeding at about 0800 h resulting in a peak of DMI and eating time ($P < 0.05$). This finding agrees with those of Beauchemin and Yang (2005), who reported similar intake patterns with decreasing PL of CS from 19.1 to 11 and then to 4.8 mm. More specifically, the researchers observed that cows fed the short variant of CS consumed more from 0100 to 0300 h, but reduced intake at the times when feed was offered (feed was offered 3 times a day in that study), demonstrating lower peaks of feed intake.

Ruminal Fermentation

Ruminal pH and VFA concentration were not affected by treatment in this study (Table 4). This result agrees with other studies (Kononoff et al., 2003a; Beauchemin and Yang, 2005; Yang and Beauchemin, 2006a), which similarly reported unchanged ruminal pH independent of dietary differences (i.e., differences in PL of CS at harvesting, fractions of particles >8 mm or contents in $\text{peNDF}_{>8}$ and $\text{peNDF}_{>8-\text{NDF}}$). However, although the contents of $\text{peNDF}_{>8}$ or $\text{peNDF}_{>8-\text{NDF}}$ in TMR were reduced in response to a decreasing PL of CS, the fraction of particles retained between the 1.18- and 8-mm screens was increased such that the difference in $\text{peNDF}_{>1.18}$ content among diets was minimized (Table 2). The greater fraction of particles retained between the 1.18- and 8-mm screens with decreasing PL of CS

Table 3. Effects of theoretical particle length (PL) of corn silage on feed intake, BW, and sorting index

Parameter	PL of corn silage			Pooled SEM	Effects ¹	
	Long	Medium	Short		Linear	Quadratic
Daily intake, kg/d						
Fresh matter	41.5	44.5	43.0	1.96	NS	NS
DM	19.0	19.7	19.7	0.98	NS	NS
NDF	6.3	6.9	6.7	0.29	NS	0.07
NFC ²	7.9	7.8	8.1	0.38	NS	NS
Particles retained on sieve ³						
19 mm	0.69	0.52	0.38	0.02	<0.01	NS
8 mm	7.29	6.36	4.52	0.27	<0.01	0.03
1.18 mm	8.00	9.80	11.30	0.49	<0.01	NS
Pan	3.05	2.95	3.40	0.18	NS	0.08
Physically effective NDF ⁴						
peNDF _{>8}	2.6	2.4	1.7	0.09	<0.01	<0.01
peNDF _{>8-NDF}	4.2	3.2	2.6	0.09	<0.01	NS
peNDF _{>1.18}	5.3	5.9	5.5	0.19	NS	0.02
BW, kg	653	660	646	19.2	NS	0.08
Sorting index ⁵						
19 mm	0.73	0.78	0.72	0.05	NS	NS
8 mm	0.95	0.96	0.95	0.01	NS	NS
1.18 mm	1.05	1.03	1.02	0.01	<0.01	NS
Pan	1.07	1.05	1.03	0.01	<0.01	NS
peNDF _{>8}	0.89	0.92	0.91	0.02	NS	NS
peNDF _{>8-NDF}	0.93	0.93	0.92	0.01	NS	NS
peNDF _{>1.18}	0.94	0.97	0.98	0.01	0.04	NS

¹Linear or quadratic effect of PL; significant at $P \leq 0.05$; tendency at $0.05 < P \leq 0.10$; NS = $P > 0.10$.

²NFC = $100 - (\% \text{ NDF} + \% \text{ CP} + \% \text{ ether extract} + \% \text{ crude ash})$.

³Particles retained on sieves of the Penn State Particle Separator (Kononoff et al., 2003b). Physically effective factor determined as the sum of particles retained on the 8-mm sieve of the Penn State Particle Separator (PSPS; Kononoff et al., 2003b).

⁴peNDF_{>8}, peNDF_{>1.18} = physically effective NDF measured as the NDF content of the diet multiplied by amount of DM retained on the 8- and 1.18-mm screens of the PSPS, respectively; peNDF_{>8-NDF} = NDF content retained on the 8-mm screen; all measurements were corrected for feed refusals.

⁵Sorting index for particles retained on different screens of the PSPS and peNDF measurements; a sorting index of 1 indicates no sorting, a sorting index of <1 indicates sorting against, and >1 indicates sorting in favor of particles on the particular screen (Silveira et al., 2007).

may have balanced the lower content in particles >8 mm, and hence contributed to leveling out the differences in ruminal pH and VFA concentration among treatments. In addition, the inclusion of the fiber-rich hay resulted in increased NDF and peNDF contents (e.g., peNDF_{>1.18} exceeded 28% on a DM basis) in TMR, which in turn might have lowered the influence of PL of CS on ruminal pH.

Decreasing PL of CS significantly decreased the proportion of propionate and increased the acetate-to-propionate ratio (Table 4), which was not expected. However, these results can be explained by a lower sorting consumption in favor of fine particles by short treatment, which presumably decreased the proportion of concentrate consumed and increased that of forages. In addition, the proportion of butyrate significantly increased, apparently at the expense of propionate because the acetate proportion remained unchanged throughout the study ($P > 0.10$; Table 4). A greater butyrate proportion at the expense of propionate was also previously observed in the rumen of dairy cows in

response to decreasing PL of CS in a TMR (Bal et al., 2000). The mechanism(s) of shifting the fermentation profile of the rumen from propionate to butyrate in response to lowering of PL of CS is not well understood. However, this finding indicates changes in the activity or number of the butyrate-producing ruminal bacteria (Diez-Gonzalez et al., 1999) or protozoa (Brossard et al., 2004) in response to the diet-related changes (i.e., less sorting consumption, increase of particles between 1.18 and 8 mm, and the intake of peNDF_{>1.18} with decreasing PL of CS). However, further investigations are warranted to elucidate this aspect.

Because no interaction was observed between sampling site (i.e., FRL vs. PARL) and dietary PL ($P > 0.10$; i.e., the response of fermentation parameters followed similar trends in FRL and PARL), only data of ruminal fermentation for FRL are presented. The only difference between PARL and FRL was that FRL had higher pH (0.49 ± 0.06 pH units; $P < 0.01$) and lower VFA concentrations (39.1 ± 4.12 mM; $P < 0.01$) compared with PARL, in agreement with our previous observations

Figure 1. Distribution of feed intake and eating time over a 24-h period for cows fed total mixed diets differing in theoretical particle length (PL) of corn silage (error bars express the pooled SEM). Arrow indicates the feeding time; *PL is significant at $P < 0.05$.

(Zebeli et al., 2008a). The lowest pH (nadir) measured was achieved at 1500 and 1800 h (i.e., 7 to 10 h after the feeding; result not shown), which corresponded to the greatest concentrations of VFA. The nadir was achieved near the range of Nordlund and Garrett (1994), who proposed to sample 4 to 8 h postfeeding in TMR-fed herds to measure ruminal pH near the nadir.

Enzyme Activities

The activities of NSP-degrading enzymes are in Table 5, which also are shown only for FRL, because no interaction was observed between sampling site (i.e., FRL vs. PARL) and dietary PL ($P > 0.10$; result not shown). In general, decreasing PL of CS linearly or quadratically (i.e., activity of mannanase) increased the lysis zone of almost all specific agar substrates investigated in this study (Table 5). A greater fibrolytic activity of rumen digesta with decreasing PL agrees with results of Zebeli et al. (2008a), who reported similar results using in vitro trials.

Significant effects of decreasing PL of CS on all NSP-degrading enzymes are not in agreement with response

of fibrolytic bacteria to dietary PL reported previously (Zebeli et al., 2007b). More specifically, the researchers showed that the counts of 2 fibrolytic bacteria (*Ruminococcus albus* and *Ruminococcus flavefaciens*), measured by 16S rDNA quantification using real-time PCR, were not affected by PL of CS ($P > 0.10$). This result suggests that decreasing PL of CS could increase the activities of NSP-degrading enzymes, although fibrolytic bacteria counts and ruminal pH remained unaffected. However, although not affected by diet, the pH values in FRL were ≥ 5.8 throughout this study (result not shown). The latter indicates that cows of this study apparently were not at risk of subacute ruminal acidosis (Beauchemin et al., 2003; Zebeli et al., 2008b), and hence the likelihood that diet may adversely affect the counts of fibrolytic microbiota in the rumen is very low (Zebeli et al., 2008b). In addition, these results suggest that if dairy cows are fed diets that do not present any apparent risk of subacute ruminal acidosis (as in this study), lowering of the PL of CS may be beneficial due to increasing fiber degradation in the rumen, particularly by stimulating the activities of NSP-degrading enzymes.

Table 4. Effects of theoretical particle length (PL) of corn silage on fermentation parameters measured in free rumen liquid (FRL)

Parameter	PL of corn silage			Pooled SEM	Effects ¹	
	Long	Medium	Short		Linear	Quadratic
Ruminal pH	6.25	6.20	6.27	0.09	NS	NS
VFA, mM	120.7	117.2	118.0	5.99	NS	NS
	mol/100 mol VFA					
Acetate (A)	55.4	56.2	57.6	1.47	NS	NS
Propionate (P)	28.4	27.2	23.8	1.56	<0.01	NS
n-Butyrate	11.4	12.0	13.9	0.88	<0.01	NS
Valerate	2.3	2.1	2.1	0.29	<0.01	NS
BCVFA ²	2.5	2.6	2.6	0.30	NS	NS
A:P	2.0	2.1	2.5	0.11	<0.01	0.06

¹Linear or quadratic effect of PL; significant at $P \leq 0.05$; tendency at $0.05 < P \leq 0.10$; NS = $P > 0.10$.

²Branched-chain fatty acids (isobutyrate and isovalerate).

In Situ and Total-Tract Digestibility

In situ and total-tract digestibilities of DM and nutrients are given in Table 6. Decreasing the PL of CS in the diet tended to increase in situ 24-h NDF disappearance ($P = 0.07$), which was also reflected in a quadratically increased total-tract NDF digestibility ($P = 0.02$). The results of in situ NDF digestibility are generally in agreement with activities of NSP-degrading enzymes in the rumen. Thus, the quadratic increase of in situ NDF digestibility with decreasing PL of CS can be explained by a greater activity of NSP-degrading enzymes of rumen microbiota. However, in terms of maximization of feed efficiency, only the moderate reduction of PL of CS was shown to be beneficial. Although DMI was similar for all diets, the medium PL had the greatest NDF and NFC digestibility. A further decrease in PL to a fine particle size decreased both NDF and NFC digestibility, which is undesirable in terms of maximizing energy efficiency.

The fact that NDF digestibility in the total digestive tract was not decreased by the short PL was unexpected, because the activity of the most NSP-degrading enzymes in the rumen was high by this treatment. How-

ever, the depression of NDF digestibility in total tract can be partly attributed to a greater consumption of forage proportion due to lower sorting consumption for cows fed the short PL. In addition, given that the difference in NDF digestibility among treatments was greater when measured in total tract than when measured in the rumen (in situ), it can be hypothesized that this difference was due to a lower fiber digestion occurring in the lower digestive tract than in the rumen. There is evidence that from total NDF digested in dairy cows fed a high DM level, 72 to 88% is degraded in reticulorumen, whereas the rest is degraded distally to the reticulorumen (Yang and Beauchemin, 2005; Silveira et al., 2007). For example, Yang and Beauchemin (2005) reported, in a similar study, unchanged NDF digestibility in the rumen (33.6, 30.8, and 32.2%) for the diets based on different PL of CS (5, 11, and 19.1 mm, respectively). In contrast, cows fed the short treatment (5 mm) showed reduced NDF digestibility in the lower digestive tract (e.g., 4.7%) compared with other treatments (e.g., 12.9 and 12.5% for 11 and 19.1 mm, respectively), which also made the difference in NDF total-tract digestibility among 3 treatments of that

Table 5. Effects of theoretical particle length (PL) of corn silage on the activities (lysis zone in cm²) of nonstarch polysaccharide-degrading enzymes¹ in ruminal digesta supernatants originating from free rumen liquid

Parameter	PL of corn silage			Pooled SEM	Effects ²	
	Long	Medium	Short		Linear	Quadratic
Carboxymethylcellulase	8.1	11.1	15.6	1.23	<0.01	NS
Galactanase	18.0	20.9	23.7	1.15	<0.01	NS
Glucanase	10.9	11.5	14.7	1.18	0.01	NS
Mannanase	8.1	10.3	8.5	0.68	NS	0.02
Polygalacturonase	4.9	5.7	6.9	0.55	<0.01	NS
Xylanase	7.4	8.2	8.8	0.71	0.01	NS

¹The activities of enzymes were measured using an agar diffusion assay (Vahjen et al., 1997).

²Linear or quadratic effect of PL; significance at $P \leq 0.05$; tendency at $0.05 < P \leq 0.10$; NS = $P > 0.10$.

Table 6. Effect of theoretical particle length (PL) of corn silage on 24-h in situ ruminal disappearance and total-tract nutrient digestibility

Parameter	PL of corn silage			Pooled SEM	Effects ¹	
	Long	Medium	Short		Linear	Quadratic
In situ disappearance, %						
DM	74.0	76.2	74.9	1.37	NS	NS
NDF	37.6	46.4	42.4	2.57	NS	0.07
Total tract, % of intake						
DM	69.6	70.8	69.4	0.53	NS	NS
OM	71.4	72.6	71.3	0.53	NS	NS
NDF	44.6	47.9	43.8	1.23	NS	0.02
ADF	40.1	42.5	37.6	1.30	NS	0.01
CP	81.1	85.3	86.2	0.73	<0.01	NS
NFC ²	89.6	89.2	87.2	0.47	<0.01	NS

¹Linear or quadratic effect of PL; significant at $P \leq 0.05$; tendency at $0.05 < P \leq 0.10$; NS = $P > 0.10$.

²NFC = 100 – (% NDF + % CP + % ether extract + % crude ash).

study. To optimize fiber digestion in the whole digestive tract of high-producing dairy cows, these observations suggest that further research is needed to elucidate effects of dietary PL and peNDF also on passage and digestion processes distally to reticulorumen.

CONCLUSIONS

Feeding short PL of CS in a TMR reduced sorting consumption. Although ruminal pH remained unaffected by dietary treatment, decreasing PL of CS significantly increased the activities of NSP-degrading enzymes in the rumen. Ruminal and total-tract degradation of fiber and NFC, in particular, were greater with the medium PL of CS. In conclusion, the results of this study showed that a moderate reduction of PL of CS in TMR also containing 10% grass hay and 50% concentrate has beneficial effects on nutrient digestion and may maximize feed efficiency and energy supply in high-yielding dairy cows.

ACKNOWLEDGMENTS

The study was funded by a grant and a PhD fellowship for B. Junck from H. W. Schaumann-Stiftung in Hamburg, Germany. The assistance of W. Vahjen from the Institute of Animal Nutrition of Free University of Berlin in adapting the agar diffusion assay for enzymatic measurements is gratefully acknowledged. We thank Sybille Suchy for her excellent lab assistance, A. Töpfer for chemical analysis of corn silage, and the staff of the research station “Meiereihof” of the University of Hohenheim (R. Funk, R. v. Schmettow, M. Schwarz, and M. Meinaß) for their assistance during corn silage and TMR preparations as well as for the care of the experimental animals.

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