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Nutrient demand interacts with legume particle length to affect digestion responses and rumen pool sizes in dairy cows

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ABSTRACT

Effects of legume particle length on dry matter intake (DMI), milk production, ruminal fermentation and pool sizes, and digestion and passage kinetics, and the relationship of these effects with preliminary DMI (pDMI) were evaluated using 13 ruminally and duodenally cannulated Holstein cows in a crossover design with a 14-d preliminary period and two 19-d treatment periods. During the preliminary period, pDMI of individual cows ranged from 22.8 to 32.4 kg/d (mean = 26.5 kg/d) and 3.5% fat-corrected milk yield ranged from 22.9 to 62.4 kg/d (mean = 35.1 kg/d). Experimental treatments were diets containing alfalfa silage chopped to (1) 19 mm (long cut, LC) or (2) 10 mm (short cut, SC) theoretical length of cut as the sole forage. Alfalfa silages contained approximately 43% neutral detergent fiber (NDF); diets contained approximately 47% forage and 20% forage NDF. Preliminary DMI, an index of nutrient demand, was determined during the last 4 d of the preliminary period, when cows were fed a common diet, and used as a covariate. Main effects of legume particle length and their interaction with pDMI were tested by ANOVA. Alfalfa particle length and its interaction with pDMI did not affect milk yield or rumen pH. The LC diet decreased milk fat concentration more per kilogram of pDMI increase than the SC diet and increased yields of milk fat and fat-corrected milk less per kilogram of pDMI increase than the SC diet, resulting in a greater benefit for LC at low pDMI and for SC at high pDMI. The LC diet tended to decrease DMI compared with the SC diet. Ruminal digestion and passage rates of feed fractions did not differ between LC and SC and were not related to level of intake. The LC diet tended to decrease the rate of ruminal turnover for NDF but increased NDF rumen pools at a slower rate than the SC diet as pDMI increased. This indicated that the faster NDF turnover rate did not counterbalance the higher DMI for SC, resulting in larger NDF rumen pools for SC than LC. As pDMI increased, LC increased ruminal digestibility of potentially digestible NDF and total NDF, and SC decreased them, but total-tract digestibilities of potentially digestible NDF, total NDF, organic matter, and dry matter were lower for LC than for SC. Ruminal digestibilities of starch and organic matter interacted quadratically with level of intake. When legume silage was the only source of forage in the diet, increasing chop length from 10 to 19 mm tended to decrease DMI but did not negatively affect productivity of cows.
Key words: particle size, alfalfa silage, digestion kinetics, rumen pool

INTRODUCTION

Optimal utilization of diets by dairy cows is influenced by the chemical composition and physical characteristics of feeds. Forage fiber, in a form that is physically effective, is necessary in dairy cow diets to promote rumen fermentation and function (Allen, 1997). Increasing forage particle size has been shown to increase chewing activity, resulting in increased saliva flow, rumen pH, acetate-to-propionate ratio, and milk fat concentration (Nørgaard, 1993; Beauchemin et al., 1997), increase ruminal retention time (Dixon and Milligan, 1985), and promote formation of the rumen mat (Grant, 1997). Although impaired rumen function and health can result when cattle are fed rations lacking in physical structure, excessive amounts of long, coarse fiber may decrease ruminal digesta passage rates and limit feed intake of lactating dairy cows when feed intake is limited by rumen fill (Allen, 2000).

Forage particle length (**FPL**) has been widely researched, but results of animal responses to FPL are inconsistent and inconclusive. These inconsistencies are likely due to large variation in dietary factors among studies, which make direct comparisons across studies difficult. Wide ranges of FPL (2 to 32 mm; Tafaj et al., 2007) and differences between FPL compared within studies (6 vs. 8 mm, Yang and Beauchemin, 2004; 24 vs. 170 mm, Randby et al., 2008) have been used to evaluate FPL. Additionally, studies often evaluate the

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effect of FPL in combination with other dietary factors including forage:concentrate ratio (Soita et al., 2000; Einarson et al., 2004), grain processing (Yang et al., 2001), grain fermentability (Krause and Combs, 2003), nonforage fiber sources (Mooney and Allen, 1997), and supplemental fat (Onetti et al., 2003). Furthermore, responses to FPL vary depending on preservation methods (hay, silage) and forage source with greater differences reported for legume and grass-based TMR compared with corn silage-based TMR (Tafaj et al., 2007). This suggests that consideration of forage family is necessary when studying the effects of particle size. Alfalfa (**AL**; *Medicago sativa*) was selected as a representative legume for use in this experiment because it is the predominant legume fed to dairy cows in the United States.

Besides dietary factors, inconsistent responses to FPL may be related to animal factors. Numerous studies have examined the effects of alfalfa FPL, but most were designed using cows at a specific stage of lactation such as early lactation (Kononoff and Heinrichs, 2003) or mid lactation (Krause and Combs, 2003). However, cows respond differently to treatments depending on their level of intake (Voelker et al., 2002; Voelker Linton and Allen, 2008) and production (Oba and Allen, 1999). Because FPL and level of intake affect ruminal passage and digestion rates and, thus, digesta fill in the rumen, the response to effects of particle size and its relationship with intake level should be assessed to determine if responses to treatment vary among cows with a wide range in DMI. We hypothesized that responses of DMI and passage rates to legume particle length are related to level of intake, and shorter particle length would permit a greater increase in passage rate than longer particle length as feed intake increases.

The objectives of this experiment were to evaluate the relationships between voluntary DMI and effects of length of cut of legume silage on DMI, milk production, ruminal fermentation and pool sizes, and digestion and passage kinetics in lactating dairy cows. This study had 3 distinctive features to improve our understanding of the role of particle size and interpret its effect on animal responses. First, it allowed effects of the interaction between FPL and preliminary DMI (**pDMI**) to be evaluated. The use of pDMI, an index of nutrient demand, allowed the evaluation of treatments on animal responses in relation to level of intake and provided an indicator to test effects of intake level independent of treatments. Second, it directly compared treatment effects of long- and short-cut legume as the sole source of forage without the confounding effects of other dietary factors. Third, ruminal passage rates of individual feed fractions, instead of entire feeds, were measured using ruminally and duodenally cannulated cows.

MATERIALS AND METHODS

Cows and Treatments

Experimental procedures were approved by the Institutional Animal Care and Use Committee at Michigan State University. Thirteen multiparous Holstein cows from the Michigan State University Dairy Cattle Teaching and Research Center were assigned randomly to treatment sequence in a crossover design experiment with one 14-d preliminary period and two 19-d experimental periods. During the preliminary period, the first 10 d were allowed for diet adaptation, and samples were collected during the final 4 d. During each experimental period, the first 12 d were allowed for diet adaptation and samples were collected during the final 7 d. Cows were 177 ± 66 (mean ± SD) DIM at the end of the preliminary period and were selected to provide a wide range and uniform distribution of pDMI and milk yield. During the final 4 d of the 14-d preliminary period, the average pDMI among cows ranged from 22.8 to 32.4 kg/d (mean = 26.5 kg/d) and 3.5% FCM yield ranged from 22.9 to 62.4 kg/d (mean = 35.1 kg/d; Table 1). Prior to calving, cows were cannulated ruminally (Bar Diamond Inc., Parma, ID) and duodenally with a gutter-type T cannula placed approximately 10 cm distal to the pylorus (Joy et al., 1997). Surgery was performed at the Department of Large Animal Clinical Science, College of Veterinary Medicine, Michigan State University.

Experimental treatments were diets containing AL silage chopped to a theoretical length of cut (**TLC**) of either (1) 19 mm (long cut, **LC**) or (2) 10 mm (short cut, **SC**) as the sole forage. These TLC were selected to provide a wide interval within the normal range of TLC to examine if animal response to FPL is affected by level of feed intake.

Alfalfa was produced at the campus farm at Michigan State University (East Lansing), harvested from the same field using a New Holland FP230 pull-type forage harvester (New Holland, Racine, WI) set according to manufacturer specifications for theoretical lengths of cut of 19 mm and 10 mm for long- and short-cut AL, respectively, and ensiled in Ag-Bags (Ag-Bag Systems Inc., St. Nazianz, WI). During the sample collection periods, long- and short-cut AL contained approximately 43% NDF (DM basis; Table 2). Diets LC and SC were formulated to contain 21% forage NDF and 18% CP. The diet fed during the preliminary period was formulated so that long- and short-cut AL each contributed 50% of forage NDF. Diets also contained dry ground corn, soybean meal (48% CP), SoyPLUS (West Central Soy Cooperative, Ralston, IA), vitamin-mineral premix, limestone, and salt (Table 3).

Table 1. Characterization of 13 cows during the final 4 d of the 14-d preliminary period, when cows were fed a common diet

Parameter	Median	Mean	SD	Minimum	Maximum
Parity	3	3.1	0.9	2	5
BW,[1] kg	609	612	61	508	750
BCS	2.75	2.7	0.5	1.9	3.6
DIM	202	177	66	50	250
Milk, kg/d	33.1	35.5	10.7	21.9	59.4
3.5% FCM, kg/d	34.4	35.1	10.4	22.9	62.4
DMI, kg/d	26.7	26.5	2.6	22.8	32.4

[1]Empty BW (ruminal digesta removed).

Data and Sample Collection

Throughout the experiment, cows were housed in tie-stalls and fed diets as TMR once daily (1130 h) at 110% of expected intake. The amounts of feed offered and refused (orts) were weighed daily for each cow. Forage samples were collected twice weekly and analyzed to adjust diets to account for DM, NDF, and CP fluctuation. Samples of all dietary ingredients (0.5 kg) and orts (12.5%) were collected daily from d 11 to 14 during the preliminary period and d 13 to 17 during each experimental period. Samples were frozen immediately after collection at −20°C and combined into one composite sample per period before analysis.

Cows were moved to an exercise lot twice daily (0230 and 1300 h) before milking in a parlor (0400 and 1430 h). Milk yield was measured and milk was sampled at each milking on d 11 to 14 of the preliminary period and on d 13 to 17 of the experimental periods. Rumen-empty BW was measured by weighing the cow after evacuation of ruminal digesta on d 14 of the preliminary period and d 19 of each experimental period. Body condition score was determined on the same days by 4 trained investigators blinded to treatments (Wildman et al., 1982; 5-point scale, where 1 = thin and 5 = fat).

Duodenal samples (900 mL), fecal samples (500 g), rumen fluid and particulate samples for microbial isolation (400 g), and rumen fluid samples for pH, concentrations of VFA, lactate, and ammonia (100 mL) were collected every 15 h from d 13 to 17 of each experimental period so that 8 samples were taken for each cow in each period, representing every 3 h of a 24-h period to account for diurnal variation. Rumen fluid and particulate matter for microbial isolation were collected from the reticulum, near the reticular-omasal orifice, transported to the laboratory, and processed. Rumen fluid for pH, VFA, lactate, and ammonia was obtained by combining digesta from 5 different sites in the rumen and straining it through nylon mesh (~1 mm pore size); fluid pH was recorded immediately. Samples were stored at −20°C.

Table 2. Chemical composition, particle size distribution, and fermentation parameters of the long-cut (19 mm) and short-cut (10 mm) alfalfa silage included in the treatment diets

	Alfalfa silage	
Item	Long	Short
Chemical composition		
DM, %	42.2	36.9
OM, % of DM	92.0	90.9
NDF, % of DM	42.3	44.0
iNDF,[1] % of DM	29.1	26.8
iNDF, % of NDF	68.8	60.9
ADF, % of DM	36.5	37.2
ADF nitrogen, % of DM	1.70	1.65
ADL, % of DM	8.91	9.05
CP, % of DM	20.4	19.6
Starch, % of DM	1.89	1.92
NDF digestibility,[2] %	34.1	35.0
Particles size distribution[3]		
Wet sieving, % DM retained		
19.0 mm	23.2	8.57
9.50 mm	39.6	20.4
4.75 mm	21.0	30.3
2.36 mm	6.29	26.6
1.18 mm	3.49	6.14
0.600 mm	2.24	3.56
0.300 mm	1.86	1.93
0.150 mm	1.20	1.19
0.075 mm	0.76	0.88
0.038 mm	0.44	0.58
Mean particle size,[4] mm	14.1	8.1
Penn State Particle Separator, % of DM retained		
>19.0 mm	33.2	10.7
19.0 to 8.0 mm	42.9	51.7
<8.0 mm	23.9	37.6
Fermentation		
pH	4.62	4.42
Acetic acid, % of DM	1.29	1.49
Propionic acid, % of DM	0.08	0.07
Butyric acid, % of DM	<0.01	<0.01
Lactic acid, % of DM	5.40	6.46
Lactic:Acetic	4.19	4.34
Ethanol, % of DM	0.10	0.08
Ammonia, m*M*	6.32	4.95

[1]iNDF = indigestible NDF.

[2]Thirty-hour in vitro NDF digestibility.

[3]Particle size distributions of silages were measured each period (n = 2).

[4]Mean particle size calculated from particle size distribution determined by wet sieving.

Table 3. Ingredients and chemical composition of preliminary and treatment diets (as analyzed) containing either long-cut (19 mm) or short-cut (10 mm) alfalfa silage as the sole source of forage

Item	Preliminary	Long	Short
Ingredients, % of DM			
Alfalfa silage, long cut	23.2	46.3	—
Alfalfa silage, short cut	23.2	—	47.0
Dry ground corn	36.4	36.4	36.0
Soybean meal (48% CP)	7.99	8.03	7.84
SoyPLUS[1]	4.00	4.02	3.92
Vitamin mineral mix[2]	4.68	4.68	4.68
Limestone	0.39	0.39	0.39
Salt	0.19	0.19	0.19
Chemical composition			
DM, %	53.1	58.8	53.5
OM, % of DM	93.4	93.4	92.8
NDF, % of DM	26.9	24.5	25.5
% forage NDF	21.7	19.6	20.7
% NDF from forage	80.9	80.0	81.1
iNDF,[3] % of DM	NA[4]	15.5	14.6
iNDF, % of NDF	NA	63.2	57.3
CP, % of DM	18.0	19.3	18.9
Starch, % of DM	30.6	30.8	30.5

[1]West Central Soy Cooperative (Ralston, IA).

[2]Vitamin mineral mix contained (DM basis) 17.1% sodium bicarbonate, 3.9% dicalcium phosphate, 2.6% magnesium oxide, 1.9% salt, 1.9% trace mineral premix, 0.4% vitamin A, 0.4% vitamin D, 0.2% vitamin E, and 71.6% dry ground corn as a carrier.

[3]iNDF = indigestible NDF.

[4]NA = no analysis for preliminary diet.

Ruminal contents were evacuated manually through the ruminal cannula 4.5 h after feeding at the beginning of d 18 (1600 h), and 41.5 h later, 2 h before feeding at the end of d 19 (0930 h) for each experimental period. Total rumen content mass and volume were determined. To ensure accurate sampling, every tenth handful of digesta (10%) was separated for a subsample throughout evacuation. This subsample was squeezed into primarily solid and liquid phases. Both phases were weighed and sampled (350 mL) for determination of nutrient pool size. All samples were stored at −20°C.

Sample Analysis and Calculations

Milk yields recorded at each milking were summed for a daily total, which were averaged for each period. Milk samples were analyzed for fat, true protein, lactose, and SNF with infrared spectroscopy by Michigan DHIA (East Lansing). Yields of 3.5% FCM and milk components were calculated using milk yield and component concentrations for each milking, summed for a daily total, and averaged for each period.

Forage samples were combined to 1 composite sample per forage per period. Particle size distribution was determined using the Penn State Particle Separator containing 2 sieves (19 and 8 mm) and a pan (Lammers et al., 1996). In addition, samples were wet sieved manually and sequentially through screens with the following aperture sizes: 19.0, 9.50, 4.75, 2.36, 1.18, 0.600, 0.300, 0.150, 0.075, and 0.038 mm. The fraction of DM retained on the screens from wet sieving was used to calculate mean particle size.

Diet ingredients, orts, and feces were lyophilized (TriPhilizer MP, FTS Systems, Stone Ridge, NY). All dried samples were ground with a Wiley mill (1-mm screen; Arthur H. Thomas, Philadelphia, PA). Dried, ground fecal samples were combined on an equal DM basis into 1 sample per cow per period. Frozen duodenal samples for each cow period (n = 8) were chopped finely using a commercial food processor (84142 Food Cutter, Hobart Manufacturing Co., Troy, OH) and subsampled in the frozen state to obtain representative samples. These duodenal subsamples and the 350 mL of ruminal solid and liquid samples were lyophilized and ground as described above. Dried ruminal solid and liquid samples were recombined according to the original ratio of solid and liquid DM.

Samples were analyzed for ash, NDF, indigestible NDF (**iNDF**), ADF, acid detergent sulfuric acid lignin (ADL), ADF nitrogen (forages only), CP, and starch. Ash concentration was determined after 5 h of combustion at 500°C in a muffle furnace. Concentrations of NDF were determined according to Mertens (2002) and ADF and ADL according to Goering and Van Soest (1970). Forage samples were analyzed for ADF nitrogen by Cumberland Valley Analytical Services Inc. (Hagerstown, MD) using ADF method 973.18 (AOAC, 2000), modified for using glass micro-fiber filter with 1.5 μm

particle retention in place of fritted glass crucible, and followed by nitrogen analysis of ADF residue using a Leco FP-528 Nitrogen Combustion Analyzer (Leco, St. Joseph, MI). Indigestible NDF was estimated as NDF residue after a 240-h in vitro fermentation (Goering and Van Soest, 1970); flasks were reinoculated at 120 h to ensure a viable microbial population. Forage NDF digestibility was determined by 30-h in vitro fermentation (Goering and Van Soest, 1970). Ruminal fluid for the in vitro incubations was collected from a nonpregnant dry cow fed dry hay only. Fraction of potentially digestible NDF (**pdNDF**) was calculated by difference (1.00 – iNDF). Crude protein was analyzed according to Hach et al. (1987). Starch was measured by an enzymatic method (Karkalas, 1985) after samples were gelatinized with sodium hydroxide. Glucose concentration was measured using a glucose oxidase method (Glucose kit #510, Sigma Chemical Co., St. Louis, MO), and absorbance was determined with a micro-plate reader (SpectraMax 190, Molecular Devices Corp., Sunnyvale, CA). Concentrations of all nutrients except DM were expressed as percentages of DM determined by drying at 105°C in forced-air oven for more than 8 h.

Duodenal digesta were analyzed for purines and ammonia to estimate microbial N (**MN**) flow and nonammonia, nonmicrobial N (**NANMN**) flow to the duodenum. Purine concentration was used as a microbial marker, and purine-to-MN ratio was estimated by analysis of microbial pellets obtained by differential centrifugation of the rumen fluid and particulate samples collected near the reticulum. Rumen fluid and particulate matter were blended, strained through nylon mesh, and the liquid portion was centrifuged at 500 × *g* for 15 min. The supernatant was centrifuged at 18,000 × *g* for 15 min, and the pellet was washed with 0.9% NaCl solution and centrifuged again at 18,000 × *g* for 15 min, resuspended in water, and lyophilized. Total purines were measured by spectrophotometer (Beckman Instruments Inc., Fullerton, CA) at 260 nm according to Zinn and Owens (1986). Ammonia concentration was determined for centrifuged duodenal and rumen fluid samples according to Broderick and Kang (1980). Rumen fluid was also analyzed for concentrations of major VFA and lactate by HPLC (Waters Corp., Milford, MA) according to Oba and Allen (2003).

Dry matter and nutrient intakes were calculated using the composition of feed offered and refused. Ruminal pool sizes (kg) of OM, NDF, iNDF, pdNDF, starch, MN, and NANMN were determined by multiplying the concentration of each component in rumen samples by the ruminal digesta DM mass (kg). Duodenal flows (kg/d) of DM, OM, total NDF, pdNDF, starch, MN, NANMN, and ammonia N were determined using iNDF as a flow marker; iNDF intake (kg/d) was multiplied by the ratio between the component and iNDF in duodenal digesta. Duodenal flow of microbial OM was determined using the purines-to-OM ratio (Oba and Allen, 2003), and true ruminally digested OM was calculated by subtracting duodenal flow of nonmicrobial OM from OM intake. Indigestible NDF was used as an internal marker to estimate nutrient digestibility in the rumen and in the total tract (Cochran et al., 1986). Turnover rate in the rumen, passage rate from the rumen, and ruminal digestion rate of each component was calculated by using the following equations:

$$\text{Turnover rate (\%/h)} = 100 \times (\text{intake of component/ruminal pool of component})/24;$$

$$\text{Passage rate (\%/h)} = 100 \times (\text{duodenal flow of component/ruminal pool of component})/24; \text{ and}$$

$$\text{Digestion rate (\%/h)} = \text{turnover rate in the rumen (\%/h)} - \text{passage rate from the rumen (\%/h)}.$$

Statistical Analysis

All data were analyzed by using the fit model procedure of JMP (version 8, SAS Institute Inc., Cary, NC). To determine differences between treatments and evaluate interactions of treatment with DMI, where pDMI (calculated as the mean of DMI values on d 11 to 14 of the 14-d preliminary period) was used as the covariate for treatment responses, data were analyzed according to the following model:

$$Y_{ijk} = \mu + C_i + P_j + T_k + PT_{jk} + pDMI + T_k pDMI + pDMI^2 + T_k pDMI^2 + e_{ijk},$$

where Y_{ijk} is the the dependent variable, μ is the overall mean, C_i is the random effect of cow ($i = 1$ to 13), P_j is the fixed effect of period ($j = 1$ to 2), T_k is the fixed effect of treatment ($k = 1$ to 2), PT_{jk} is the interaction of period and treatment, pDMI is the linear effect of pDMI, T_kpDMI is the interaction of treatment and pDMI (linear), $pDMI^2$ is the quadratic effect of pDMI, $T_k pDMI^2$ is the interaction of treatment and pDMI (quadratic), and e_{ijk} is the residual error. Statistical significance for T_kpDMI and $T_k pDMI^2$ indicated that treatment differences were related to pDMI. Covariate and interaction terms were removed stepwise from the model if $P > 0.20$. Treatment effects and their interaction (linear and quadratic relationships) were declared significant at $P \leq 0.05$ and $P \leq 0.10$, respectively. Ten-

dencies for treatment effects and their interactions were declared at $P \leq 0.10$ and $P \leq 0.15$, respectively.

Sixteen cows started the experiment; however, 2 cows were removed during the experiment (one cow injured a teat and the other cow went off feed). Additionally, data from one cow were excluded before statistical analysis because she ate sporadically and had inconsistent DMI, which ranged from 12.3 to 21.7 kg/d during the 4 d collection of the preliminary period and from 2.7 to 26.0 kg/d and 17.5 to 24.2 kg/d during the 5-d collection of the first and second experimental periods, respectively. Data from 13 cows were statistically analyzed for all response variables except those associated with N metabolism (Table 11), which included 12 cows. One cow (with the highest pDMI) was considered an outlier based on large Cook's distance values (Cook and Weisberg, 1982) for response variables for MN flux and microbial efficiency only. This indicated a problem with the partitioning of NANMN and MN due to purine concentration for this cow; however, all N data from this cow were removed.

RESULTS AND DISCUSSION

Comparison of Forages and Diets

Physical characteristics of AL are listed in Table 2. Forages chopped to a TLC of 19 and 10 mm had mean particle sizes of 14.1 and 8.1 mm, respectively. The proportion of particles >19 mm was 22.5 percentage units higher (33.2 vs. 10.7%) and that of particles <8 mm was 13.7 percentage units lower (23.9 vs. 37.6%) for long-cut than for short-cut AL, respectively.

Chemical analyses (Table 2) showed that AL with different lengths of cut had similar concentrations of OM, ADF, ADL, CP, and starch. Long-cut AL had higher DM concentration than short-cut AL due to the longer wilting time for long-cut AL as the silages were sequentially harvested, and long-cut AL was mowed, chopped, and ensiled last. Indigestible NDF expressed as a percentage of total NDF was high for both silages. Despite AL silages being harvested from the same field on the same day, the proportion of iNDF of total NDF was 7.9 percentage units higher for long-cut than short-cut because concentration of total NDF was 1.7 percentage units lower and iNDF was 2.3 percentage units higher for long-cut AL compared with short-cut AL. Although the iNDF concentration was higher for long-cut AL compared with short-cut AL, in vitro NDF digestibility (30 h) of long-cut AL was only 0.9 percentage units lower than that of short-cut AL. It is possible that the drier, longer particles of long-cut AL did not pack as densely as the wetter, shorter particles of short-cut AL, which might have affected fermentation and storage; however, this was not evident based on the chemical analyses or fermentation profile. The ADF nitrogen concentrations, a measure of indigestible compounds formed by chemically linked protein and carbohydrate used as an indicator of heat-damaged protein, were low and similar for both cuts of AL, and they appeared to undergo favorable fermentation and be well preserved based on the low pH and the production of mainly lactic acid.

Diet ingredients and chemical composition are shown in Table 3. The preliminary diet contained similar proportions of forage NDF from long- and short-cut AL. Both treatment diets had a 47:53 forage:concentrate ratio, contained approximately 20% forage NDF and had similar OM, CP, and starch composition, which was mathematically calculated according to the proportion of each feed ingredient in the diet and its respective analytical values. The LC diet had approximately 1 percentage unit lower total NDF and higher iNDF than the SC diet, which resulted in the proportion of iNDF of total dietary NDF being 5.9 percentage units higher for LC. Differences in DM concentration in diets were because of the different DM concentrations of the forages. The calculated concentrations of total NDF in LC and SC and forage NDF in LC were slightly lower than the formulated targets but similar to NRC (2001) minimum requirements. In both diets, forage NDF provided nearly 80% of the total diet NDF.

Effects of Legume FPL and pDMI

Results of AL particle length and its interaction with pDMI on milk yields and composition are shown in Table 4. Response of milk fat concentration to FPL was related to pDMI, as indicated by a significant interaction between FPL and pDMI (interaction $P = 0.01$); LC decreased milk fat concentration more per kilogram of pDMI increase than did SC (Figure 1). This effect on concentration of milk fat influenced other treatment by pDMI interactions, including milk fat yield (interaction $P = 0.006$), FCM yield (interaction $P = 0.03$), and efficiency (FCM/DMI; interaction $P = 0.06$); LC increased these responses less per kilogram of pDMI increase than did SC. The aforementioned interactions resulted in a greater benefit for LC for cows with low pDMI and a greater benefit for SC for cows with high pDMI.

The LC diet tended to decrease DMI (26.3 vs. 27.2 kg/d; $P = 0.10$, Table 4) compared with the SC diet. We expected LC to be more filling than SC, causing greater rumen distention and potentially limiting DMI, particularly in cows with high DMI for which ruminal distension is more likely to limit feed intake (Allen, 1996). Oba and Allen (1999) and Voelker et al. (2002)

Table 4. Milk production and composition, feed intake, and BW change of cows fed treatment diets containing either long-cut (19 mm) or short-cut (10 mm) alfalfa silage as the sole source of forage

	Treatment LSM			*P*-value[1]					
Item	Long	Short	SE	Trt	Trt × Period	pDMI	Trt × pDMI	pDMI × pDMI	Trt × pDMI × pDMI
Yield, kg/d									
Milk	37.7	37.4	3.3	0.67	NS[2]	0.03	NS	0.04	NS
FCM (3.5%)	38.8	38.8	3.2	0.88	NS	0.03	0.03	0.06	NS
Milk fat	1.28	1.29	0.09	0.57	0.08	0.11	0.006	NS	NS
Milk protein	1.20	1.19	0.08	0.71	NS	0.008	NS	0.04	NS
Milk lactose	1.85	1.83	0.18	0.66	NS	0.03	NS	0.03	NS
SNF	3.01	2.99	0.26	0.67	NS	0.02	NS	0.03	NS
Milk composition, %									
Fat	3.77	3.80	0.15	0.58	NS	0.08	0.01	0.05	NS
Protein	3.24	3.26	0.13	0.38	NS	0.32	NS	0.09	NS
Lactose	4.86	4.87	0.07	0.41	NS	0.003	NS	0.005	NS
SNF	7.98	8.01	0.09	0.14	NS	0.18	NS	NS	NS
DMI, kg/d	26.3	27.2	0.5	0.10	NS	<0.001	0.34	0.03	0.18
3.5% FCM/DMI	1.36	1.34	0.09	0.40	NS	0.08	0.06	0.07	NS
BW change, kg/19 d	15.3	11.2	2.2	0.22	0.009	0.15	0.19	NS	NS
BCS change/19 d	0.11	0.07	0.06	0.68	NS	NS	NS	NS	NS

[1]*P*-values for treatment (Trt), Trt by period interaction (Trt × Period), preliminary DMI (pDMI), Trt by pDMI interaction (Trt × pDMI), quadratic effect of pDMI (pDMI × pDMI), and Trt by quadratic effect of pDMI (Trt × pDMI × pDMI).
[2]Nonsignificant, with $P > 0.20$; term was removed from the statistical model.

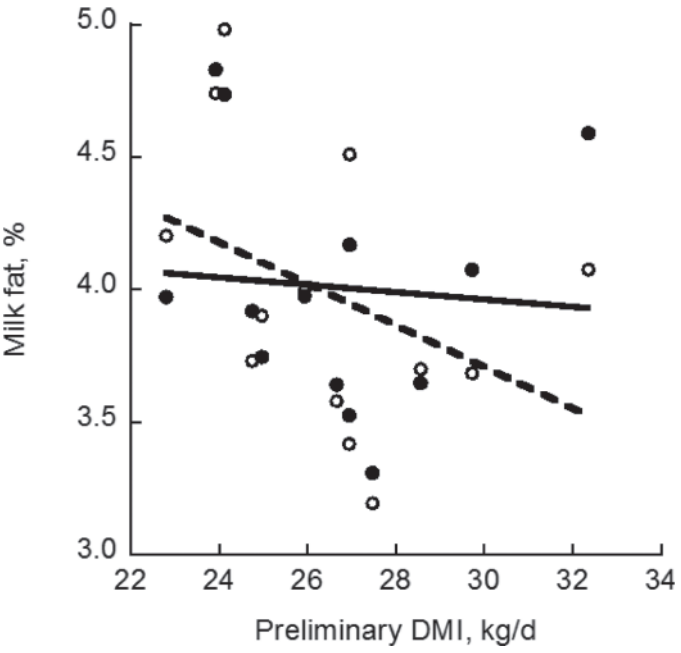

Figure 1. Interaction of long (19 mm; open circles, dashed line) and short (10 mm; closed circles, solid line) alfalfa particle length with preliminary DMI for milk fat concentration (interaction: $P = 0.01$ linear; long: $P = 0.18$, $R^2 = 0.15$; short: $P = 0.80$, $R^2 = 0.01$). The preliminary DMI on the x-axis are the mean DMI of individual cows during the final 4 d of the preliminary period when all cows were fed a common diet. The best-fit lines are drawn to demonstrate the significant interaction even if the individual relationships are not significant.

found DMI responses to a more filling diet varied by production level, which is generally correlated with DMI (NRC, 2001), where DMI was increasingly limited by high-fill diets compared with low-fill diets as milk yield increased. We expected LC to slow rates of ruminal passage but FPL and its interaction with pDMI did not affect the rates of pdNDF, iNDF, or starch passage from the rumen (Table 5).

The LC diet tended to decrease the rate of ruminal turnover of NDF (4.68 vs. 4.91%/h; $P = 0.09$, Table 5) compared with the SC diet. Despite the slower turnover rate of NDF for LC, the rumen pool of NDF was less for LC compared with SC except for cows with the lowest or highest DMI (interaction $P = 0.08$, quadratic; Figure 2A). This indicated that the faster NDF turnover rate was not sufficient to counterbalance the higher DMI for SC, resulting in larger NDF rumen pools for SC than LC. Additionally, rumen digesta wet weight (interaction $P = 0.03$, quadratic; Figure 2B) and volume (interaction $P = 0.006$, quadratic; Figure 2C) were related to pDMI (Table 6); rumen digesta wet weight and volume were less for LC compared with SC except for cows at the low and high ends of the pDMI range. Although the effect of treatment on DMI was not related to pDMI (interaction $P \geq 0.18$), a visual examination of a graph with pDMI and DMI (Figure 2D) illustrated that the difference in DMI between LC and SC was small for cows with low pDMI but the difference became greater as pDMI increased and then narrowed for cows with high pDMI. Because LC had less rumen digesta mass and volume than SC for cows

Table 5. Rumen kinetics of cows fed treatment diets containing either long-cut (19 mm) or short-cut (10 mm) alfalfa silage as the sole source of forage

	Treatment LSM			*P*-value[1]					
Item	Long	Short	SE	Trt	Trt × Period	pDMI	Trt × pDMI	pDMI × pDMI	Trt × pDMI × pDMI
Ruminal turnover rate, %/h									
DM	11.0	10.8	0.3	0.59	0.03	NS[2]	NS	NS	NS
OM	11.2	11.0	0.3	0.61	0.04	NS	NS	NS	NS
NDF	4.68	4.91	0.16	0.09	0.08	NS	NS	NS	NS
pdNDF[3]	13.8	16.7	2.3	0.41	NS	NS	NS	NS	NS
Starch	54.3	52.2	4.8	0.73	0.03	NS	NS	NS	NS
Ruminal turnover time, h									
DM	9.28	9.36	0.26	0.77	0.02	NS	NS	NS	NS
OM	9.09	9.16	0.27	0.81	0.03	NS	NS	NS	NS
NDF	21.9	20.6	0.7	0.10	0.07	NS	NS	NS	NS
pdNDF	7.58	6.75	0.58	0.34	NS	0.24	NS	0.15	NS
iNDF[4]	30.3	31.0	1.2	0.55	0.16	NS	NS	NS	NS
Starch	2.08	2.11	0.15	0.85	0.006	NS	NS	NS	NS
Ruminal passage rate, %/h									
pdNDF	1.44	1.99	0.45	0.33	0.16	NS	NS	NS	NS
iNDF	3.39	3.28	0.13	0.36	0.17	NS	NS	NS	NS
Starch	23.6	23.4	3.1	0.97	NS	NS	NS	NS	NS
Ruminal digestion rate, %/h									
pdNDF	12.3	14.7	2.3	0.51	NS	NS	NS	NS	NS
Starch	33.3	31.4	3.4	0.56	0.05	0.62	NS	0.18	NS

[1]*P*-values for treatment (Trt), Trt by period interaction (Trt × Period), preliminary DMI (pDMI), Trt by pDMI interaction (Trt × pDMI), quadratic effect of pDMI (pDMI × pDMI), and Trt by quadratic effect of pDMI (Trt × pDMI × pDMI).

[2]Nonsignificant, with $P > 0.20$; term was removed from the statistical model.

[3]pdNDF = potentially digestible NDF.

[4]iNDF = indigestible NDF.

with the greatest reduction in DMI, it is unlikely that DMI for LC was limited by rumen fill.

Feeding behavior was measured in another experiment of similar design (Kammes and Allen, 2012) evaluating the effects of grass FPL. Similarly, cows consumed 0.9 kg/d less (21.8 vs. 22.7 kg/d) when fed diets with long-cut compared with short-cut grass silage. Total chewing time was greater for cows consuming long-cut than short-cut grass silage diets, such that cows consuming long-cut grass silage were approaching maximum chewing times reported in the literature (Tafaj et al., 2007). Feeding behavior was not measured in this study, but DMI for cows consuming LC might have been limited by chewing time.

Treatment interacted quadratically with pDMI to affect site of starch digestion (Table 7). True ruminal starch digestion (kg/d, interaction $P = 0.13$, quadratic) and digestibility (%, interaction $P = 0.09$, quadratic; Figure 3A) were lower for LC compared with SC for cows with low and high pDMI. As a result, starch flux from the rumen to duodenum (interaction $P = 0.05$, quadratic; Figure 3B) was greater for LC compared with SC for cows with low and high pDMI. Postruminal starch digestion (kg/d, interaction $P = 0.05$, quadratic) followed a similar pattern as starch flux, and postruminal starch digestibility (%, interaction $P = 0.08$, quadratic; Figure 3C) had a pattern that was the inverse of true ruminal starch digestibility. Although the cow at each end of the pDMI range (<23 and >32 kg of DM/d) was not identified as an outlier based on Cook's distance values (Cook and Weisberg, 1982), both cows amplified the quadratic effects. Therefore, data were statistically reanalyzed after the 2 cows were removed. Removal did not eliminate the quadratic effects or notably change results and conclusions so data from both cows were included; however, caution should be used when making inferences.

As pDMI increased, LC increased ruminal digestibility of pdNDF and SC decreased it (interaction $P = 0.10$; Table 8). Ruminal digestibility of NDF was lower for LC compared with SC ($P = 0.01$; Table 8), and the differences were greater for cows with lower pDMI (interaction $P = 0.09$; Figure 4). Total-tract digestibilities of pdNDF (63.5 vs. 78.0%, $P = 0.009$) and NDF (23.7 vs. 34.0%, $P < 0.001$) were lower for LC than for SC (Table 8). The lower digestibility of NDF for LC may be due, in part, to the higher concentration of iNDF for long-cut AL than for short-cut AL (Table 2), despite being harvested from the same field and having similar ensiling characteristics as previously discussed.

Total-tract digestibilities of NDF (and pdNDF) were lower than ruminal digestibility because negative

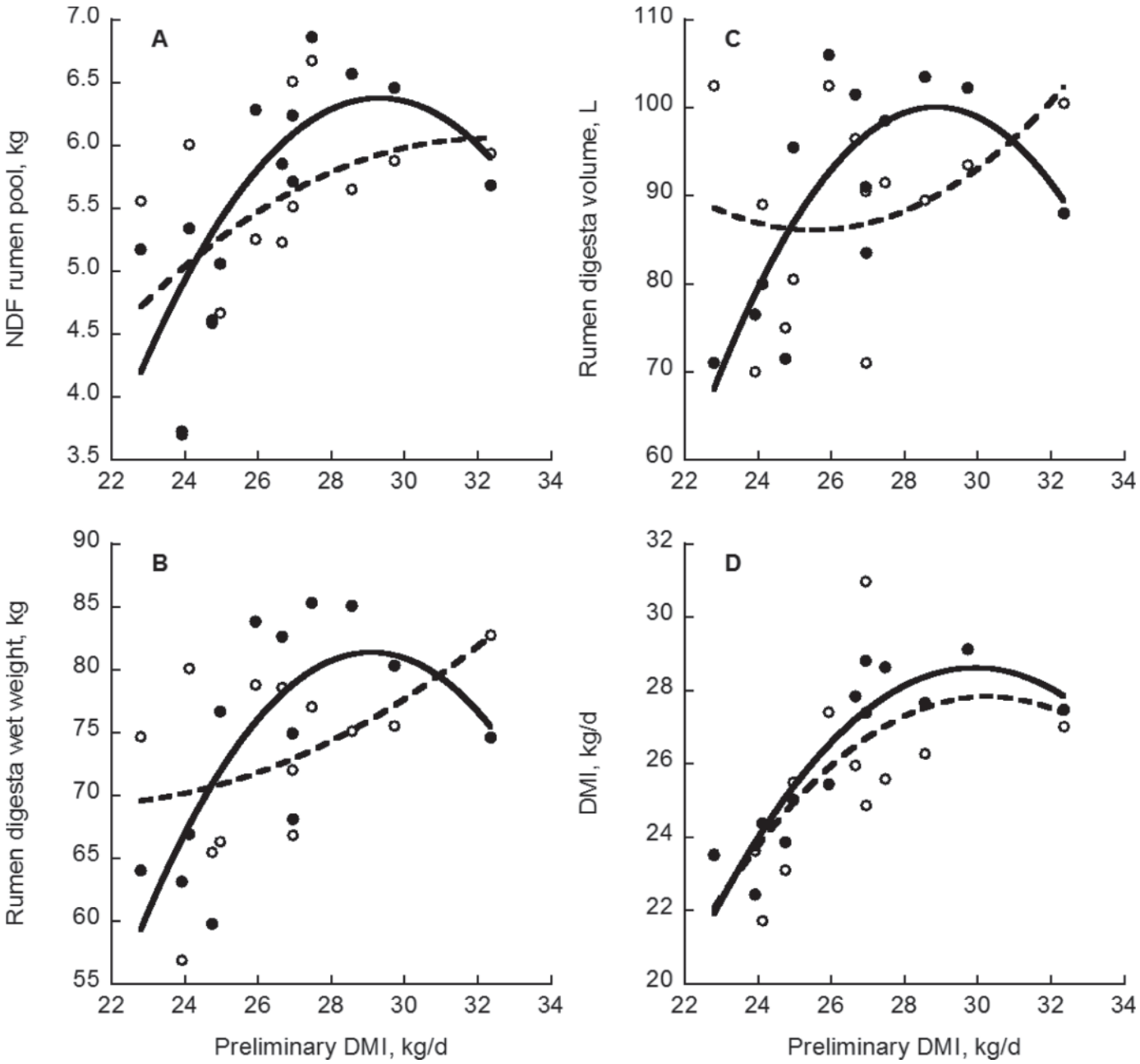

Figure 2. Interaction of long (19 mm; open circles, dashed line) and short (10 mm; closed circles, solid line) alfalfa particle length with preliminary DMI for (A) NDF rumen pool (interaction: $P = 0.08$ quadratic; long: $P = 0.25$, $R^2 = 0.24$; short: $P = 0.02$, $R^2 = 0.57$), (B) rumen digesta wet weight (interaction: $P = 0.03$ quadratic; long: $P = 0.25$, $R^2 = 0.24$; short: $P = 0.02$, $R^2 = 0.55$), (C) rumen digesta volume (interaction: $P = 0.006$ quadratic; long: $P = 0.43$, $R^2 = 0.15$; short: $P = 0.009$, $R^2 = 0.61$), and (D) DMI (interaction: not significant; long: $P = 0.04$, $R^2 = 0.48$; short: $P < 0.001$, $R^2 = 0.82$). The preliminary DMI on the x-axis are the mean DMI of individual cows during the final 4 d of the preliminary period when all cows were fed a common diet. The best-fit lines are drawn to demonstrate the significant interaction even if the individual relationships are not significant.

postruminal digestibilities were calculated for NDF (and pdNDF) in the present experiment. We evaluated Cr_2O_3 [5 g dosed through the ruminal cannula at 8-h intervals (total of 15 g of Cr_2O_3 /d) from d 6 to d 17 with a priming dose of 2× on d 6], ADL, and acid detergent peroxide lignin (Cochran et al., 1988) as alternative flow markers. Based on comparisons of calculated flow data using different markers, iNDF provided the most reasonable results and was used as flow marker. The lower digestibility for total tract than in the rumen is

Table 6. Rumen pools of cows fed treatment diets containing either long-cut (19 mm) or short-cut (10 mm) alfalfa silage as the sole source of forage

	Treatment LSM			*P*-value[1]					
Item	Long	Short	SE	Trt	Trt × Period	pDMI	Trt × pDMI	pDMI × pDMI	Trt × pDMI × pDMI
Wet weight, kg	72.6	77.6	2.4	0.05	NS[2]	0.03	0.07	0.32	0.03
Volume, L	86.8	95.1	3.6	0.02	NS	0.07	0.01	0.44	0.006
Density, kg/L	0.84	0.82	0.02	0.30	0.18	0.96	0.14	0.71	0.15
Rumen pool, kg									
DM	10.2	10.8	0.4	0.07	0.13	0.02	0.33	0.16	0.15
OM	9.35	9.85	0.41	0.11	0.16	0.02	0.22	0.17	0.15
NDF	5.57	5.98	0.25	0.04	NS	0.02	0.09	0.19	0.08
pdNDF[3]	0.74	0.85	0.06	0.27	NS	0.004	NS	NS	NS
iNDF[4]	4.89	5.18	0.24	0.12	0.19	0.04	0.29	0.08	0.09
Starch	0.72	0.72	0.06	0.97	0.01	0.09	NS	NS	NS

[1]*P*-values for treatment (Trt), Trt by period interaction (Trt × Period), preliminary DMI (pDMI), Trt by pDMI interaction (Trt × pDMI), quadratic effect of pDMI (pDMI × pDMI), and Trt by quadratic effect of pDMI (Trt × pDMI × pDMI).

[2]Nonsignificant, with $P > 0.20$; term was removed from the statistical model.

[3]pdNDF = potentially digestible NDF.

[4]iNDF = indigestible NDF.

due to a net gain of fiber from the duodenum to the feces, which has previously been reported with both the gutter-type T duodenal cannula (Huhtanen and Jaakkola, 1993; Poore et al., 1993), the type used in this study, and closed T-type duodenal cannula (Stensig and Robinson, 1997). The underestimation of duodenal NDF flow or duodenal iNDF:NDF ratio using iNDF as a marker creates inaccuracies of estimated flow of duodenal fiber and postruminal digestibility. These errors may be related to unrepresentative digesta sampling due to differential separation of fluid and particles relative to the true material flowing out of the duodenum or analytical problems in fiber determination of duodenal samples possibly due to a component in the duodenal digesta that interferes with the analysis. Although absolute values are not biologically reasonable, relative comparisons between treatments within the same experiment are useful.

Due to NDF digestibility, LC decreased total-tract digestibility of DM (65.6 vs. 67.6%, $P = 0.04$) and OM (66.6 vs. 68.6%, $P = 0.03$) and total-tract digestion of DM (17.3 vs. 18.2 kg/d, $P = 0.01$) and OM (16.4 vs. 17.2 kg/d, $P = 0.02$) compared with SC (Table 9). Interaction of treatment and pDMI for true ruminal

Table 7. Starch digestion of cows fed treatment diets containing either long-cut (19 mm) or short-cut (10 mm) alfalfa silage as the sole source of forage

	Treatment LSM			*P*-value[1]					
Item	Long	Short	SE	Trt	Trt × Period	pDMI	Trt × pDMI	pDMI × pDMI	Trt × pDMI × pDMI
Intake, kg/d	8.49	8.51	0.18	0.92	NS[2]	<0.001	0.53	0.03	0.14
Apparent ruminal digestion									
kg/d	5.35	4.94	0.35	0.43	NS	0.01	0.98	0.004	0.14
%	62.9	57.8	4.0	0.43	NS	0.11	0.81	0.008	0.10
True ruminal digestion									
kg/d	5.54	5.12	0.35	0.43	NS	0.009	0.99	0.004	0.13
%	65.1	59.9	4.0	0.43	NS	0.11	0.78	0.01	0.09
Passage to duodenum, kg/d	3.14	3.57	0.33	0.40	NS	0.99	0.83	0.03	0.05
Apparent postruminal digestion									
kg/d	2.57	3.09	0.33	0.32	NS	0.66	0.78	0.03	0.05
% of intake	30.5	36.7	4.1	0.36	NS	0.08	0.76	0.01	0.08
% of duodenal passage	83.3	84.0	1.9	0.80	NS	0.10	NS	0.18	NS
Apparent total-tract digestion									
kg/d	7.92	8.03	0.13	0.45	NS	<0.001	0.32	0.009	0.04
%	93.6	94.1	0.5	0.52	NS	0.12	NS	NS	NS

[1]*P*-values for treatment (Trt), Trt by period interaction (Trt × Period), preliminary DMI (pDMI), Trt by preliminary pDMI interaction (Trt × pDMI), quadratic effect of pDMI (pDMI × pDMI), and Trt by quadratic effect of pDMI (Trt × pDMI × pDMI).

[2]Nonsignificant, with $P > 0.20$; term was removed from the statistical model.

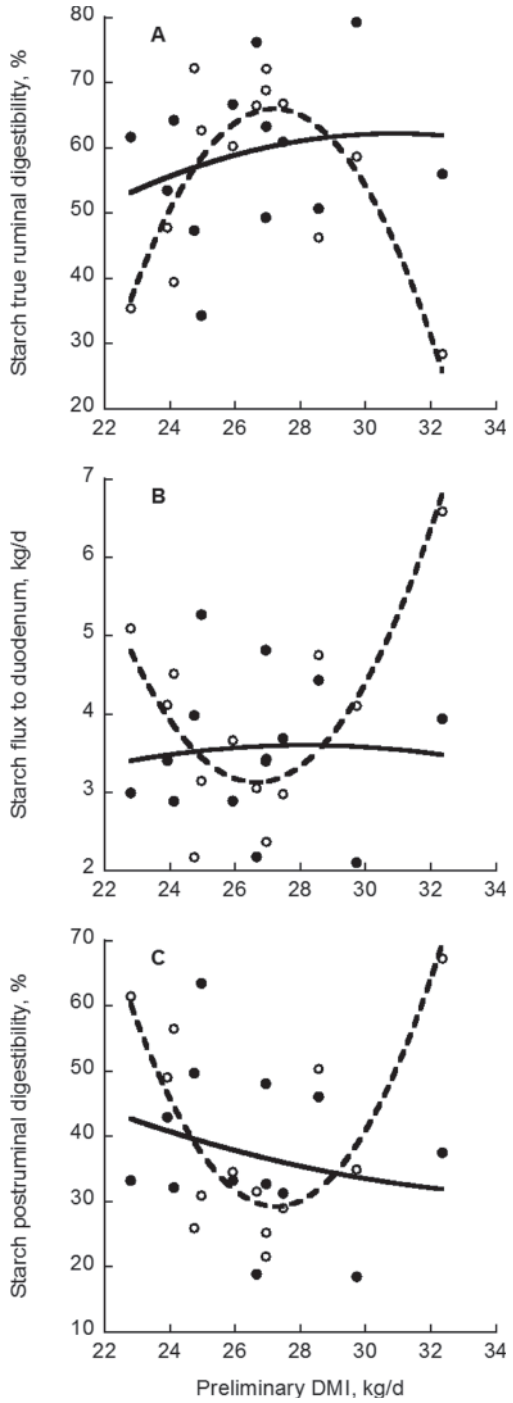

Figure 3. Interaction of long (19 mm; open circles, dashed line) and short (10 mm; closed circles, solid line) alfalfa particle length with preliminary DMI for starch (A) true ruminal digestibility (interaction: $P = 0.09$ quadratic; long: $P = 0.002$, $R^2 = 0.71$; short: $P = 0.77$, $R^2 = 0.05$), (B) flux from the rumen to duodenum (interaction: $P = 0.05$ quadratic; long: $P = 0.002$, $R^2 = 0.72$; short: $P = 0.98$, $R^2 = 0.004$), and (C) postruminal digestibility (interaction: $P = 0.08$ quadratic; long: $P = 0.002$, $R^2 = 0.70$; short: $P = 0.74$, $R^2 = 0.06$). The preliminary DMI on the x-axis are the mean DMI of individual cows during the final 4 d of the preliminary period when all cows were fed a common diet. The best-fit lines are drawn to demonstrate the significant interaction even if the individual relationships are not significant.

OM digestion (kg/d, interaction $P = 0.11$, quadratic) and digestibility (%, interaction $P = 0.03$, quadratic; Table 9) were due to effects on starch digestion (Table 7, Figure 3).

Although differences in ruminal digestion were detected, FPL and its interaction with pDMI did not affect rumen pH ($P > 0.19$), which was 6.26 for LC and SC, or total VFA concentration ($P > 0.48$; Table 10). However, LC tended to increase concentrations of butyrate (19.2 vs. 18.3 m*M*, $P = 0.06$) and valerate (2.32 vs. 2.20 m*M*, $P = 0.06$) compared with SC (Table 10).

Effects of pDMI on Ruminal Passage Rates

Experimental data on rates of passage from the rumen, particularly for individual feed fractions, are scarce. Given the effect of passage on ruminal digestibility and pool sizes and microbial growth, quantitative knowledge on rates of nutrient passage from the rumen are needed to better understand nutrient availability in ruminants and improve nutrition models. Furthermore, because passage rates from the rumen generally increase with increased intake, measurements of ruminal passage rates of nutrients over a wide range of DMI are necessary. We measured the effects of DMI on rates of passage of feed fractions from the rumen using the pool and flux method (Robinson et al., 1987).

We expected ruminal passage rates to increase with pDMI, but passage rates of pdNDF, iNDF, and starch were not related to level of intake either independent of or dependent upon treatment (Table 5). These results are consistent with the previously mentioned experiment of similar design evaluating the effects of grass FPL (Kammes and Allen, 2012). Although passage rates were not related to pDMI in either study, rates of ruminal digestion of starch and pdNDF were related to pDMI in that study, which were not observed in the present experiment.

Effects of Treatment and pDMI on N Flux and Microbial Efficiency

Flux of NANMN passed from the rumen to the duodenum was related to pDMI, which increased at a slower rate for LC than for SC (interaction $P = 0.03$; Figure 5). The increase in NANMN flux contributed to increased NAN flux as pDMI increased (interaction $P = 0.02$; Table 11), as level of intake did not affect MN flux. Despite the increase in NAN flux with greater intake, postruminal digestion (g/d) and digestibility (%) were not related to pDMI (interaction $P \geq 0.21$; Table 11). When expressed as a percentage of duodenal NAN, NANMN and MN fluxes to the duodenum were related to pDMI (interaction $P = 0.008$; Table 11). As

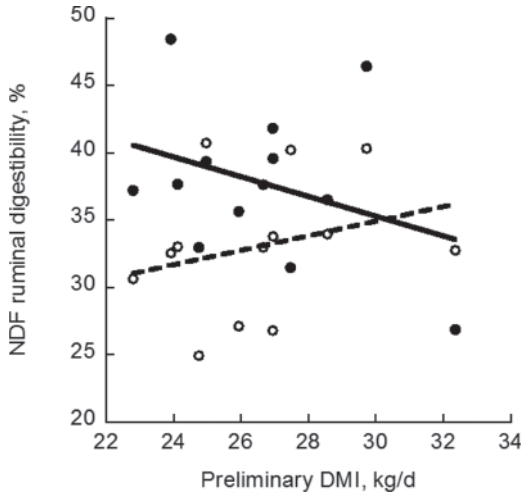

Figure 4. Interaction of long (19 mm; open circles, dashed line) and short (10 mm; closed circles, solid line) alfalfa particle length with preliminary DMI for NDF ruminal digestibility (interaction: $P = 0.09$ linear; long: $P = 0.36$, $R^2 = 0.08$; short: $P = 0.27$, $R^2 = 0.11$). The preliminary DMI on the x-axis are the mean DMI of individual cows during the final 4 d of the preliminary period when all cows were fed a common diet. The best-fit lines are drawn to demonstrate the significant interaction even if the individual relationships are not significant.

pDMI increased, LC decreased NANMN flux and SC increased it (Figure 6A), and the reverse was observed for MN flux (Figure 6B). Microbial N flux was highly related to the rate of ruminal digestion of starch for LC ($P = 0.004$, $R^2 = 0.71$) but not SC ($P = 0.78$, $R^2 = 0.05$; Figure 7A). Similarly, MN flux was highly related to true ruminal OM digestion (kg/d) for LC ($P = 0.002$, $R^2 = 0.75$) but not SC ($P = 0.25$, $R^2 = 0.26$; Figure 7B). Microbial efficiency was not related to level of intake either independent of or dependent upon treatment ($P \geq 0.25$; Table 11). The LC diet tended to increase ruminal ammonia concentration (16.4 vs. 14.3 mg/dl, $P = 0.06$; Table 11) compared with SC. Despite being drier, long-cut AL had higher ammonia (6.32 vs. 4.95 m*M*, Table 2) than short-cut AL, which may be the source for the greater ruminal ammonia concentration observed for cows consuming the LC diet.

CONCLUSIONS

In addition to treatment differences in particle length, forages differed in iNDF concentration (iNDF as a proportion of total NDF was 7.9 percentage units higher for long-cut AL than for short-cut AL) for unknown reasons, despite our efforts to prevent potentially con-

Table 8. Neutral detergent fiber digestion of cows fed treatment diets containing either long-cut (19 mm) or short-cut (10 mm) alfalfa silage as the sole source of forage

	Treatment LSM			*P*-value[1]					
Item	Long	Short	SE	Trt	Trt × Period	pDMI	Trt × pDMI	pDMI × pDMI	Trt × pDMI × pDMI
NDF									
Intake, kg/d	6.17	6.72	0.13	<0.001	0.16	0.001	NS[2]	0.08	NS
Ruminal digestion									
kg/d	1.99	2.50	0.09	<0.001	0.02	0.13	NS	NS	NS
%	33.1	38.1	1.3	0.01	0.08	0.90	0.09	NS	NS
Passage to duodenum, kg/d	4.03	4.07	0.12	0.78	NS	0.02	0.15	NS	NS
Postruminal digestion									
kg/d	−0.58	−0.27	0.14	0.09	NS	0.37	0.15	NS	NS
Total-tract digestion									
kg/d	1.40	2.22	0.07	<0.001	NS	NS	NS	NS	NS
%	23.7	34.0	1.2	<0.001	NS	0.07	NS	NS	NS
Potentially digestible NDF									
Intake, kg/d	2.31	2.95	0.05	<0.001	0.006	<0.001	0.03	0.04	0.10
Ruminal digestion									
kg/d	1.99	2.50	0.09	<0.001	0.02	0.13	NS	NS	NS
%	87.7	87.1	3.2	0.88	NS	0.95	0.10	NS	NS
Passage to duodenum, kg/d	0.28	0.37	0.08	0.43	NS	0.64	0.10	NS	NS
Postruminal digestion									
kg/d	−0.58	−0.27	0.14	0.09	NS	0.37	0.15	NS	NS
Total-tract digestion									
kg/d	1.40	2.22	0.07	<0.001	NS	NS	NS	NS	NS
%	63.5	78.0	3.1	0.009	NS	0.10	NS	NS	NS
Indigestible NDF									
Intake, kg/d	3.84	3.79	0.08	0.56	NS	0.001	NS	0.07	NS

[1]*P*-values for treatment (Trt), Trt by period interaction (Trt × Period), preliminary DMI (pDMI), Trt by pDMI interaction (Trt × pDMI), quadratic effect of pDMI (pDMI × pDMI), and Trt by quadratic effect of pDMI (Trt × pDMI × pDMI).

[2]Nonsignificant, with $P > 0.20$; term was removed from the statistical model.

Table 9. Dry matter and OM digestion of cows fed treatment diets containing either long-cut (19 mm) or short-cut (10 mm) alfalfa silage as the sole source of forage

	Treatment LSM			*P*-value[1]					
Item	Long	Short	SE	Trt	Trt × Period	pDMI	Trt × pDMI	pDMI × pDMI	Trt × pDMI × pDMI
DM									
Intake, kg/d	26.3	27.2	0.5	0.10	NS[2]	<0.001	0.34	0.03	0.18
Apparent total-tract digestion									
kg/d	17.3	18.2	0.3	0.01	0.13	<0.001	NS	0.02	NS
%	65.6	67.6	0.6	0.04	NS	0.07	NS	NS	NS
OM									
Intake, kg/d	24.6	25.3	0.5	0.18	NS	<0.001	0.36	0.03	0.18
Apparent ruminal digestion									
kg/d	11.6	11.8	0.4	0.65	NS	0.002	NS	<0.001	NS
%	47.8	45.8	1.8	0.47	NS	0.17	0.32	0.004	0.18
True ruminal digestion									
kg/d	15.2	14.9	0.6	0.68	NS	0.005	0.51	0.006	0.11
%	61.8	58.9	1.9	0.31	NS	0.21	0.21	0.01	0.03
Passage to duodenum, kg/d	12.9	13.7	0.5	0.23	NS	0.09	0.25	0.24	0.09
Apparent postruminal digestion									
kg/d	4.62	5.74	0.45	0.10	NS	0.76	0.21	0.03	0.14
% of intake	20.1	21.7	1.6	0.45	NS	0.06	NS	0.005	NS
Apparent total-tract digestion									
kg/d	16.4	17.2	0.2	0.02	0.11	<0.001	NS	0.01	NS
%	66.6	68.6	0.5	0.03	NS	0.05	NS	NS	NS

[1]*P*-values for treatment (Trt), Trt by period interaction (Trt × Period), preliminary DMI (pDMI), Trt by pDMI interaction (Trt × pDMI), quadratic effect of pDMI (pDMI × pDMI), and Trt by quadratic effect of pDMI (Trt × pDMI × pDMI).
[2]Nonsignificant, with $P > 0.20$; term was removed from the statistical model.

founding errors. Legume particle length and its interaction with pDMI did not affect milk yield or rumen pH. The LC diet decreased milk fat concentration more per kilogram of pDMI increase and increased yields of milk fat and fat-corrected milk less per kilogram of pDMI increase than did the SC diet. The LC diet tended to decrease DMI compared with the SC diet. Ruminal digestion and passage rates of feed fractions did not differ between LC and SC and were not related to level of intake. The LC diet tended to decrease rate of ruminal turnover for NDF but increased NDF rumen pools at a slower rate than the SC diet as pDMI increased. This indicated that the faster NDF turnover rate was not sufficient to counterbalance the higher DMI for SC, resulting in larger NDF rumen pools for SC than LC. As pDMI increased, LC increased ruminal digestibilities

Table 10. Ruminal VFA concentrations and pH of cows fed treatment diets containing either long-cut (19 mm) or short-cut (10 mm) alfalfa silage as the sole source of forage

	Treatment LSM			*P*-value[1]					
Item	Long	Short	SE	Trt	Trt × Period	pDMI	Trt × pDMI	pDMI × pDMI	Trt × pDMI × pDMI
Total VFA, m*M*	139	138	2	0.48	NS[2]	0.15	NS	NS	NS
Acetate	84.4	84.8	1.3	0.57	NS	NS	NS	NS	NS
Propionate	29.9	29.3	0.6	0.40	0.03	0.04	NS	NS	NS
Butyrate	19.2	18.3	0.4	0.06	0.05	0.11	NS	NS	NS
Lactate	0.37	0.81	0.24	0.21	NS	NS	NS	NS	NS
Isobutyrate	1.37	1.39	0.07	0.89	NS	0.43	0.19	0.49	0.19
Valerate	2.32	2.20	0.05	0.06	NS	NS	NS	NS	NS
Isovalerate	2.10	2.13	0.09	0.78	NS	0.55	0.15	NS	NS
Branched-chain VFA[3]	3.54	3.50	0.12	0.82	NS	NS	NS	NS	NS
Acetate:Propionate	2.85	2.90	0.04	0.32	0.008	0.08	NS	NS	NS
Ruminal pH	6.26	6.26	0.04	0.99	0.01	0.06	0.94	0.86	0.19

[1]*P*-values for treatment (Trt), Trt by period interaction (Trt × Period), preliminary DMI (pDMI), Trt by pDMI interaction (Trt × pDMI), quadratic effect of pDMI (pDMI × pDMI), and Trt by quadratic effect of pDMI (Trt × pDMI × pDMI).
[2]Nonsignificant, with $P > 0.20$; term was removed from the statistical model.
[3]Branched-chain VFA include isobutyrate and isovalerate.

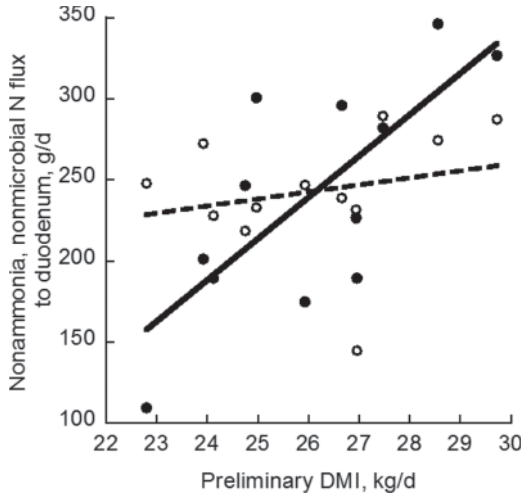

Figure 5. Interaction of long (19 mm; open circles, dashed line) and short (10 mm; closed circles, solid line) alfalfa particle length with preliminary DMI for nonammonia, nonmicrobial N flux from the rumen to duodenum (interaction: $P = 0.03$ linear; long: $P = 0.48$, $R^2 = 0.05$; short: $P = 0.007$, $R^2 = 0.53$). The preliminary DMI on the x-axis are the mean DMI of individual cows during the final 4 d of the preliminary period when all cows were fed a common diet. The best-fit lines are drawn to demonstrate the significant interaction even if the individual relationships are not significant.

of pdNDF and total NDF and SC decreased them, but total-tract digestibilities of pdNDF, total NDF, OM, and DM were lower for LC than SC. When legume silage was the only source of forage in the diet, increasing the chop length from 10 to 19 mm tended to decrease DMI but did not negatively affect productivity of cows.

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Table 11. Nitrogen metabolism of cows fed treatment diets containing either long-cut (19 mm) or short-cut (10 mm) alfalfa silage as the sole source of forage

	Treatment LSM			*P*-value[1]					
Item	Long	Short	SE	Trt	Trt × Period	pDMI	Trt × pDMI	pDMI × pDMI	Trt × pDMI × pDMI
N intake, g/d	793	790	13	0.86	0.13	0.001	NS[2]	NS	NS
Ruminal ammonia, mg/dL	16.4	14.3	0.8	0.06	NS	NS	NS	NS	NS
Flow to duodenum									
Ammonia N, g/d	12.4	11.7	0.6	0.42	NS	NS	NS	NS	NS
NAN									
g/d	559	568	21	0.71	NS	0.02	NS	NS	NS
% of N intake	70.2	71.9	1.8	0.58	NS	NS	NS	NS	NS
NANMN[3]									
g/d	243	241	13	0.90	NS	0.03	0.03	NS	NS
% of N intake	30.9	30.3	1.9	0.78	NS	0.39	0.04	NS	NS
% of duodenal NAN	42.0	43.1	3.5	0.68	NS	0.63	0.008	0.79	0.13
Microbial N									
g/d	316	328	23	0.60	NS	0.40	0.20	NS	NS
% of duodenal NAN	58.0	56.9	3.5	0.68	NS	0.63	0.008	0.79	0.13
g/kg TRDOM[4]	22.5	22.9	1.2	0.77	NS	NS	NS	NS	NS
NAN apparent postruminal digestion									
g/d	297	319	19	0.38	NS	NS	NS	NS	NS
% of N intake	37.4	40.5	2.0	0.29	NS	NS	NS	NS	NS
% of duodenal passage	52.8	56.1	1.7	0.21	NS	NS	NS	NS	NS
N apparent total-tract digestion									
g/d	531	541	11	0.37	0.06	0.02	NS	NS	NS
%	67.1	68.6	0.9	0.27	NS	0.17	NS	NS	NS

[1]*P*-values for treatment (Trt), Trt by period interaction (Trt × Period), preliminary DMI (pDMI), Trt by pDMI interaction (Trt × pDMI), quadratic effect of pDMI (pDMI × pDMI), and Trt by quadratic effect of pDMI (Trt × pDMI × pDMI).

[2]Nonsignificant, with $P > 0.20$; term was removed from the statistical model.

[3]NANMN = nonammonia, nonmicrobial nitrogen.

[4]TRDOM = true ruminally digested OM.

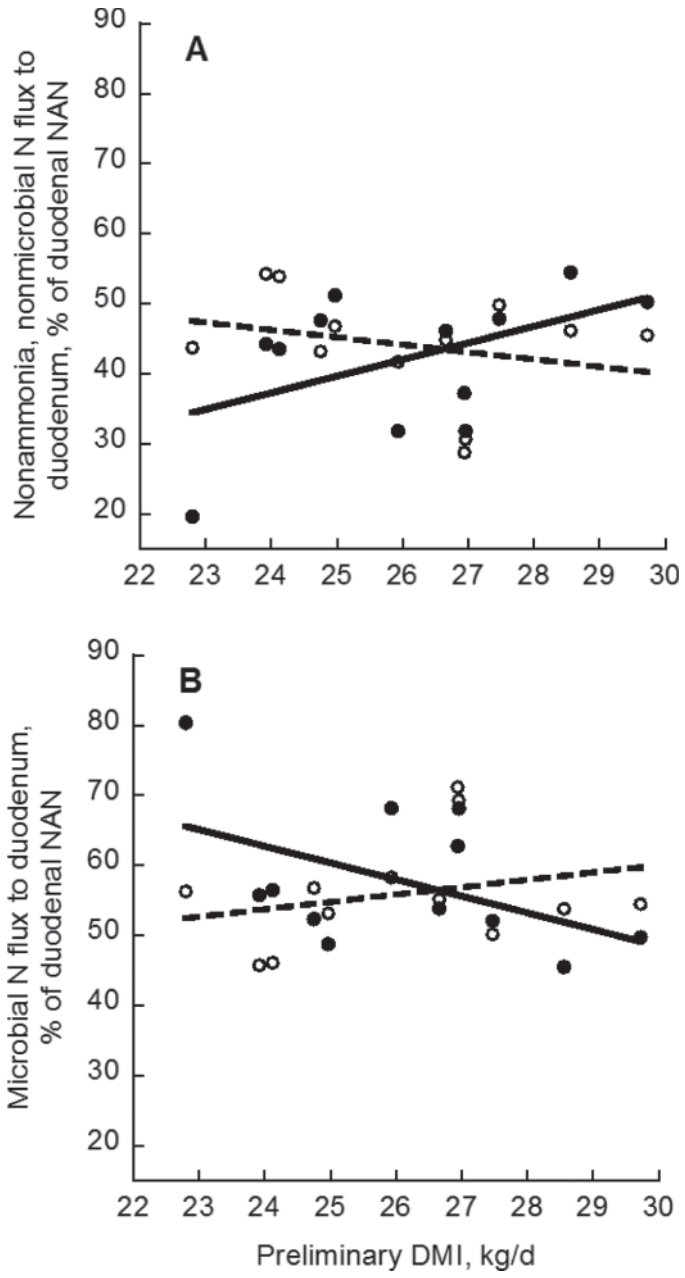

Figure 6. Interaction of long (19 mm; open circles, dashed line) and short (10 mm; closed circles, solid line) alfalfa particle length with preliminary DMI for (A) nonammonia, nonmicrobial N (interaction: $P = 0.008$ linear; long: $P = 0.39$, $R^2 = 0.07$; short: $P = 0.12$, $R^2 = 0.23$) and (B) microbial N (interaction: $P = 0.008$ linear; long: $P = 0.39$, $R^2 = 0.07$; short: $P = 0.12$, $R^2 = 0.23$) fluxes from the rumen to duodenum expressed as percent of duodenal NAN. The preliminary DMI on the x-axis are the mean DMI of individual cows during the final 4 d of the preliminary period when all cows were fed a common diet. The best-fit lines are drawn to demonstrate the significant interaction even if the individual relationships are not significant.

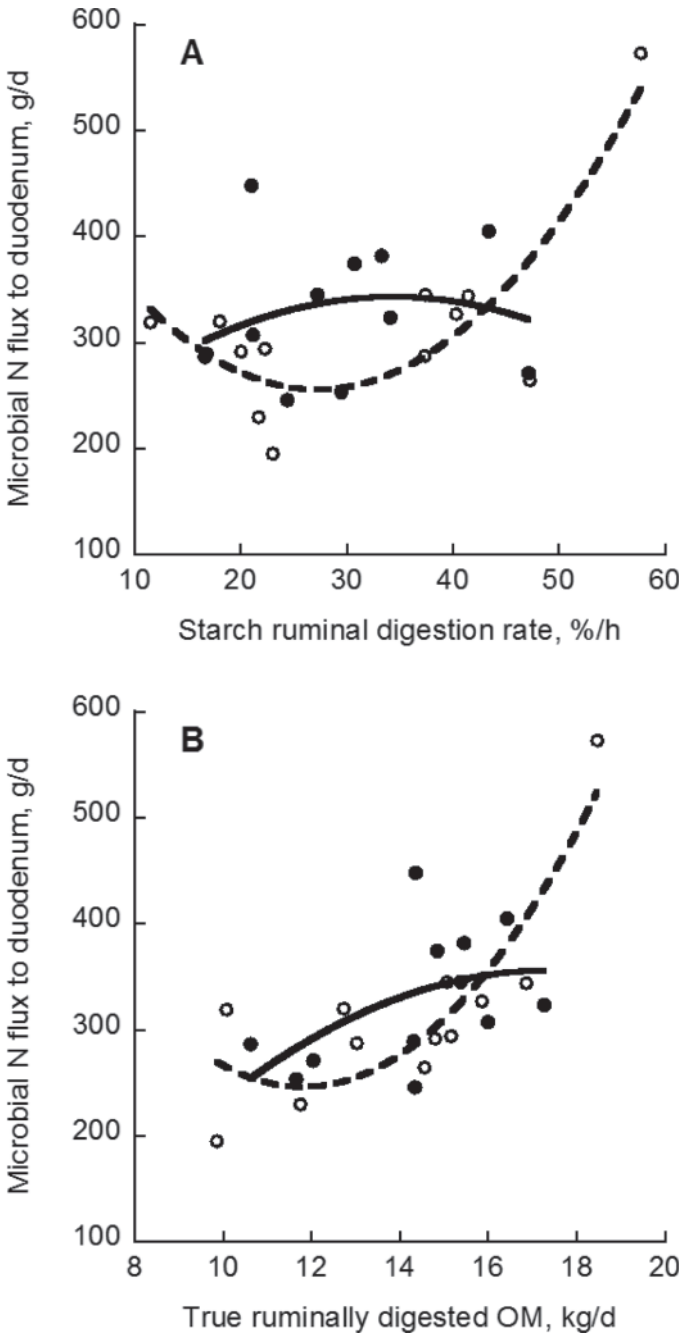

Figure 7. (A) Relationship between starch ruminal digestion rate (k_d) and microbial N (MN) flux from rumen to duodenum for long (open circles, dashed line; MN flux, g/d = {205 + (1.76 × starch ruminal k_d, %/h) + [0.306 × (starch ruminal k_d, %/h − 30.1)^2]}; $P = 0.004$, $R^2 = 0.71$) and short (closed circles, solid line; $P = 0.78$, $R^2 = 0.05$) alfalfa particle length. (B) Relationship between true ruminally digested OM (TRDOM) and MN flux from rumen to duodenum for long (MN flux, g/d = {−145 + (30.0 × TRDOM, kg/d) + [6.27 × (TRDOM, kg/d − 14.2)^2]}; $P = 0.002$, $R^2 = 0.75$) and short ($P = 0.25$, $R^2 = 0.26$) alfalfa particle length.

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