EFFECTS OF FORAGE CHARACTERISTICS AND VOLUNTARY FEED INTAKE ON RUMINAL PASSAGE OF DIGESTA FRACTIONS IN LACTATING DAIRY COWS

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ABSTRACT

EFFECTS OF FORAGE CHARACTERISTICS AND VOLUNTARY FEED INTAKE ON RUMINAL PASSAGE OF DIGESTA FRACTIONS IN LACTATING DAIRY COWS

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Passage from the rumen is a dynamic, complicated process that involves numerous animal and feed factors. Ruminal passage rates affect intake, digestibility, and the amount and type of fermentation endproducts and protein available to dairy cows. The success of existing nutrition models is limited by the lack of data for passage rate of individual digesta fractions, and the inability to account for effects of feed intake and forage characteristics of passage rates of specific feed fractions. These limitations can be addressed using the pool and flux method, which allows measurement of rates of passage for fractions within feeds, and an experimental design optimized to assess the interactions of diet characteristics and dry matter intake (DMI).

Five experiments were conducted to evaluate the effects of level of feed intake on the response of passage rate of digesta fractions in dairy cows to forage characteristics. All experiments utilized ruminally and duodenally cannulated cows with a wide range of DMI in a crossover design, where DMI was measured during a preliminary period (pDMI), and rates of digestion and passage of feed fractions were determined by the pool and flux method. The use of pDMI, an index of nutrient demand, allowed the evaluation of treatments on animal responses in relation to level of intake and effects of intake level independent of treatments. Level of intake ranged from approximately 20 to 30 kg dry matter per day among cows on the five experiments, and each experiment evaluated individual pairs of forage treatments including: 1) legume particle size, 2) grass particle size, 3) legume maturity, 4) grass maturity, and 5) forage family. Alfalfa and orchardgrass were selected as a representative legume and grass, respectively, and were the

sole source of forage used in diets, which were formulated to contain a similar forage neutral detergent fiber concentration among diets within experiment. We hypothesized that the normal variation in diet characteristics related to forage alters both the passage rates of feed fractions and the extent to which passage rates of these fractions are affected by voluntary DMI.

In general, it has been accepted that ruminal passage rates, microbial nitrogen flow to the duodenum, and efficiency of microbial synthesis increase with DMI; however, this is likely an oversimplification based on our research. Results from these experiments demonstrated passage rates of individual digesta fractions from the rumen were highly variable at a given level of voluntary DMI across cow periods, depended upon the forage characteristic being evaluated, and were inconsistent among the forage treatments evaluated. Additionally, microbial efficiency was not related to level of intake but was related to other factors, which varied among the experiments and included passage rates of starch and potentially digestible neutral detergent fiber, digestion rate of starch, and amount of true ruminally digested organic matter. Furthermore, there was no evidence that the filling effect of diets affected feed intake differently for cows with high intake compared to cows with low intake for any of the forage treatments evaluated. Finally, this research illustrated the complexity of ruminal passage and emphasized the difficulty involved in accurately predicting ruminal passage and digestibility.

Although the effects of DMI on passage rates are not consistent, these experiments provide absolute passage rates of digesta fractions for use in the development of equations to predict ruminal digesta passage and the foundation for additional research in this area. Data obtained from these experiments and others using the pool and flux method can be compiled and used in a meta-analysis, with the potential to discover relationships. The results will improve the accuracy of nutrition models to predict nutrient intake, passage, and utilization in dairy cows.

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KEY TO ABBREVIATIONS

ADF	Acid detergent fiber
ADL	Acid detergent lignin
AL	Alfalfa
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemists
BCS	Body condition score
BW	Body weight
CLA	Conjugated linoleic acid
CNCPS	Cornell Net Carbohydrate Protein System
CP	Crude protein
DM	Dry matter
DIM	Days in milk
DMI	Dry matter intake
DHIA	Dairy Herd Improvement Association
EARLY	Diet containing early maturity forage
FCM	Fat-corrected milk
FPL	Forage particle length
HPLC	High performance liquid chromatography
iNDF	Indigestible neutral detergent fiber
k_d	Rate of digestion
k_p	Rate of passage

k_r	Rate of particle size reduction
L	Large particle (≥ 2.36 mm)
LATE	Diet containing late maturity forage
LONG	Diet containing forage with theoretical length of cut of 19 mm
LSM	Least squares mean
MFD	Milk fat depression
MN	Microbial nitrogen
MUN	Milk urea nitrogen
NAN	Nonammonia nitrogen
NANMN	Nonammonia, nonmicrobial nitrogen
NDF	Neutral detergent fiber
NDFD	Neutral detergent fiber digestibility
NRC	National Research Council
NS	Not significant
OG	Orchardgrass
OM	Organic matter
pDMI	Preliminary dry matter intake
pdNDF	Potentially digestible neutral detergent fiber
R^2	Coefficient of determination
S	Small particle (< 2.36 mm)
SD	Standard deviation
SHORT	Diet containing forage with theoretical length of cut of 10 mm
SNF	Solids-not-fat

TDN	Total digestible nutrient
TLC	Theoretic length of cut
TMR	Total mixed ration
TRDOM	True ruminally digested organic matter
VFA	Volatile fatty acids

CHAPTER 1

A Review of Literature

OVERVIEW

Passage from the rumen is a dynamic, complicated process that involves numerous animal and feed factors. Passage of liquid and particulate matter from the rumen occurs through the reticular-omasal orifice, and the flow of particles can be restricted by particle size, particle density, and particle entrapment within the rumen mat. Ruminal passage rates of nutrients affect feed intake, nutrient digestibility, and the amount and type of fermentation endproducts and protein available to dairy cows. The accuracy of existing nutrition models are hampered by limited availability of accurate passage rate data, especially for feed fractions, and lack of information on how passage rates are affected by dry matter intake (DMI) and dietary characteristics. Although the importance of digesta passage and its role in the performance of lactating dairy cows is recognized, a limited understanding of the process and lack of accurate quantitative measures of passage rates for feed fractions greatly hinders the ability of ruminant nutrition models to accurately predict ruminal digestion and passage.

RUMINAL DIGESTION AND PASSAGE

Feeds that enter the rumen can disappear by two processes: digestion or passage (Figure 1). Rates of digestion and passage are important determinants of ruminal digestibility (Waldo et al., 1972), which is calculated using the following equation: ruminal digestibility (%) = $[k_d/(k_d+k_p)]*100$; where k_d = rate of digestion (%/h) and k_p = rate of passage (%/h). This equation is applicable to homogenous feed fractions only as both digestion rate and passage rate

can vary widely among fractions within an individual feed ingredient. The processes of digestion and passage compete against one another affecting digestibility; digestibility increases as digestion rate increases or as passage rate decreases (i.e. retention time in the rumen increases). Retention time is the reciprocal of fractional rate of passage. Given the impact of passage on feed intake, ruminal digestibility, and microbial growth, a greater understanding of the influence of animal and dietary characteristics and their interactions on ruminal passage of particulate matter and quantitative knowledge of rates of nutrient passage from the rumen are necessary.

CONSTRAINTS TO PASSAGE FROM THE RUMEN

Passage rate of fibrous particles largely depends on the reduction of particle size and the increase in particle specific gravity, which are required for particles to escape the rumen mat, sink to the ventral rumen, and exit the rumen via the reticular-omasal orifice (Sutherland, 1988). Particle size reduction is mainly a result of chewing during eating and ruminating (Kennedy, 1985). Although digestion has little direct effect on particle breakdown (Murphy and Nicoletti, 1984), it affects the rate of reduction from chewing by increasing tissue fragility (Chai et al., 1984). Differences in fragility of forage particles affect particle size reduction rate and retention time in the rumen (McLeod and Minson, 1988).

Particle Size

Resistance to flow from the rumen increases with particle size (Poppi et al., 1980; Dixon and Milligan, 1985), and passage rate from the rumen increases exponentially with decreasing particle size (Lechner-Doll et al., 1991). Poppi et al. (1980) suggested a threshold size in which particles greater than the defined size have increased resistance to passage, and this was

determined to be particles retained on sieves with apertures of 1.18 mm for sheep (Poppi et al., 1980) and 3 to 4 mm for dairy cows (Cardoza and Mertens, 1986) and steers (Dixon and Milligan, 1985). However, the threshold size likely varies with physical form of forages, DMI, and neutral detergent fiber (NDF) concentration in the diet (Van Soest et al., 1988).

Particle Density

Although particle size reduction is a prerequisite to flow from the reticulorumen, it is not always a constraint because most of the particulate matter in the rumen is smaller than the maximum particle size of the feces (Allen, 1996). This suggests that particle size reduction is not the only requirement for particles to flow from the rumen. Particle density is also a constraint to flow; a negative relationship between particle density and retention time in the rumen exists (Allen, 1996). Ruminal retention time decreased from 91 to 19 h as density of inert particles increased from 0.9 to 1.5 g/ml across experiments reported in the literature (Lechner-Doll et al., 1991). Despite a true specific gravity of 1.3 to 1.5 (Siciliano-Jones and Murphy, 1991), most digesta particles in the rumen are buoyant (Sutherland, 1988) which decreases their probability of escape. Buoyancy is the result of retention of fermentation gases in and on particles (Hooper and Welch, 1985). Its effect lessens as digestion progresses (Hooper and Welch, 1985; Nocek and Kohn, 1987; Siciliano-Jones and Murphy, 1991) because the digestible portion of the particle or the ability of the cells to retain gas or both are diminished (Jung and Allen, 1995), which increases the likelihood of passage. Forages differ greatly in buoyancy over time because of differences in both concentration and digestion rate of potentially digestible NDF (pdNDF; Jung and Allen, 1995) and because of differences in their anatomic structure that affect their ability to retain gases (Wilson et al., 1989).

Ruminal Mat

The distribution of particles, including stratification of long fibrous particles into layers, in the rumen forms a mat that functions effectively as a retaining mechanism (Sutherland, 1988) with less dense, longer material on top and more dense, smaller particles sinking to the bottom. The ruminal mat has high selectivity for large particles (Sutherland, 1988), and long forage particles entrap smaller particles from other feeds increasing ruminal retention time and digestibility (Grant, 1997). Escape from the ruminal mat has been identified as a rate-limiting step to the passage of forage particles (Poppi et al., 2001).

Selective Retention

Particles within the rumen can be defined as potentially escapable or non-escapable based on size and density, and the rate at which a non-escapable particle becomes potentially escapable depends on the rate of particle size reduction and change in density and buoyancy (Allen and Mertens, 1988). This suggests that passage is not random. Instead, it is mediated by selective retention of undigested fibrous particles in the rumen, a phenomenon that evolved in ruminants to maximize ruminal fiber digestion (Allen and Mertens, 1988), and involves the ruminal mat previously discussed. Although fibrous particles contain both indigestible and digestible fractions, it is reasoned that the likelihood of particles to escape the rumen should increase as the particle increases in indigestible material; therefore, the passage rate of indigestible NDF (iNDF) should be greater than that of pdNDF (Huhtanen et al., 1995). Most studies have reported selective retention of pdNDF compared to iNDF (Tamminga et al., 1989; Rinne et al., 2002; Huhtanen et al., 2007; Lund et al., 2007). However, Stensig and Robinson (1997) reported a faster rate of passage of iNDF than that of pdNDF for timothy but not alfalfa. Passage rates of

pdNDF and iNDF might vary with the distribution of these fractions within plant parts and with differences in buoyancy over time (Firkins et al, 1998).

There is no single rate-limiting component to flow from the rumen, and more research is needed to expand our knowledge in particle dynamics and their effects on passage rate. This was explored further in a comparison of alfalfa and orchardgrass reported in Chapter 7 of this dissertation.

RUMINANT NUTRITION MODELS

A basic goal of ruminant research has been to improve animal production and reduce excretion of waste, which requires unraveling the mechanisms that influence nutrient utilization by the animal. Therefore, extensive research has been conducted over several decades to increase our knowledge in areas including, but not limited to, feeds and feeding management, intake, digestion and passage rates, escape of dietary protein, and microbial growth efficiency to estimate energy, nutrient requirements and supply, and feed utilization for various feeding situations. This research provided a large volume of scientific knowledge, and mathematical models were generated to integrate various aspects of nutrient utilization in an effort to advance diet formulation, predict animal responses, and explain biological mechanisms.

Ruminant nutrition models have evolved for nearly 55 years to predict requirements and supply of energy and nutrients based on animal characteristics, diet composition, and environmental factors. Nutrition models have been developed over time for different purposes, such as research, diet formulation, and evaluation of management strategies (Illius and Allen, 1994). Recent advances in modeling nutrient utilization in ruminants are reported by Kebreab et al. (2009). Models vary in complexity and may be classified as empirical or mechanistic as well

as static or dynamic; however, the accuracy of each is greatly affected by its ability to model digestion in and passage from the rumen. Existing mechanistic models have limited prediction accuracy, particularly across a wide range of conditions (Firkins et al., 1998; Offner and Sauvant, 2004; Tedeschi et al., 2005), and they must be improved, especially with an accurate prediction of passage rate, before they will be capable of contributing to the improvement of nutrient utilization (Allen, 2011).

Although discussion of the numerous models available and their specifics are beyond the scope of this literature review, two major ruminant nutrient models used for diet formulation, namely the Nutrient Requirements of Dairy Cattle (NRC, 2001) and the Cornell Net Carbohydrate Protein System (CNCPS; Fox et al., 2004), are briefly described here as they are referred to later in the review. The seventh edition of the NRC was published 10 years ago and provides a summary of current reference information for dairy cattle nutrition, which has been included in a ration evaluation program. The current CNCPS model was designed to formulate farm specific feeding programs and evaluate nutrient excretion and efficiency of use of nutrients. Both models contain a stand-alone computer software program.

Limitations of Ruminant Nutrition Models

Major limitations contributing to the inability of models to accurately predict passage rates of digesta fractions (e.g. starch, indigestible fiber, and digestible fiber) have been outlined by Allen (2011) and include the lack of available data for passage rates of feed fractions and the effects of voluntary DMI and dietary characteristics on passage rates of individual fractions. These limitations and methods to obtain accurate, quantitative information needed to further the development of models are discussed.

MEASUREMENTS OF RUMINAL RATES OF DIGESTION AND PASSAGE

Models of nutrient digestion and utilization in dairy cows need to predict the ruminal digestibility of specific nutrients. The ruminal digestibility of any homogeneous nutrient fraction can be calculated by dividing its rate of digestion by its total rate of disappearance from the rumen, which is the sum of its rate of digestion and rate of passage. Both digestion rate and passage rate can vary widely among fractions within a single feed ingredient. Because the various fractions are digested and utilized at different rates and through different mechanisms, digestibility and digestion rate are measured for feed fractions rather than entire feeds, but data for passage rates of feed fractions are lacking (Allen, 2011).

Rate of digestion of individual feed fractions is often determined by measuring nutrient disappearance *in vitro* or *in situ* at a number of time points, and data are readily available in the literature. However, these data are not useful for models because they provide relative measurements, not absolute measurements of digestion rates of feed fractions, which are required to predict ruminal digestibility (Allen, 2011). The estimation of rate of passage is done through an independent procedure based on using external markers applied to intact forages and concentrates because of the cost and difficulty involved in directly determining passage rate, thus standard values are often used. Because these passage rates are inaccurate and apply to entire feeds, they are not useful in models to predict digestibility of fractions within feeds across a wide range of conditions (Allen, 2011).

Current models, such as NRC and CNCPS, use the digestion rates of individual fractions but passage rates of entire feeds derived from experiments using external markers to predict digestibility of feed fractions because passage rate of fractions are not available. This is problematic because differences in fraction solubility, particle size, and buoyancy result in

different passage rates for the various fractions within a feed (Allen, 2011). The use of the same overall passage rate for all fractions within feeds will overestimate ruminal digestibility of soluble fractions and small particles with faster rates of passage and will underestimate ruminal digestibility of large particles with much slower rates of passage (Allen, 2011).

In an attempt to improve model prediction, the NRC (2001) and CNCPS (Seo et al., 2006) use separate passage rate prediction equations for concentrates and forages, and NRC made further distinctions in passage rates for dry or wet forages. Mean passage rates of dry forages, wet forages, and concentrates were 4.5, 5.2, and 6.7%/h, respectively, based on rare earth markers (Seo et al., 2006, Table 1). These passage rates for forages were twice as high as the mean passage rate of pdNDF (2.4%/h), and the passage rate for concentrates was less than one half the mean passage rate for starch (15.3%/h; Table 1) based on 315 records in 11 experiments using the pool and flux method (Voelker Linton, 2006). The similar passage rates obtained for forages and concentrates when using rare earth markers is likely because markers migrate from labeled feeds (Teeter et al. 1984; Combs et al., 1992), preferentially bind to small particles (Erdman and Smith, 1985), and therefore do not accurately represent the passage rate of the feed originally labeled (Allen, 2011). The pool and flux method is the only method capable of accurately measuring passage rates of individual feed fractions and is discussed below.

Krizsan et al. (2010) reported the NRC and CNCPS models overestimated ruminal passage rate (i.e. underestimated retention time of particulate matter in the rumen) based on a meta-analysis of 172 treatment means from 49 studies that fed a range of forages and used the pool and flux method. Evaluation of the NRC and CNCPS models with the observed data resulted in both significant ($P < 0.001$) mean biases of -2.40 and -1.70%/h and linear biases of -0.59 and -0.53, respectively (Krizsan et al., 2010). The above discrepancies stress the need for

accurate data for incorporation into models to improve accuracy of predictions over a wide range of conditions. Because the digestibility of most nutrients in the rumen is limited by their retention times, which vary widely across feeding situations, approaches that integrate the kinetics of digestion with the kinetics of passage are required.

Pool and Flux Method

Although accurate passage rates of feed fractions are not currently available for use in models, these rates can be accurately measured using the pool and flux method (Robinson et al., 1987). This method allows the simultaneous measurements of rate of digestion and rate of passage of individual feed fractions. Passage rate is calculated by dividing duodenal flux of an individual digesta fraction by its ruminal pool size. Additionally, the total rate of disappearance (turnover rate) of a digesta fraction from the rumen at steady state can be calculated by dividing its rate of intake by its ruminal pool size. The digestion rate of the fraction can then be calculated by subtracting its passage rate from its total rate of disappearance in the rumen. Because digestion and passage rates of each fraction are calculated simultaneously, these rates are consistent with both pool size and ruminal digestibility (Allen, 2011). Therefore, the information is compatible with models that partition feeds into fractions with more uniform rates of digestion and passage. Data using this method are scarce because it requires duodenally and ruminally cannulated cows and data collection and sample analysis are labor intensive. However, the pool and flux method can be used to measure passage rates of feed fractions and provide the data needed to improve the various models of nutrient availability in ruminants. This method was used for all research presented in the following chapters of this dissertation.

EFFECTS OF DMI ON PASSAGE RATE

Passage rate from the rumen increases with DMI (Riewe and Lippke, 1970). This likely occurs through effects of increased distention on the rate of reticular contractions (Dado and Allen, 1995) and on the amplitude and duration of reticular contractions (Okine and Mathison, 1991a). The proportion of large particles in the rumen increases with higher DMI (Okine and Mathison, 1991b), probably because ruminating time per unit of DM consumed decreases as DMI increases (Welch and Smith, 1969; Luginbuhl et al., 1989). Additionally, fecal particle size increased with increasing DMI in several experiments suggesting that increased ruminal fill from greater DMI probably increases passage rate from the rumen through regulation at the reticular-omasal orifice (Allen, 1996).

Diet digestibility generally decreases as a result of the increased passage rate. However, the effects of feeding level on digestibility are not constant but vary with diet composition. As feeding level increased, high concentrate diets decreased ruminal retention time more than low concentrate diets (Colucci et al., 1982, 1990) and concentrate particles had a faster passage rate than forage particles (Colucci et al., 1990; Wylie et al., 2000). An indirect comparison showed effects of feeding level on digestibility are greater for corn silage-based diets (Gabel et al., 2003) than for grass silage-based diets (Volden, 1999). Diets with a high digestibility at maintenance exhibited greater depressions in digestibility with increased intake (Huhtanen et al., 2009). Depression in digestibility with increased DMI is greater for higher concentrate diets because the rapid digestion of concentrates (primarily starch) can lower ruminal pH and subsequently reduce fiber digestion (NRC, 2001). Additionally, recent reports of the relative decrease in organic matter digestibility per multiple of maintenance feeding were 2.0% (Huhtanen et al., 2009) and 2.5% (Yan et al., 2002) for grass silage-based diets and 3.2% (Gabel et al., 2003) for primarily

corn silage-based diets. These values are smaller than the 4% reported by Tyrrel and Moe (1975), which was adopted by NRC (2001).

Predictions for passage rate from a mechanic model developed to predict rate of passage of forage particles in dairy cattle was most sensitive to intake (Seo et al., 2009). This indicates the importance of including accurate DMI in models, but the inconsistencies on the extent of depression in digestibility mentioned above in response to increased feeding level are difficult to account for in nutrition models. Some models have adjusted energy values for the level of feeding to improve predictions, but Huhtanen et al. (2009) reported the NRC and CNCPS models overestimated the total digestible nutrient (TDN) discount for dairy cows with DMI greater than maintenance intake based on a meta-analysis comprised of 497 treatment means from 92 studies that fed mainly grass silage-based diets. Residual analysis of the NRC and CNCPS models resulted in both significant ($P < 0.001$) mean biases of -0.77 and -0.55 kg/d and linear biases of -0.55 and -0.41, respectively (Huhtanen et al., 2009).

Data are lacking for the effect of nutrient intake above 4X maintenance on digestibility and passage rate. These data are needed because nutrient intake at this level is now common among dairy cows (Vandehaar, 1998; NRC, 2001), and the linear relationship between DMI and passage rate should not be extrapolated beyond the limits of the DMI in the existing data (NRC, 2001). In fact, the decline in digestibility with increasing DMI may not be linear but rather digestibility might decrease at a decreasing rate as DMI increases (Van Soest et al., 1992). Fiber digestibility is not only affected by forage source and retention time in the rumen but also by the fermentability of other carbohydrate sources in the ration. Therefore, any measurement of the effects of diet characteristics on ruminal passage rate and the resulting effects on digestibility must also account for the interactions between DMI and the effects of diet on passage rate.

EFFECTS OF FEED CHARACTERISTICS ON PASSAGE RATE

Rate of passage of individual feed fractions are not constant and are greatly affected by other dietary and animal factors and their interactions. This is well summarized by Nousiainen et al. (2009) who stated that digestibility of dairy cow diets is influenced by intrinsic digestion characteristics of the diet and extrinsic factors that influence the extent to which the intrinsic digestion potential is achieved. The NRC subcommittee recognized that intrinsic properties of feeds affect passage rate but concluded "data are too sparse to make adjustments for those factors" (NRC, 2001). Evaluation of the effects of specific forage and concentrate factors on ruminal digestibility in studies where only one specific factor varied demonstrated that, in addition to the effects of feeding level, many other factors had a significant effect on digestibility (Nousiainen et al., 2009) suggesting the need to include other factors in models for accurate prediction.

Forages and Rumen Fill

The primary constraints to passage from the rumen vary greatly among forages. Not only is forage fiber retained in the rumen longer and more filling than other feed components because of its bulkiness, fibrous forage particles also form a rumen mat capable of sequestration of other feed particles and increase their retention time. The physical filling effect of diets is related to forage NDF concentration and the digestion characteristics of forage NDF (Allen, 2000), including the proportion of iNDF fraction, the rate of digestion of pdNDF fraction, and the retention time of feed particles in the rumen. Most of the variation in organic matter digestibility for cows consuming feed above maintenance level was related to changes in the concentration and digestibility of the NDF fraction in the diet (Nousiainen et al., 2009). Enhanced NDF

digestibility within a forage family decreases gut fill and has greater potential effect on DMI as milk yield increases (Allen, 2000). Neutral detergent fiber from cool-season grasses, although more digestible than NDF from legumes, is generally more filling and has a greater benefit when DMI is not limited by rumen distention (Voelker Linton and Allen, 2007).

Rumen fill can limit feed intake, especially for high producing cows and cows fed high forage diets (Allen et al., 2005). Although most studies report a significant decrease in DMI as forage NDF increased, the DMI response is variable, depending upon the degree to which intake is limited by ruminal fill. Dry matter intake of high producing cows (earlier lactation, higher nutrient demand) is limited by the filling effects of diets to a greater extent than for low producing cows (later lactation, lower nutrient demand) consuming the same diet (Voelker et al., 2002). As lactation proceeds and milk yield declines, feed intake is increasingly dominated by metabolic signals rather than rumen fill (Allen et al., 2009). Highly fermentable diets often decrease feed intake in mid to late lactation, likely from stimulation of hepatic oxidation by propionate (Allen et al., 2009). When fill limits intake, passage rate can increase as a mechanism allowing the animal to increase its intake even though ruminal and total tract digestibility may be reduced (Jung and Allen, 1995).

Forages and Chewing Activity

Concentrations of NDF and its source are associated with chewing activities, which can affect ruminal passage. Forage NDF is positively related to total chewing time, which stimulates the flow of saliva necessary to neutralize fermentation acids and maintain adequate pH levels for proper rumen function (Allen, 1997). As rumen pH decreases, ruminal motility (Ash and Dobson, 1963) and rate of fiber digestion (Oba and Allen, 2003) are reduced. Thus, low ruminal pH exerts negative effects on digestion and passage, rumen fill, and possibly intake.

Additionally, rumination contributes to particle size reduction and increases specific gravity of forages (Welch and Hooper, 1988) increasing their probability of passage.

Because forages have a greater effect on digesta passage rate than most other feeds, the effect of forage characteristics on ruminal passage rate is the focus of this dissertation and specific forage characteristics are discussed later.

Experimental Model

The response of dietary characteristics, and the extent to which physical and metabolic factors affect DMI, are dependent on individual energy balance (Mertens, 1994; Allen, 1996). Therefore, testing only overall treatment mean differences may mask important responses in intake, digestion, and production (Allen, 2000). Because cows are now frequently grouped and fed according to milk yield, models that predict effects of feed intake level on response to diet are necessary.

An experimental model to evaluate effects of preliminary DMI or milk yield, indicators of nutrient demand, on animal responses to dietary treatments has been developed and used for more than a decade (Oba and Allen, 1999a; Burato et al., 2001; Voelker et al., 2002; Harvatine and Allen, 2002; Bradford and Allen, 2004; Voelker Linton and Allen, 2007, 2008). The design is a crossover model with a preliminary period utilizing a group of cows with a wide range and uniform distribution of preliminary DMI or milk yield. A common, intermediate diet is fed during the preliminary period, which is used to determine preliminary DMI. This experimental model uses relatively short periods to minimize period effects and decrease the possibility of a treatment by period interaction while allowing sufficient time for diet adaptation. Post peak lactation cows only are used in this model because the rapid rise in milk yield and DMI during the first 50-60 days after calving can cause treatment by period interactions. After peak milk

production, a gradual decline in milk yield occurs which is not expected to result in a treatment by period interaction. This model was used for all research presented in the following chapters of this dissertation.

FORAGE FACTORS AFFECTING DIGESTA PASSAGE RATES

Diet characteristics related to forage type and to forage harvesting and management practices that are expected to have the greatest effect on passage rate of digesta fractions are discussed here. Forage NDF concentration, forage NDF digestibility, forage particle size, forage maturity, and forage family are the chosen topics for this section; however, many other dietary factors influence passage rates.

Forage NDF Concentration

Forage NDF concentration is a major factor that affects intake and production of dairy cows because fiber is the least digestible component in feeds and its bulkiness occupies more space in the rumen than other feed constituents. The effect of NDF concentration on DMI depends on the mechanism that is controlling intake. A summarization of 15 studies by Allen (2000) showed a general decline in DMI with increasing NDF concentrations when diets exceeded 25% NDF. Neutral detergent fiber has rumen filling characteristics, and high forage NDF concentration in a diet increases rumen distention (Dado and Allen, 1995), which can limit voluntary DMI (Allen, 2000). Additionally, Voelker et al. (2002) found high forage diets limit voluntary DMI to a greater extent as milk yield increases. Because the filling effect of a diet is highly dependent on the rates of digestion and passage, research including measurements of ruminal kinetics are necessary to determine the underlying reason for the decrease in DMI observed when high NDF diets are fed.

Two experiments have been conducted to evaluate the effects of forage NDF concentration on passage rates of feed fractions using the pool and flux method. One study compared low and high dietary NDF diets (29% and 38% NDF, respectively) by feeding a corn silage-based diet using either brown midrib or normal corn silage; the low NDF diet increased the passage rates of pdNDF (3.49 vs. 2.43%/h, $P < 0.001$) and starch (14.5 vs. 9.00%/h, $P < 0.001$) but did not affect the passage rate of iNDF compared with the high NDF diet (Oba and Allen, 2000). Another study compared low and high dietary NDF diets (24% and 31% NDF, respectively) by feeding a 2:1 mixture of corn silage and alfalfa silage-based diet that varied in forage:concentrate ratio (45:55 and 61:39, respectively); the low NDF diet increased the passage rates of iNDF (4.84 vs. 4.36%/h, $P < 0.01$) and starch (25.4 vs. 18.8%/h, $P < 0.01$) and tended to increase the passage rate of pdNDF (1.57 vs. 1.04%/h, $P = 0.06$) compared with the high NDF diet (calculated from data reported in Voelker Linton and Allen, 2007). The inconsistent results on the effects of forage NDF concentration on passage rate of iNDF might be because of differences in forage sources or NDF concentrations between these experiments. In general, low NDF diets increased passage rates compared with high NDF diets based on these two studies, which may allow greater DMI for cows fed low NDF diets than high NDF diets. Additional research is needed to confirm this generalization.

Forage NDF Digestibility

Digestibility of NDF is an important forage parameter that influences intake and performance of dairy cows. Because fiber fractions ferment slower and are retained in the rumen longer, they have a greater effect on ruminal fill than nonfiber fractions. However, ruminal digestibility of forage NDF is highly variable ranging from less than 35% to over 75% for various forage types (Nocek and Russell, 1988). Oba and Allen (1999b) compiled treatment

means for 13 sets of forage comparisons differing in NDF digestibility (NDFD) from several experiments reported in the literature. They concluded that enhanced NDFD of forage within forage family improved DMI and milk yield; a one-unit increase in NDFD *in vitro* or *in situ* was associated with a 0.17 kg increase in DMI and a 0.25 kg increase in 4% fat corrected milk yield. Measurements of ruminal kinetics are needed to explain the fundamental reason for the increase in DMI and milk production observed when high NDFD forages are fed.

Effects of forage NDFD on passage rates of feed fractions using the pool and flux method have been evaluated. In a comparison of brown midrib corn silage and normal corn silage with a 9.4 percentage unit difference in NDF digestibility, the brown midrib corn silage with higher NDFD increased the passage rates of iNDF (3.64 vs. 3.20%/h, $P < 0.001$) and starch (12.9 vs. 10.6%/h, $P = 0.02$) but did not affect passage rate of pdNDF compared to the normal corn silage with lower NDFD (Oba and Allen, 2000). Overall, high NDFD forage increased passage rates compared with the low NDFD forage based on this single study. The faster passage rates, especially for iNDF, might permit greater DMI for cows fed high NDFD forage compared with low NDFD diet; however, more research is required to verify this statement. Although it is difficult to conduct experiments evaluating forage NDFD that are not confounded by differences in fiber source or forage:concentrate ratio, this research is needed.

Forage Particle Size

Sufficient amounts of forage in both chemical and physical forms are necessary for proper rumen function in dairy cows. The current NRC (2001) guidelines provide recommendations for minimum chemical concentrations of total NDF and forage NDF but do not account for physical form or particle size of feeds. Forage fiber, in a form that is physically effective, is necessary in dairy cow diets to promote rumen health and cow performance.

Insufficient particle size may reduce rumen pH, impair fiber digestion, and increase incidence of health disorders including ruminal acidosis and displaced abomasum. Increasing forage particle size increases ruminal retention time (Dixon and Milligan, 1985) and promotes formation of the rumen mat (Grant, 1997). Although impaired rumen fermentation and function can result when cattle are fed rations lacking in physical structure, excessive amounts of long, coarse fiber may limit intake (Allen, 2000).

Effects of particle size were evaluated for two forages including a legume (alfalfa) and a grass (orchardgrass) because of differences in anatomical structure and digestion characteristics affecting particle size reduction and passage (Allen, 1996) and are reported in Chapters 2 and 3 of this dissertation. Greater ruminal distention caused by longer forage particles is more likely to affect passage rate and DMI when feed intake is more limited by fill.

Forage Maturity

Rates of digestion, passage, and particle size reduction in the rumen are influenced by the fiber content of forage and the potential digestibility of the fiber (Mertens, 1993), which change during the development and growth of the plant. Because fiber has been related to the filling properties of feeds and forage fiber increases but its digestibility decreases as plants mature, the importance of physical rumen fill in limiting feed intake increases when feeding dairy cows a more mature forage compared to a less mature forage. Previous research showed highly lignified forages (i.e. more mature) remained in the rumen longer due to its slow rate of digestion, and possibly increased buoyancy, and resulted in lower DMI (Jung and Allen, 1995; Allen, 2000).

Effects of forage maturity on passage rates of feed fractions using the pool and flux method have been evaluated previously. In a comparison of grass silages harvested at four stages (1 week intervals), the passage rate of pdNDF tended to increase linearly and

quadratically ($P < 0.10$) and the passage rate of iNDF increased linearly ($P < 0.01$) as maturity increased (Rinne et al., 2002). According to Poppi et al. (1981) and Ulyatt (1983), increased maturity of grasses at harvest increased the rate of particle size reduction by chewing because of greater fragility. Thus, the increasing passage rate as maturity increased noted here might be because of greater fragility due to increased lignification.

Effects of maturity were evaluated for two forages including a legume (alfalfa) and a grass (orchardgrass) because of differences in anatomical structure and digestion characteristics affecting particle size reduction and passage (Allen, 1996) and are reported in Chapters 4 and 5 of this dissertation. Greater ruminal distention caused by increased maturity is more likely to affect passage rate and DMI when feed intake is more limited by fill.

Forage Family

Fiber particles from different forage families (legumes and grasses) have different chemical compositions, anatomical characteristics, and digestion characteristics that affect the rate and extent of digestion (Allen, 1996; Wilson and Kennedy, 1996). Cows fed grass-based diets had lower DMI and milk yield than cows fed legume-based diets despite greater NDF digestibility for grass (Oba and Allen, 1999b). Voluntary DMI is probably more limited for grass forage because of its filling effect caused by slow particle breakdown or slow passage rate. Grass NDF is digested more slowly than alfalfa NDF, and its cell walls are more resistant to particle breakdown than are alfalfa cell walls (Wilson and Hatfield, 1997). Because grass NDF is more digestible but also more slowly digested, digestion is extended over a greater period of time for grass particles, increasing their buoyancy over time compared to alfalfa particles (Allen, 1996). Because passage rate increases as density increases and as particle size decreases, alfalfa

particles probably escape the rumen more quickly than grass particles possibly allowing for greater DMI.

Evaluation of the effects of forage family on passage rates of fiber fractions, measured using the pool and flux method, yielded opposing results for relative passage rates of iNDF and pdNDF. An experiment comparing alfalfa silage to orchardgrass silage-based diets formulated to similar forage NDF concentrations reported alfalfa tended to increase iNDF passage rate compared to orchardgrass (2.9 vs. 2.4%/h, $P = 0.06$) but there was no difference in pdNDF passage rate between the two species (Voelker Linton and Allen, 2008). In a comparison of alfalfa silage and timothy silage-based diets, alfalfa increased pdNDF passage rate compared with timothy (2.4 vs. 1.8%/h, $P = 0.005$) but iNDF passage rates were similar (Stensig and Robinson, 1997). The reason for this discrepancy in passage rates is not apparent. Effects of forage family on passage rate were evaluated further and are reported in Chapter 6 of this dissertation.

RESEARCH OBJECTIVES AND HYPOTHESIS

The research reported in this dissertation was designed to determine the effect of voluntary DMI on ruminal passage rates of digesta fractions and how it is altered by forage characteristics. Five experiments were conducted using the pool and flux method and experimental model described in the literature review. The specific objective was to determine the effects of voluntary DMI on the response of passage rate of individual feed fractions to legume particle size (Chapter 2), grass particle size (Chapter 3), legume maturity (Chapter 4), grass maturity (Chapter 5), and forage family (legume vs. grass; Chapter 6). An additional objective of the forage family experiment was to the evaluate the relationships between

voluntary DMI and effects of forage family on rates of particle size reduction in, and particle passage from, the rumen (Chapter 7). Each experiment allowed the effects of the interaction between the treatment (forage characteristic) and DMI to be evaluated, direct comparison of treatment effects, and measurement of ruminal passage rates for individual feed fractions. We hypothesized that the normal variation in diet characteristics related to forages, including forage particle size, forage maturity, and forage family, alters both the passage rates of feed fractions and the extent to which passage rates of these fractions are affected by voluntary DMI.

APPENDIX

Table 1. Rate of passage of feeds determined by excretion pattern of rare earth markers in feces or of feed fractions by the pool and flux method

	Mean	Range
Rare earth markers[1]		
Dry forage	4.5	3.4 – 5.7
Wet forage	5.2	3.9 – 6.3
Concentrate	6.7	3.6 – 9.2
Pool and flux[2]		
Indigestible NDF	3.2	1.2 – 5.3
Potentially digestible NDF	2.4	0.2 – 4.3
Starch	15.3	3.4 – 33.9
Pool and flux[3]		
Indigestible NDF	2.6	1.1 – 5.1

[1]Seo et al., 2006; 319, 63, and 139 treatment means for dry forage, wet forage, and concentrate, respectively, from 275 published experiments.

[2]Voelker Linton, 2006; 315 cow periods from 11 experiments conducted in Allen laboratory at Michigan State University. Pool and flux calculations based on rumen pool size of indigestible NDF (iNDF) and iNDF flux to duodenum.

[3]Krizsan et al., 2010; 172 treatment means from 49 published experiments. Pool and flux calculations based on rumen pool size of iNDF and a combination of flux of iNDF from intake or fecal output.

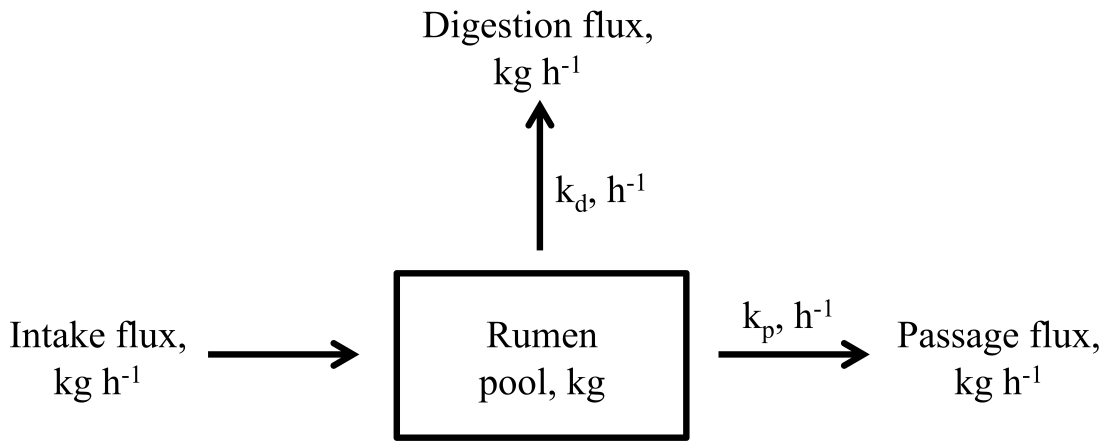

Figure 1. Simple first-order model of digestion and passage in the rumen for a feed fraction. The total rate of escape is the sum of the rate of digestion (k_d) and the rate of passage (k_p). The fraction digested (D) is calculated by dividing the rate of digestion by the total rate of escape ($D = k_d/(k_d+k_p)$); the fraction passed (P) is calculated by dividing the rate of passage by the total rate of escape ($P = k_p/(k_d+k_p)$). This is the basic subunit of many digestion and passage models, and it is applied to multiple feed fractions with similar rates of digestion and passage.

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CHAPTER 2

Nutrient Demand Interacts with Legume Particle Length to Affect Digestion Responses and Rumen Pool Sizes in Dairy Cows[1]

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 either a) 19 mm (LONG) or b) 10 mm (SHORT) theoretical length of cut as the sole forage. Alfalfa silages contained ~43% neutral detergent fiber (NDF); diets contained ~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. LONG decreased milk fat concentration more per kilogram pDMI increase than SHORT and increased yields of milk fat and fat-corrected milk less per kilogram pDMI increase than SHORT, resulting in a greater benefit for LONG at low pDMI and for SHORT at high pDMI. LONG tended to decrease DMI compared to SHORT. Ruminal digestion and passage rates of

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feed fractions did not differ between LONG and SHORT and were not related to level of intake. LONG tended to decrease the rate of ruminal turnover for NDF but increased NDF rumen pools at a slower rate than SHORT as pDMI increased. This indicated the faster NDF turnover rate did not counterbalance the higher DMI for SHORT resulting in higher NDF rumen pools for SHORT than LONG. As pDMI increased, LONG increased ruminal digestibility of potentially digestible NDF and total NDF and SHORT decreased them, but total tract digestibilities of potentially digestible NDF, total NDF, organic matter and dry matter were lower for LONG than SHORT. 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.

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 1983; 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 and 24 vs. 170 mm; Randby et al., 2008) have been used to evaluate FPL. Additionally, studies often evaluate 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), non-forage 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 to corn silage-based TMR (Tafaj et al., 2007). This suggests consideration of forage family when studying the effects of particle size is necessary. 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 will 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 three 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 2). 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 either a) 19 mm (LONG) or b) 10 mm (SHORT) theoretical length of cut (TLC) 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 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 ~43% NDF (DM basis; Table 3). Diets LONG and SHORT 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, Ralston, IA), vitamin-mineral premix, limestone, and salt (Table 4).

Data and Sample Collection

Throughout the experiment, cows were housed in tie-stalls and fed diets as total mixed rations once daily (1130 h) at 110% of expected intake. The amount of feed offered and refused (orts) was 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 to one composite sample per period prior to analysis.

Cows were moved to an exercise lot twice daily (0230 and 1300 h) prior to 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 was collected from the reticulum, near the reticular-omasal orifice, transported to the laboratory, and processed. Rumen fluid for pH, VFA, lactate, and ammonia were obtained by combining digesta from five 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.

Ruminal contents were evacuated manually through the ruminal cannula at 4.5 h after feeding at the beginning of d 18 (1600 h) and 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 yield recorded at both milkings 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 one 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 (Tri-Philizer 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 one

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 lignin (ADL), ADF nitrogen (forages only), CP, and starch. Ash concentration was determined after 5 h 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 (St. Joseph, MI). Indigestible NDF was estimated as NDF residue after 240 h in vitro fermentation (Goering and Van Soest, 1970); flasks were reinoculated at 120 h to insure 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 was blended, strained through nylon mesh, and the liquid portion was centrifuged at 500 x *g* for 15 min. The supernatant was centrifuged at 18,000 x *g* for 15 min, and the pellet was washed with 0.9% NaCl solution and centrifuged again at 18,000 x *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 concentration 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 ratio of purines to OM (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 the following equations:

Turnover rate (%/h) = 100 x (Intake of component / Ruminal pool of component) / 24

Passage rate (%/h) = 100 x (Duodenal flow of component / Ruminal pool of component) / 24

Digestion rate (%/h) = Turnover rate in the rumen (%/h) – Passage rate from the rumen (%/h).

Statistical Analysis

All data were analyzed by using the fit model procedure of JMP (Version 8, SAS Institute, 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: $Y_{ijk} = \mu + C_i + P_j + T_k + PT_{jk} + pDMI + T_kpDMI + pDMI^2 + T_kpDMI^2 + e_{ijk}$ where 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_kpDMI^2 is the interaction of treatment and pDMI (quadratic), and e_{ijk} is the residual error. Statistical significance for T_kpDMI and T_kpDMI^2 indicated 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. Tendencies 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, two cows were removed during the experiment (one cow injured a teat and the other cow went off feed). Additionally, data from one cow was excluded prior to 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 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, which included 12 cows. One cow (with 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 was removed.

RESULTS AND DISCUSSION

Comparison of Forages and Diets

Physical characteristics of AL are listed in Table 3. 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 particles < 8 mm was 13.7 percentage units lower (23.9 vs. 37.6%) for long than short cut AL, respectively.

Chemical analyses (Table 3) 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 percent 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 compared to short cut AL. Although the iNDF concentration was higher for long cut AL compared to short cut AL, in vitro NDF digestibility (30 h) of long cut AL was only 0.9 percentage unit lower than that of short cut AL. It is possible 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 4. 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 ~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. LONG had ~1 percentage unit lower total NDF and higher iNDF than SHORT, which resulted in the proportion of iNDF of total dietary NDF 5.9 percentage units higher for LONG. Differences in DM concentration in diets were because of the different DM concentrations of the forages. The calculated

concentrations of total NDF in LONG and SHORT and forage NDF in LONG 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 5. Response of milk fat concentration to FPL was related to pDMI, as indicated by a significant interaction between FPL and pDMI ($P = 0.01$); LONG decreased milk fat concentration more per kilogram pDMI increase than SHORT (Figure 2). This effect on concentration of milk fat influenced other treatment by pDMI interactions including milk fat yield ($P = 0.006$), FCM yield ($P = 0.03$), and efficiency (FCM/DMI, $P = 0.06$); LONG increased these responses less per kilogram pDMI increase than SHORT. The aforementioned interactions resulted in a greater benefit for LONG for cows with low pDMI and a greater benefit for SHORT for cows with high pDMI.

LONG tended to decrease DMI (26.3 vs. 27.2 kg/d, $P = 0.10$, Table 5) compared to SHORT. We expected LONG to be more filling than SHORT causing greater rumen distention and potentially limiting DMI, particularly in cows with high DMI for which ruminal distention is more likely to limit feed intake (Allen, 1996). Oba and Allen (1999) and Voelker et al. (2002) 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 to low fill diets as milk yield increased. We expected LONG to slow rates of ruminal passage but FPL and its interaction with pDMI did not affect the rates that pdNDF, iNDF, or starch passed from the rumen (Table 6).

LONG tended to decrease the rate of ruminal turnover of NDF (4.68 vs. 4.91%/h, $P =$

0.09, Table 6) compared to SHORT. Despite the slower turnover rate of NDF for LONG, the rumen pool of NDF was less for LONG compared to SHORT except for cows with the lowest or highest DMI (interaction $P = 0.08$, quadratic, Figure 3A). This indicated that the faster NDF turnover rate was not sufficient to counterbalance the higher DMI for SHORT resulting in larger NDF rumen pools for SHORT than LONG. Additionally, rumen digesta wet weight ($P = 0.03$, quadratic, Figure 3B) and volume ($P = 0.006$, quadratic, Figure 3C) were related to pDMI (Table 7); rumen digesta wet weight and volume were less for LONG compared to SHORT 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 ($P \geq 0.18$), a visual examination of a graph with pDMI and DMI (Figure 3D) illustrated the difference in DMI between LONG and SHORT was small for cows with low pDMI but the difference became greater as pDMI increased and then narrowed for cows with high pDMI. Because LONG had less rumen digesta mass and volume than SHORT for cows with the greatest reduction in DMI, it is unlikely that DMI for LONG was limited by rumen fill.

Feeding behavior was measured in another experiment of similar design (Kammes and Allen, accepted) 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 to 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 it is possible that DMI for cows consuming LONG was limited by chewing time.

Treatment interacted quadratically with pDMI to affect site of starch digestion (Table 8). True ruminal starch digestion (kg/d, interaction $P = 0.13$) and true ruminal starch digestibility (%, interaction $P = 0.09$, Figure 4A) were lower for LONG compared to SHORT for cows with

low and high pDMI. As a result, starch flux from the rumen to duodenum (interaction $P = 0.05$, Figure 4B) was greater for LONG compared to SHORT for cows with low and high pDMI. Postruminal starch digestion (kg/d, interaction $P = 0.05$) followed a similar pattern as starch flux, and postruminal starch digestibility (%, interaction $P = 0.08$, Figure 4C) 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 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 was statistically reanalyzed after the two 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, LONG increased ruminal digestibility of pdNDF and SHORT decreased it ($P = 0.10$, Table 9). Ruminal digestibility of NDF was lower for LONG compared to SHORT ($P = 0.01$, Table 9) and the differences were greater for cows with lower pDMI (interaction $P = 0.09$, Figure 5). Total tract digestibilities of pdNDF (63.5 vs. 78.0%, $P < 0.001$) and NDF (23.7 vs. 34.0%, $P < 0.001$) were lower for LONG than SHORT (Table 9). The lower digestibility of NDF for LONG may in part be due to the higher concentration of iNDF for long cut AL than short cut AL (Table 3) despite being harvested from the same field and having similar ensiling characteristics as previously discussed.

Total tract digestibilities of NDF (and pdNDF) are lower than ruminal digestibility because negative 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 Cr_2O_3 /d) from d 6 to d 17 with a priming dose of 2X on d 6], ADL, and ADL peroxide (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 the flow marker. The higher digestibility for total tract than in the rumen is 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), which is 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. While absolute values are not biologically reasonable, relative comparisons between treatments within the same experiment are useful.

As a result of NDF digestibility, LONG 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 to SHORT (Table 10). Interaction of treatment and pDMI for true ruminal OM digestion (kg/d, $P = 0.11$) and digestibility (%, $P = 0.03$, Table 10) were because of effects on starch digestion (Table 8, Figure 4).

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 LONG and SHORT, or total VFA concentration ($P > 0.48$, Table 11). However, LONG tended to increase concentrations of butyrate (19.2 vs. 18.3 mM, $P = 0.06$) and valerate (2.32 vs. 2.20 mM, $P = 0.06$) compared with SHORT (Table 11).

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 impact 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 7). These results are consistent with the previously mentioned experiment of similar design evaluating the effects of grass FPL (Kammes and Allen, accepted). 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 LONG than SHORT ($P = 0.03$, Figure 6). The increase in NANMN flux contributed to increased NAN flux as pDMI increased ($P = 0.02$, Table 12), 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 ($P \geq 0.21$, Table 12). When expressed as a percent of duodenal NAN, NANMN and MN fluxes to the duodenum were related to pDMI ($P = 0.008$, Table 12). As pDMI increased, LONG decreased NANMN flux and

SHORT increased it (Figure 7A), and the reverse was observed for MN flux (Figure 7B). Microbial N flux was highly related to the rate of ruminal digestion of starch for LONG (P = 0.004, R^2 = 0.71) but not SHORT (P = 0.78, R^2 = 0.05, Figure 8A). Similarly, MN flux was highly related to true ruminal OM digestion (kg/d) for LONG (P = 0.002, R^2 = 0.75) but not SHORT (P = 0.25, R^2 = 0.26, Figure 8B). Microbial efficiency was not related to level of intake either independent of or dependent upon treatment ($P \geq 0.25$, Table 12). LONG tended to increase ruminal ammonia concentration (16.4 vs. 14.3 mg/dl, P = 0.06, Table 12) compared to SHORT. Despite being drier, long cut AL had higher ammonia (6.32 vs. 4.95 mM, Table 3) than short cut AL, which may be the source for the greater ruminal ammonia concentration observed for cows consuming LONG.

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 short cut AL) for unknown reasons, despite our efforts to prevent potentially confounding errors. Legume particle length and its interaction with pDMI did not affect milk yield or rumen pH. LONG decreased milk fat concentration more per kilogram pDMI increase and increased yields of milk fat and fat-corrected milk less per kilogram pDMI increase than SHORT. LONG tended to decrease DMI compared to SHORT. Ruminal digestion and passage rates of feed fractions did not differ between LONG and SHORT and were not related to level of intake. LONG tended to decrease rate of ruminal turnover for NDF but increased NDF rumen pools at a slower rate than SHORT as pDMI increased. This indicated the faster NDF turnover

rate was not sufficient to counterbalance the higher DMI for SHORT resulting in larger NDF rumen pools for SHORT than LONG. As pDMI increased, LONG increased ruminal digestibilities of pdNDF and total NDF and SHORT decreased them, but total tract digestibilities of pdNDF, total NDF, OM and DM were lower for LONG than SHORT. 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.

APPENDIX

Table 2. 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	Standard deviation	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).

Table 3. Chemical composition, particle size distribution, and fermentation parameters of the long (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, % DM	92.0	90.9
NDF, % DM	42.3	44.0
iNDF[1], % 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[2], % of DM	8.91	9.05
CP, % DM	20.4	19.6
Starch, % DM	1.89	1.92
IV NDF digestibility[3], %	34.1	35.0
Particles size distribution[4]		
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[5], mm	14.1	8.1
Penn State Particle Separator, % 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, % DM	1.29	1.49
Propionic acid, % DM	0.08	0.07
Butyric acid, % DM	<0.01	<0.01
Lactic acid, % DM	5.40	6.46
Lactic:Acetic	4.19	4.34
Ethanol, % DM	0.10	0.08
Ammonia, mM	6.32	4.95

[1] iNDF = indigestible NDF.

Table 3 (cont'd)

[2]ADL = acid detergent lignin.
[3]30 h in vitro NDF digestibility.
[4]Particle size distributions of silages were measured each period (n = 2).
[5]Mean particle size calculated from particle size distribution determined by wet sieving.

Table 4. Ingredients and chemical composition of preliminary and treatment diets (as analyzed) containing either long (19 mm) or short cut (10 mm) alfalfa silage as the sole source of forage

	Preliminary	Long	Short
Ingredients, % 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®	4.00	4.02	3.92
Vitamin mineral mix[1]	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, % DM	93.4	93.4	92.8
NDF, % DM	26.9	24.5	25.5
% forage NDF	21.7	19.6	20.7
% NDF from forage	80.9	80.0	81.0
iNDF[2], % DM	NA[3]	15.5	14.6
iNDF, % of NDF	NA	63.2	57.3
CP, % DM	18.0	19.3	18.9
Starch, % DM	30.6	30.8	30.5

[1]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.

[2]iNDF = indigestible NDF.

[3]NA = no analysis for preliminary diet.

Table 5. Milk production and composition, feed intake, and BW change of cows fed treatment diets containing either long (19 mm) or short cut (10 mm) alfalfa silage as the sole source of forage

	Treatment LSM[1]			*P*[2]					
	Long	Short	SE	Trt	Trt x Period	pDMI	Trt x pDMI	pDMI x pDMI	Trt x pDMI x pDMI
Yield, kg/d									
Milk	37.7	37.4	3.3	0.67	NS	0.03	NS[3]	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	NS	0.11	0.006	0.20	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]Treatment least squares means.

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

[3]NS = not significant ($P > 0.20$); term removed from statistical model.

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

	Treatment LSM[1]			*P*[2]					
	Long	Short	SE	Trt	Trt x Period	pDMI	Trt x pDMI	pDMI x pDMI	Trt x pDMI x pDMI
Ruminal turnover rate, %/h									
DM	11.0	10.8	0.3	0.59	0.03	NS[3]	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[4]	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[5]	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	NS	NS	0.18	NS

[1]Treatment least squares means.

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

Table 6 (cont'd)

[3]NS = not significant ($P > 0.20$); term removed from statistical model.
[4]pdNDF = potentially digestible NDF.
[5]iNDF = indigestible NDF.

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

	Treatment LSM[1]			*P*[2]					
	Long	Short	SE	Trt	Trt x Period	pDMI	Trt x pDMI	pDMI x pDMI	Trt x pDMI x pDMI
Wet weight, kg	72.6	77.6	2.4	0.05	NS[3]	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[4]	0.74	0.85	0.06	0.27	NS	0.004	NS	NS	NS
iNDF[5]	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]Treatment least squares means.

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

[3]NS = not significant ($P > 0.20$); term removed from statistical model.

[4]pdNDF = potentially digestible NDF.

[5]iNDF = indigestible NDF.

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

	Treatment LSM[1]			*P*[2]					
	Long	Short	SE	Trt	Trt x Period	pDMI	Trt x pDMI	pDMI x pDMI	Trt x pDMI x pDMI
Starch									
Intake, kg/d	8.49	8.51	0.18	0.92	NS[3]	<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]Treatment least squares means.

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

[3]NS = not significant ($P > 0.20$); term removed from statistical model.

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

	Treatment LSM[1]			*P*[2]					
	Long	Short	SE	Trt	Trt x Period	pDMI	Trt x pDMI	pDMI x pDMI	Trt x pDMI x pDMI
NDF									
Intake, kg/d	6.17	6.72	0.13	<0.001	0.16	0.001	NS[3]	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]Treatment least squares means.

Table 9 (cont'd)

[2]*P*-values for treatment (Trt), treatment by period interaction (Trt x Period), preliminary DMI (pDMI), treatment by preliminary DMI interaction (Trt x pDMI), quadratic effect of preliminary DMI (pDMI x pDMI), and treatment by quadratic effect of preliminary DMI (Trt x pDMI x pDMI).
[3]NS = not significant ($P > 0.20$); term removed from statistical model.

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

	Treatment LSM[1]			*P*[2]					
	Long	Short	SE	Trt	Trt x Period	pDMI	Trt x pDMI	pDMI x pDMI	Trt x pDMI x pDMI
DM									
Intake, kg/d	26.3	27.2	0.5	0.10	NS[3]	<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]Treatment least squares means.

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

Table 10 (cont'd)

[3]NS = not significant ($P > 0.20$); term removed from statistical model.

Table 11. Ruminal VFA concentrations and pH of cows fed treatment diets containing either long (19 mm) or short cut (10 mm) alfalfa silage as the sole source of forage

	Treatment LSM[1]			*P*[2]					
	Long	Short	SE	Trt	Trt x Period	pDMI	Trt x pDMI	pDMI x pDMI	Trt x pDMI x pDMI
Total VFA, mM	139	138	2	0.48	NS[3]	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
Branch chain VFA	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]Treatment least squares means.

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

[3]NS = not significant ($P > 0.20$); term removed from statistical model.

Table 12. Nitrogen metabolism of cows fed treatment diets containing either long (19 mm) or short cut (10 mm) alfalfa silage as the sole source of forage

	Treatment LSM[1]			*P*[2]					
	Long	Short	SE	Trt	Trt x Period	pDMI	Trt x pDMI	pDMI x pDMI	Trt x pDMI x pDMI
N intake, g/d	793	790	13	0.86	0.13	0.001	NS[3]	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[4]									
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[5]	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] Treatment least squares means.

Table 12 (cont'd)

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

[3]NS = not significant ($P > 0.20$); term removed from statistical model.

[4]NANMN = nonammonia, nonmicrobial nitrogen.

[5]TRDOM = true ruminally digested OM.

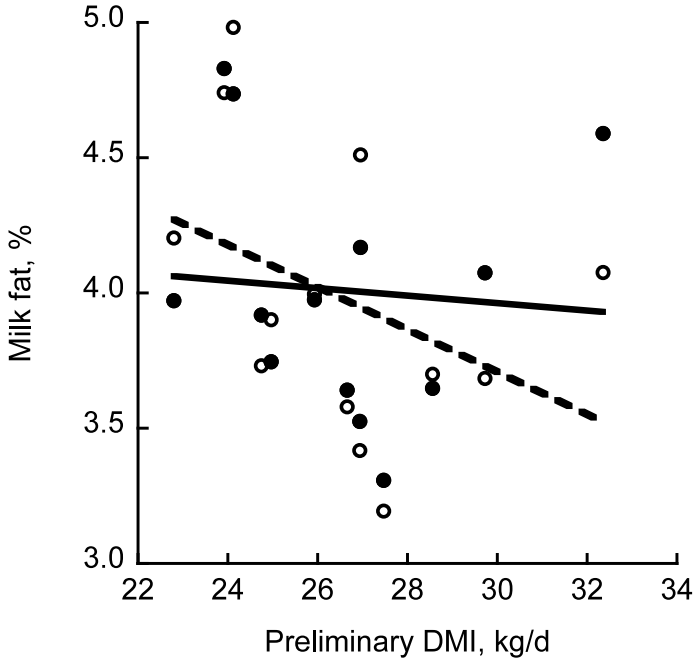

Figure 2. Interaction of long (19 mm; open circles, dashed line) and short (10 mm; closed circles, solid line) alfalfa particle length with preliminary DMI for milk fat concentration (P = 0.01). The preliminary DMI on the x-axis are the mean DMI of individual cows during the final 4 d of the preliminary period when all cows were fed a common diet.

Figure 3

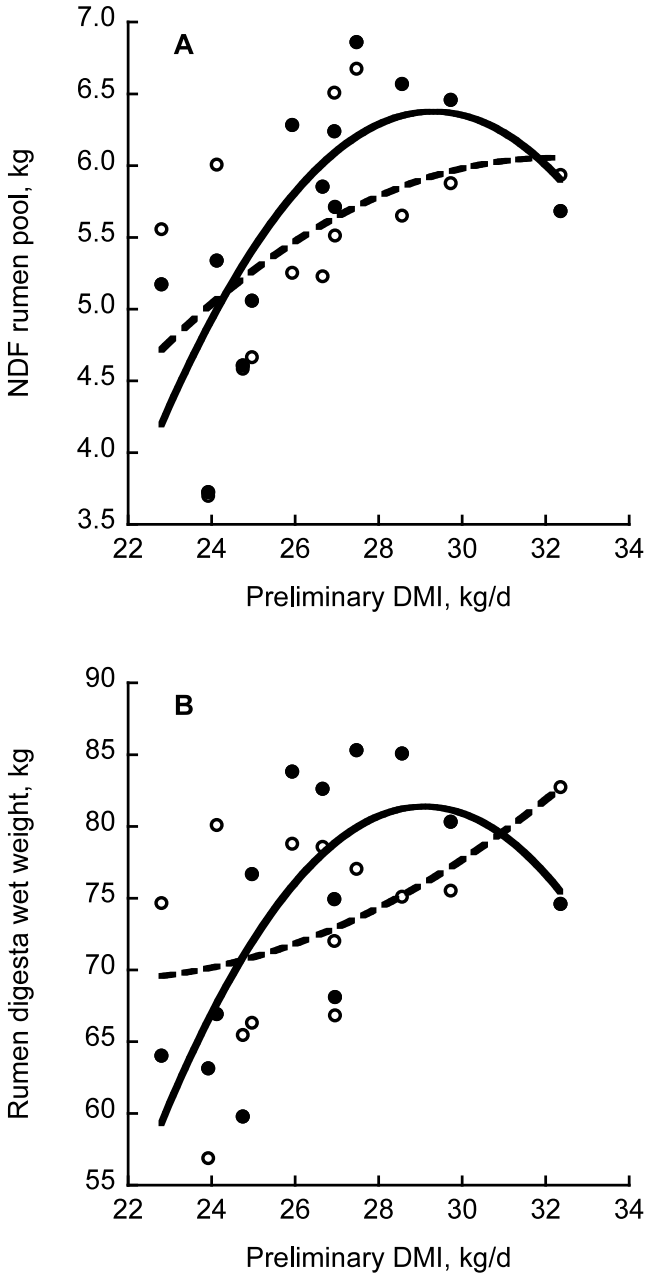
A
NDF rumen pool, kg
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
22
24
26
28
30
32
34
Preliminary DMI, kg/d
B
Rumen digesta wet weight, kg
90
85
80
75
70
65
60
55
22
24
26
28
30
32
34
Preliminary DMI, kg/d

Figure 3 (cont'd)

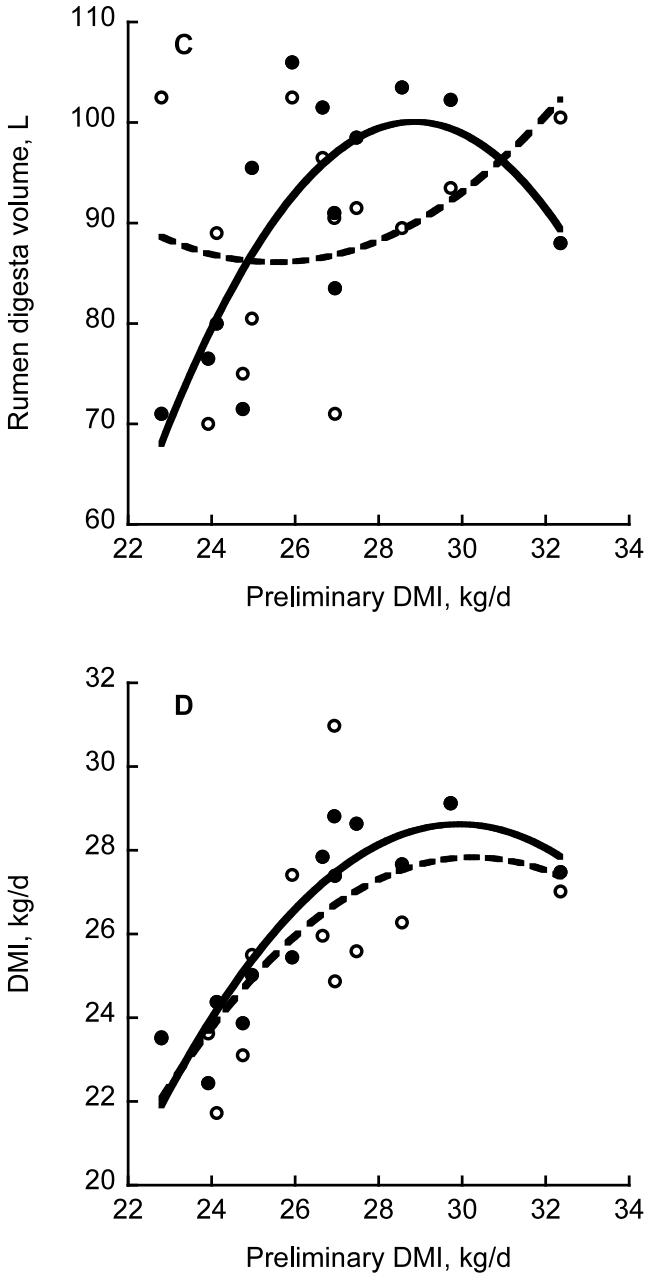

Figure 3. Interaction of long (19 mm; open circles, dashed line) and short (10 mm; closed circles, solid line) alfalfa particle length with preliminary DMI for A) NDF rumen pool (P = 0.08), B) rumen digesta wet weight (P = 0.03), C) rumen digesta volume (P = 0.006), and D) DMI (interaction not significant). 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.

Figure 4

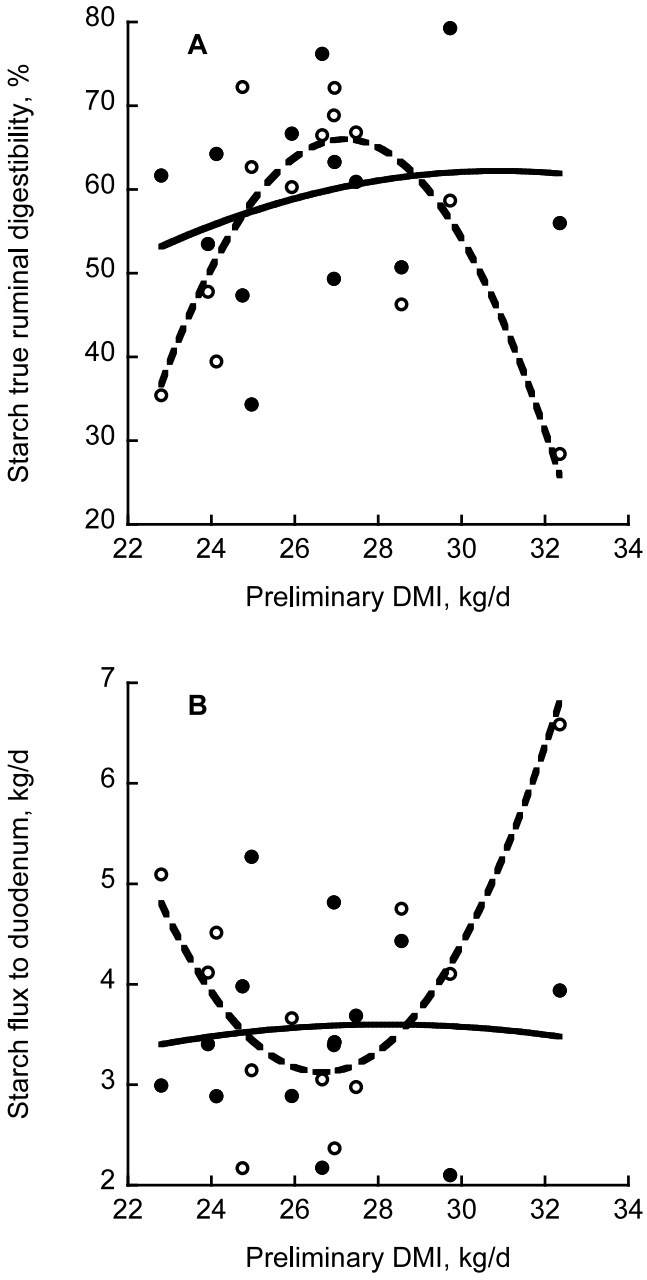
A
Starch true ruminal digestibility, %
80
70
60
50
40
30
20
22
24
26
28
30
32
34
Preliminary DMI, kg/d
B
Starch flux to duodenum, kg/d
7
6
5
4
3
2
22
24
26
28
30
32
34
Preliminary DMI, kg/d

Figure 4 (cont'd)

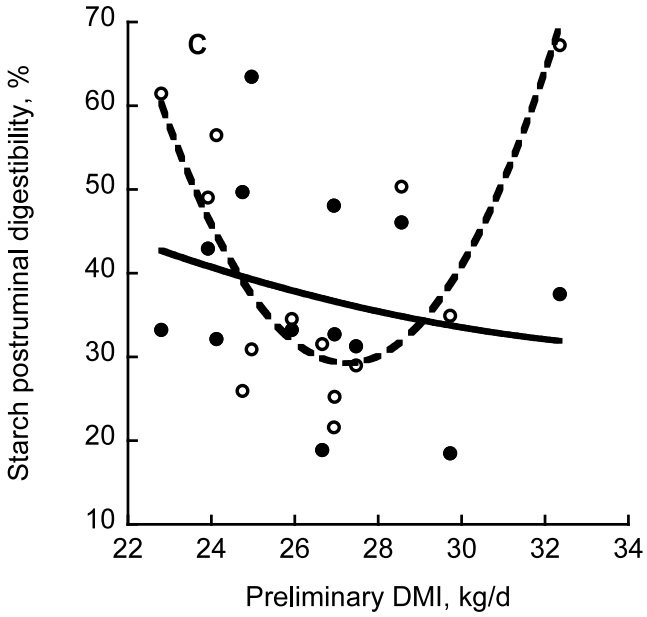

Figure 4. Interaction of long (19 mm; open circles, dashed line) and short (10 mm; closed circles, solid line) alfalfa particle length with preliminary DMI for starch A) true ruminal digestibility ($P = 0.09$), B) flux from the rumen to duodenum ($P = 0.05$), and C) postruminal digestibility ($P = 0.08$). 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.

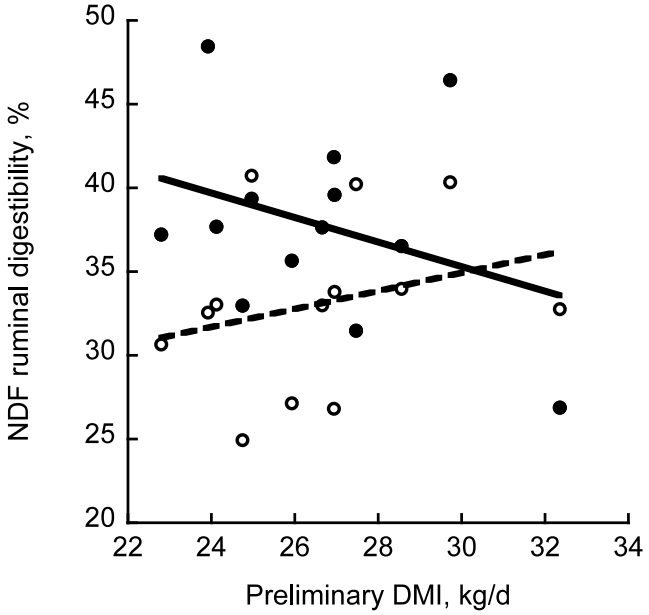

Figure 5. Interaction of long (19 mm; open circles, dashed line) and short (10 mm; closed circles, solid line) alfalfa particle length with preliminary DMI for NDF ruminal digestibility ($P = 0.09$). The preliminary DMI on the x-axis are the mean DMI of individual cows during the final 4 d of the preliminary period when all cows were fed a common diet.

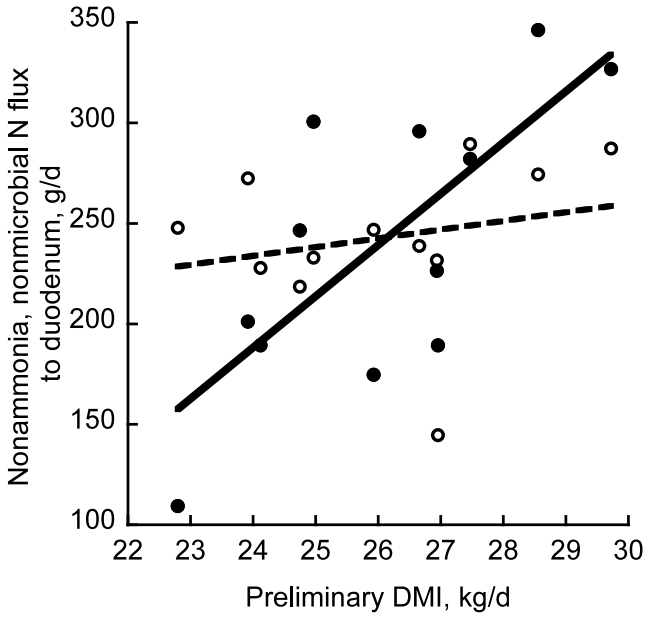

Figure 6. Interaction of long (19 mm; open circles, dashed line) and short (10 mm; closed circles, solid line) alfalfa particle length with preliminary DMI for nonammonia, nonmicrobial N flux from the rumen to duodenum ($P = 0.03$). The preliminary DMI on the x-axis are the mean DMI of individual cows during the final 4 d of the preliminary period when all cows were fed a common diet.

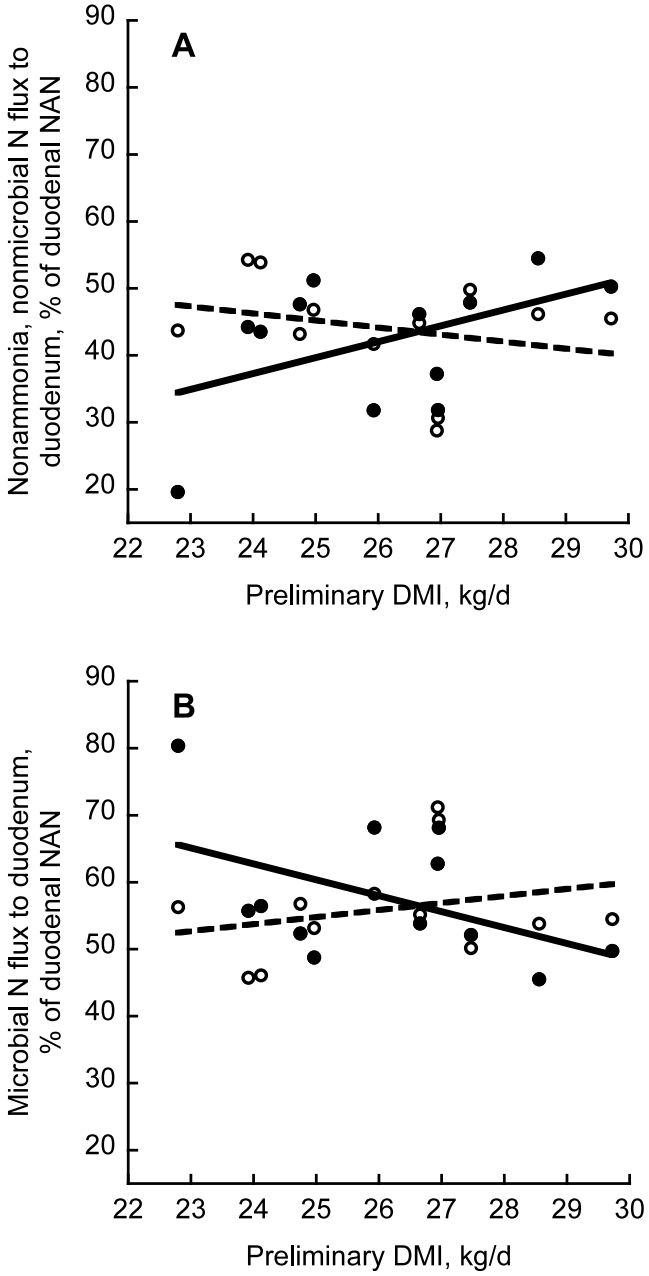

Figure 7. Interaction of long (19 mm; open circles, dashed line) and short (10 mm; closed circles, solid line) alfalfa particle length with preliminary DMI for A) nonammonia, nonmicrobial N ($P = 0.008$) and B) microbial N ($P = 0.008$) flux from the rumen to duodenum expressed as percent of duodenal 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.

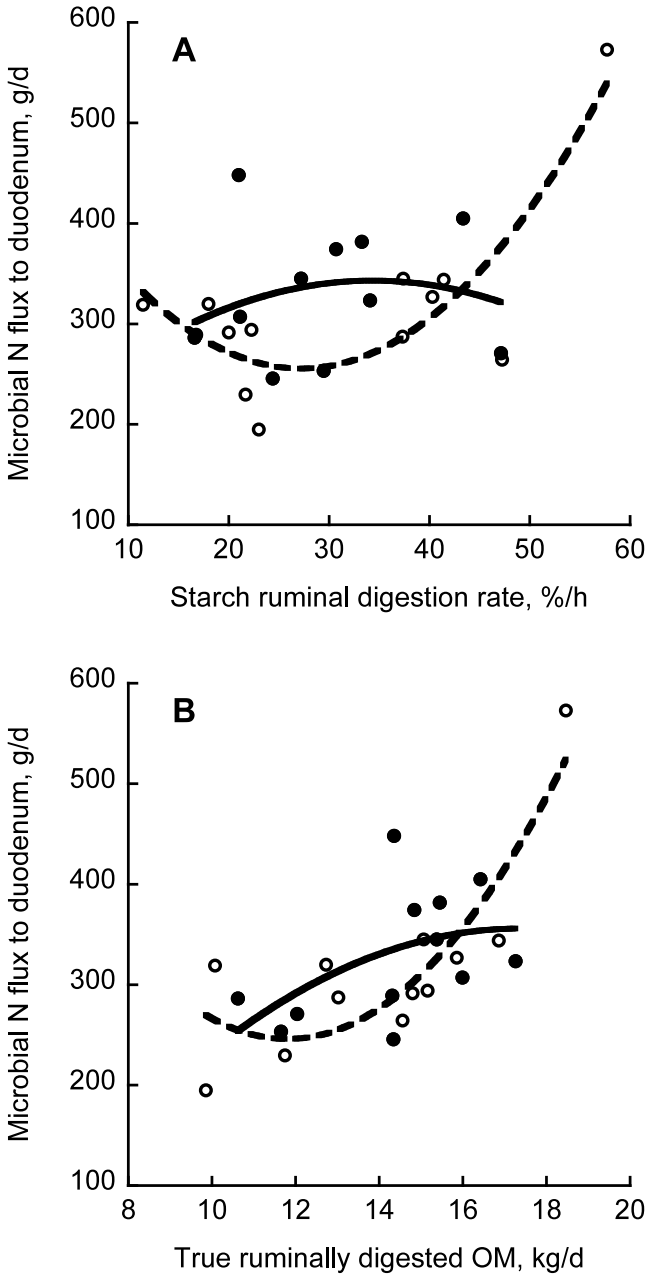

Figure 8. A) Relationship between starch ruminal digestion rate (k_d) and microbial N (MN) flux from rumen to duodenum for long (open circles, dashed line; MN flux, g/d = [205 + (1.76 x starch ruminal k_d, %/h) + (0.306 x (starch ruminal k_d, %/h – 30.1)^2)]; $P = 0.004$, $R^2 = 0.71$) and short (closed 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 x TRDOM, kg/d) + (6.27 x (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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CHAPTER 3

Nutrient Demand Interacts with Grass Particle Length to Affect Digestion Responses and Chewing Activity in Dairy Cows[1]

ABSTRACT

Effects of grass particle length on dry matter intake (DMI), milk production, ruminal fermentation and pool sizes, digestion and passage kinetics, and chewing activity and the relationship of these effects with preliminary DMI (pDMI) were evaluated using 15 ruminally and duodenally cannulated Holstein cows in a crossover design with a 14-d preliminary period and two 18-d treatment periods. During the preliminary period, pDMI of individual cows ranged from 22.6 to 29.8 kg/d (mean = 25.8 kg/d) and 3.5% fat-corrected milk yield ranged from 29.2 to 56.9 kg/d (mean = 41.9 kg/d). Experimental treatments were diets containing orchardgrass silage chopped to either a) 19 mm (LONG) or b) 10 mm (SHORT) theoretical length of cut as the sole forage. Grass silages contained ~46% neutral detergent fiber (NDF); diets contained 50% forage, 23% forage NDF, and 28% total 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 grass particle length and their interaction with pDMI were tested by ANOVA. Grass particle length and its interaction with pDMI did not affect milk yield, milk composition, or rumen pH. LONG tended to decrease DMI compared to SHORT, which might have been limited by rumen fill or chewing time or both. Passage rates of feed fractions did not differ between LONG and SHORT and were not related to level of intake. As pDMI increased, LONG decreased ruminal digestion rate of pdNDF at a faster rate than SHORT.

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As a result, LONG decreased or tended to decrease rates of ruminal turnover for NDF, organic matter, and dry matter and increased their rumen pools compared to SHORT for cows with high pDMI. LONG increased eating time, which affected cows with high intake to the greatest extent, and total chewing time compared to SHORT. As intake increased, ruminal digestion (kg/d) and digestibility (%) of starch decreased, rumen pool size of starch increased, and post ruminal digestion and digestibility of starch increased quadratically. When grass 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, which were fed adequate fiber.

INTRODUCTION

Forage particle size impacts various aspects of rumen function and digestion kinetics. Ruminal digesta passage rates decrease with increasing particle size due to greater retention time in the rumen (Dixon and Milligan, 1985) and mat formation by long forage particles, which increases digestibility of smaller particles (Grant, 1997). Greater ruminal distention caused by longer forage particles is more likely to affect passage rate and feed intake of lactating dairy cows when feed intake is more limited by rumen fill. Decreasing particle size permits rapid removal of digesta from the rumen allowing increased feed intake when intake is limited by distention, but ruminal pH might be reduced for several reasons including a reduction in buffer capacity of ruminal digesta mass, a reduction in rate of VFA absorption from decreased motility, and a reduction in salivary buffer secretion from decreased rumination.

Forage particle length (FPL) has been widely researched, but the effects of FPL on animal responses are inconsistent and inconclusive. Some of the inconsistency on responses to particle size may be due to forage type. Tafaj et al. (2007) reported that effects of forage particle

size were less when corn silage was included in the TMR and greater for grass silage-based TMR. Furthermore, grasses and legumes differ in *in vitro* cell wall digestion rates (Smith et al., 1972; Robles et al. 1980) and anatomical structure and digestion characteristics affecting particle size reduction and passage (Allen and Mertens, 1988). These differences suggest consideration of forage family is necessary when evaluating the effects of particle size. Orchardgrass (OG; *Dactylis glomerata L.*) was selected as a representative cool-season grass for use in this experiment.

Besides dietary factors, another reason for inconsistent responses to FPL may be related to animal factors. Cows respond differently to treatments depending on their level of intake (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 digesta passage rates to grass particle length are related to level of intake and shorter particle length will 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 grass silage on DMI, milk production, ruminal fermentation and pool sizes, digestion and passage kinetics, and chewing behavior in lactating dairy cows. This study had three unique 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 OG 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. Fifteen 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 18-d experimental periods. The first 10 d of each period were allowed for diet adaptation and samples were collected during the final 4 d of the preliminary period and 8 d of each experimental period. Cows were 164±56 (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 22.6 to 29.8 kg/d (mean = 25.8 kg/d) and 3.5% FCM yield ranged from 29.2 to 56.9 kg/d (mean = 41.9 kg/d; Table 13). 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 OG silage chopped to either a) 19 mm

(LONG) or b) 10 mm (SHORT) theoretical length of cut (TLC) 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.

Orchardgrass (Baridana cultivar, Barenbrug USA, Tangent, OR) was produced at the campus farm at Michigan State University (East Lansing), chopped from the same field, and ensiled in Ag-Bags (Ag-Bag Systems Inc., St. Nazianz, WI). During the sample collection periods, long and short cut OG contained ~46% NDF (DM basis; Table 14). Diets LONG and SHORT were formulated to contain 21% forage NDF, 28% total NDF, and 18% CP. The diet fed during the preliminary period was formulated so that long and short cut OG each contributed 50% of forage NDF. Diets also contained dry ground corn, soybean meal (48% CP), SoyPlus® (West Central Soy, Ralston, IA), vitamin-mineral premix, and limestone (Table 15).

Data and Sample Collection

Throughout the experiment, cows were housed in tie-stalls and fed diets as total mixed rations once daily (1130 h) at 110% of expected intake. The amount of feed offered and refused (orts) was 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 11 to 15 during each experimental period. Samples were frozen immediately after collection at −20°C and combined to one composite sample per period prior to analysis.

Cows were moved to an exercise lot twice daily (0230 and 1300 h) prior to 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 11 to 15 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 18 of each experimental period. Body condition score was determined on the same days by 3 trained investigators blinded to treatments (Wildman et al., 1982; 5-point scale where 1 = thin and 5 = fat). Chewing activity was monitored and recorded by observation every 5 min for 24 h on d 16 of each experimental period. Activity was noted as eating, ruminating, drinking, or idle for each cow at each time.

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 11 to 15 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 was 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 five 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.

Ruminal contents were evacuated manually through the ruminal cannula 4 h after feeding at the beginning of d 17 (1530 h) and 2 h before feeding at the end of d 18 (0930) 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 yield recorded at both milkings were summed for a daily total, which were averaged for each period. Milk samples were analyzed for fat, true protein, lactose, SNF, and MUN 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 one 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). Due to the high moisture content of the silages, the particles tended to cling together and remain on the top sieve during the shaking process yielding inaccurate measurements. Therefore, samples were dried to a constant weight with forced air (no added heat) prior to separation. 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 (Tri-Philizer 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 one 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 lignin (ADL), CP, and starch. Ash concentration was determined after 5 h 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). Indigestible NDF was estimated as NDF residue after 240 h in vitro fermentation (Goering and Van Soest, 1970); flasks were reinoculated at 120 h to insure a viable microbial population. 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 was blended, strained through nylon mesh, and the liquid portion was centrifuged at 500 x *g* for 15 min. The supernatant was centrifuged at 18,000 x *g* for 15 min, and the pellet was washed with 0.9% NaCl solution and centrifuged again at 18,000 x *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 concentration of major VFA and lactate by HPLC (Waters Corp., Milford, MA) according to Oba and Allen (2003a).

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 ratio of purines to OM (Oba and Allen, 2003a), and true ruminally degraded 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 the following equations:

Turnover rate (%/h) = 100 x (Intake of component / Ruminal pool of component) / 24

Passage rate (%/h) = 100 x (Duodenal flow of component / Ruminal pool of component) / 24

Digestion rate (%/h) = Turnover rate in the rumen (%/h) – Passage rate from the rumen (%/h).

Manually observed chewing activity was summarized by a logic script in Igor Pro (Version 6.12, WaveMetrics Inc., Lake Oswego, OR) to generate meal and rumination bout information according to previously established criteria (Dado and Allen, 1994). Variables

determined included frequency of meal bouts per day, interval between meals, frequency of ruminating bouts per day, interval between ruminating bouts, eating time per day, ruminating time per day, and total chewing time per day.

Statistical Analysis

All data were analyzed by using the fit model procedure of JMP (Version 8, SAS Institute, 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: $Y_{ijk} = \mu + C_i + P_j + T_k + PT_{jk} + pDMI + T_kpDMI + pDMI^2 + T_kpDMI^2 + e_{ijk}$ where μ is the overall mean, C_i is the random effect of cow (i = 1 to 15), 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_kpDMI^2 is the interaction of treatment and pDMI (quadratic), and e_{ijk} is the residual error. Statistical significance for T_kpDMI and T_kpDMI^2 indicated 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. Tendencies 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, one cow went off feed during the second experimental period 2 d prior to the start of sample collection and was removed. Thus, data from 15 cows were statistically analyzed.

RESULTS AND DISCUSSION

Comparison of Forages and Diets

Physical characteristics of OG are listed in Table 14. Forages chopped to a TLC of 19 and 10 mm had mean particle sizes of 15.3 and 11.3 mm, respectively. The proportion of particles > 19 mm was 20 percentage units higher (46 vs. 26%) and particles < 8 mm was 17 percentage units lower (25 vs. 44%) for long than short cut OG, respectively. Particle size distribution of short cut OG was similar to the guidelines recommended by Heinrichs (1996) that the portion of haylage retained on the 19 mm sieve, the 8 mm sieve, and the bottom pan of the Penn State Particle Separator should be 15-25%, 30-40%, and 40-50%, respectively.

Chemical analyses (Table 14) showed that OG with different lengths of cut had similar concentrations of OM, total NDF, pdNDF, iNDF, ADF, ADL, CP, and starch. Both silages were wetter than expected, and long cut OG had lower DM concentration than short cut OG due to the shorter wilting time for long cut OG as the silages were sequentially harvested and long cut OG was mowed, chopped, and ensiled first. Both OG silages underwent favorable fermentation and were well preserved based on the low pH and high lactic acid concentrations. However, the concentrations of acetic acid were higher than that typical for grass silages, which is likely due to the high moisture content of both OG (Kung and Shaver, 2001).

Diet ingredients and chemical composition are shown in Table 15. The preliminary diet contained similar proportions of forage NDF from long and short cut OG. Both treatment diets

had a 50:50 forage:concentrate ratio, contained 23% forage NDF and had similar chemical composition, which was mathematically calculated according to the proportion of each feed ingredient in the diet and its respective analytical values. The calculated percent forage NDF in the diet was slightly higher than the formulated target but was similar for both LONG and SHORT and above NRC (2001) minimum requirements. In both diets, forage NDF provided over 82% of the total diet NDF. Differences in DM concentration in diets were because of the different DM concentrations of the forages.

Effects of Grass FPL and pDMI

Forage particle length and its interaction with pDMI did not affect yields of milk or milk components or milk composition (Table 16). LONG increased MUN concentrations ($P < 0.001$; Table 16) compared to SHORT. This is consistent with higher ruminal ammonia concentration and flow of ammonia N to the duodenum ($P < 0.01$; Table 17) for LONG than SHORT. Although long cut OG silage was wetter, similar ammonia concentrations (Table 14) were measured in both silages and, therefore, long cut OG silage was not the source for increased ammonia observed in cows fed LONG.

LONG tended to increase starch ruminal rate of digestion (19.0 vs.14.9%/h, $P = 0.07$, Table 18) and true ruminal digestibility (60.3 vs. 49.8%, $P = 0.09$, Table 19) and tended to decrease starch flux from the rumen to the duodenum (2.84 vs. 3.51 kg/d, $P = 0.09$, Table 19) compared to SHORT. The mechanism for increased starch digestion rate is unclear but one explanation is that longer forage particles may promote greater numbers or activity of starch digesting bacteria in the rumens of cows consuming LONG. Some starch-digesting bacteria in the rumen (e.g. *Streptococcus bovis*) also have high proteolytic activity (Russell et al., 1981) resulting in deamination of amino acids and production of ammonia, which could contribute to

the increased ammonia concentrations for LONG.

LONG tended to decrease DMI (21.8 vs. 22.7 kg/d, $P = 0.06$, Table 16) compared to SHORT. We expected LONG to be more filling than SHORT causing greater rumen distention and potentially limiting DMI particularly in cows with high DMI for which ruminal distention is more likely to limit feed intake (Allen, 1996). Oba and Allen (1999) and Voelker et al. (2002) found DMI responses varied by production level, which is generally correlated with DMI (NRC, 2001), where DMI was increasingly limited by high fill diets compared to low fill diets as milk yield increased. Although we expected the longer particles of LONG to slow rates of ruminal passage, FPL and its interaction with pDMI did not affect the rates that pdNDF, iNDF, or starch passed from the rumen (Table 18). However, rate of ruminal digestion of pdNDF was related to pDMI, which decreased at a faster rate for LONG than SHORT as pDMI increased (interaction $P = 0.08$, Figure 9A). As a result, LONG decreased or tended to decrease rates of ruminal turnover of pdNDF, NDF (interaction $P = 0.07$, Figure 9B), OM, and DM (Table 18) and increased rumen pools of NDF (interaction $P = 0.04$, Figure 9C), OM, and DM (Table 20) compared to SHORT for cows with high pDMI, but effect of treatment on DMI was not related to pDMI. Although the treatment by pDMI interaction was not significant (interaction $P > 0.40$), a visual examination of a graph with pDMI and DMI (Figure 9D) illustrated the differences in DMI between LONG and SHORT were small for cows with low pDMI but the difference became greater as pDMI increased with the greatest divergence for cows with high pDMI. Therefore, rumen fill as a constraint limiting DMI for cows with high intake fed LONG compared to SHORT is possible but results did not provide conclusive evidence.

Chewing activity results (Table 21) suggest that DMI for LONG was possibly limited by chewing time. LONG increased meal length (39.1 vs. 33.0 min/bout, $P = 0.008$) and meal size

(2.52 vs. 2.28 kg DM/meal, $P = 0.05$) resulting in greater eating time (16.5 vs. 15.1 min/kg DMI, $P = 0.02$) and ruminating time (25.4 vs. 23.2 min/kg DMI, $P = 0.05$) and thus increased total chewing time (42.0 vs. 38.3 min/kg DMI and 867 vs. 827 min/d, $P = 0.02$) compared to SHORT. LONG increased total time spent chewing to reduce particle size compared to SHORT, but the effect of FPL on eating time was related to pDMI. LONG increased and SHORT decreased eating time expressed as min/kg DMI, min/kg NDF intake (interaction $P = 0.006$, Figure 9E), and min/kg forage NDF intake as pDMI increased. LONG increased eating time expressed as min/d with increasing pDMI compared to SHORT, which remained constant for SHORT across the range of pDMI (interaction $P = 0.004$, Figure 9F). Because the total amount of time spent chewing per day is likely limited (Van Soest, 1994), cows with high intake consuming LONG might have reached the upper limit for time spent chewing. In this study, LONG had greater time chewing (mean = 867 min/d) compared to the mean chewing time (694 min/d) across 72 treatments and 19 experiments (Tafaj et al. 2007), and total chewing time for individual cows ranged from 735 to 1055 min/d. Cows consuming LONG spent 42 min/kg DM and 152 min/kg NDF, which were approaching the maximum chewing time per unit of DM and NDF (47 and 160 min, respectively) reported by Tafaj et al. (2007).

Forage particle length and its interaction with pDMI did not affect ruminal pH (Table 22). We expected LONG to potentially increase ruminal pH through greater chewing and salivary buffer flow (Allen, 1997), but this was not observed. This might be because there were no main effects of treatment on rumen pool sizes (Table 20), total rumination time (Table 21), or OM truly digested in the rumen (Table 23). Differences were detected for concentrations of VFA (Table 22), but these differences were quite small and likely not biologically significant.

LONG decreased rumen empty BW for cows with pDMI < 26 kg/d but increased BW for

cows with pDMI > 26 kg/d compared to SHORT (interaction $P = 0.02$, Table 16). The BW gain for LONG with high pDMI occurred despite digesting less DM in total tract than SHORT (interaction $P = 0.04$, Table 23). The reason for the BW changes observed in relation to FPL and pDMI is not clear.

Direct comparisons of animal responses across individual studies evaluating the effects of FPL should be interpreted with caution. There are multiple reasons for this, which may help explain why results from particular experiments may or may not be in agreement. There is a wide range of FPL (2 to 32 mm) reported for studies from 1997 to 2005 (Tafaj et al., 2007) and differences between FPL compared within studies (6 vs. 8 mm; Yang and Beauchemin, 2004 and 24 vs. 170 mm; Randby et al., 2008). Furthermore, the lack of a consistent method of measuring and reporting physical characteristics complicates interpretation because similar TLC and physically effective NDF do not necessarily yield the same particle size distribution (Beauchemin and Rodes, 1998). Responses to FPL vary depending on forage source with greater differences reported for grass-based TMR compared to corn silage-based TMR (Tafaj et al., 2007). Additionally, feeding conditions differ from offering forage and concentrate separately and limit fed (Zebeli et al., 2007) to numerous studies where cows are fed total mixed rations ad libitum. Studies often evaluate effect of FPL in combination with other dietary factors including, but not limited to, forage:concentrate ratio (Soita et al., 2000; Einarson et al., 2004), grain processing (Yang et al., 2001), grain fermentability (Krause and Combs, 2003), non-forage fiber sources (Mooney and Allen, 1997), and supplemental fat (Onetti et al., 2003).

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 impact of passage on ruminal degradation 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 nutrients from the rumen using the pool and flux method (Robinson et al., 1987).

Although we expected ruminal passage rates to increase with pDMI, passage rates of pdNDF, iNDF, and starch were not related to level of intake independent of or dependent upon treatment (Table 18). However, rate of starch digestion decreased quadratically (interaction P = 0.02, Figure 10A) and true ruminal digestibility of starch tended to decrease quadratically (interaction P = 0.13, Figure 10B) as pDMI increased. Two cows with high pDMI (> 29 kg DM/d) amplified the quadratic effects. The reductions in starch rate of digestion and digestibility are consistent with increased starch rumen pool (interaction P = 0.11, Figure 10C) and likely related to the increased liquid dilution rate associated with increased intake (not measured) decreasing populations of starch degrading microbes. Concentrations of ruminal ammonia (P = 0.002, Figure 10D) and branch chained VFA (interaction P = 0.05, Figure 10E), which are derived from degradation of branched chain amino acids, also decreased with increased pDMI and are consistent with less proteolytic activity in the rumen due to greater removal of ruminal microbes through passage and lysis. As pDMI increased, ammonia N flux to the duodenum (P = 0.05, Figure 10F) increased and MUN concentration (P = 0.07, Table 16) tended to increase.

Additionally, the rate of pdNDF digestion decreased as pDMI increased (Table 18) and had a greater effect on LONG than SHORT (interaction P = 0.08, Figure 9A) as previously

mentioned. The reduction in pdNDF digestion rate is likely related to differences in mechanical processing (TLC) and mastication, which reduce particle size and increase the surface area available for microbial attachment and enzymatic attack (Bowman and Firkins, 1993). The proportion of large particles in the rumen increases with higher DMI (Okine and Mathison, 1991) because ruminating time per unit of DM consumed decreases as DMI increases (Welch and Smith, 1969). In this experiment, cows tended to decrease amount of time spent ruminating per unit of forage NDF consumed as level of intake increased ($P = 0.06$, Figure 11A). As a result of the reduction in pdNDF digestion rate, cows tended to decrease ruminal pdNDF digestibility (%, $P = 0.06$, Figure 11B) and increase pdNDF flux from the rumen to the duodenum ($P = 0.02$, Figure 11C) as pDMI increased. Furthermore, ruminal acetate concentration decreased as pDMI increased ($P = 0.02$, Figure 11D), and this is consistent with lower ruminal pdNDF digestibility because acetate is the predominant VFA produced from fiber digestion.

Effects of pDMI on N Flux and Microbial Efficiency

Fluxes of NAN and NANMN from the rumen to the duodenum tended to increase as pDMI increased, which increased or tended to increase postruminal NAN digestion (g/d) and digestibility (%) with increased pDMI ($P \leq 0.10$, Table 17). Microbial N flux and efficiency (g of MN produced per kg true ruminally digested OM) were not related to level of intake (Table 17) or quantity of OM truly digested in the rumen (not shown). However, a positive relationship was observed between microbial efficiency and ruminal passage rates of starch ($P < 0.001$, $R^2 = 0.44$, Figure 12A) and pdNDF ($P < 0.001$, $R^2 = 0.52$, Figure 12B). This indicated that energy from ruminal fermentation was more efficiently utilized for microbial growth as passage rates for starch and pdNDF increased, and the greater passage rates possibly decreased microbial lysis and

turnover in the rumen because many microbial organisms flow from the rumen attached to fibrous particles.

Microbial N flux and efficiency are low for both treatments in the present experiment. This is unlikely associated with the method used because this same method was used in other experiments that reported higher MN flux and efficiency (Oba and Allen, 2003b; Taylor and Allen, 2005). Microbial N flux has not been consistent among studies comparing orchardgrass and alfalfa with MN flux ranging from low (Voelker Linton and Allen, 2009) to high (Kammes and Allen, submitted). This indicates low microbial yield is not specific to orchardgrass. The reason for low MN production in this study is not clear but appears to be related to the treatments.

CONCLUSIONS

Grass particle length and its interaction with pDMI did not affect milk yield, milk composition, or rumen pH. Increasing grass particle length tended to decrease DMI, which might be limited by rumen fill or chewing time or both. Passage rates of feed fractions did not differ between LONG and SHORT and were not related to level of intake. As pDMI increased, LONG decreased ruminal digestion rate of pdNDF at a faster rate than SHORT. As a result, LONG decreased or tended to decrease rates of ruminal turnover for NDF, OM, and DM and increased their rumen pools compared to SHORT for cows with high pDMI. LONG increased eating time, which affected cows with high intake to the greatest extent, and total chewing time compared to SHORT. Ruminal starch digestibility decreased, starch rumen pool increased, and post ruminal starch digestibility increased quadratically as feed intake increased. Sorting of feed particles was minimal in this experiment due to the wet forages and individual feeding of cows,

but sorting would likely increase for cows fed diets using drier forages or group fed. When grass silage was the only source of forage in diet, increasing chop length from 10 to 19 mm tended to decrease DMI but did not negatively affect productivity of cows, which were fed adequate fiber.

APPENDIX

Table 13. Characterization of 15 cows during the final 4 d of the 14-d preliminary period, when cows were fed a common diet

Parameter	Median	Mean	Standard deviation	Minimum	Maximum
Parity	3	3.1	1.2	2	6
BW[1], kg	581	579	58	469	687
BCS	2.1	2.3	0.7	1.5	3.7
DIM	168	164	56	83	258
Milk, kg/d	43.1	41.5	10.6	24.2	62.2
3.5% FCM, kg/d	40.6	41.9	8.9	29.2	56.9
DMI, kg/d	25.4	25.8	2.1	22.6	29.8

[1]Empty BW (ruminal digesta removed).

Table 14. Chemical composition, particle size distribution, and fermentation parameters of the long (19 mm) and short cut (10 mm) orchardgrass silage included in the treatment diets

	Orchardgrass silage	
Item	Long	Short
Chemical composition		
DM, %	26.5	31.4
OM, % DM	88.2	88.4
NDF, % DM	46.9	45.2
iNDF[1], % DM	12.2	11.6
iNDF, % of NDF	26.1	25.7
ADF, % of DM	31.3	32.1
ADL[2], % of DM	2.93	2.99
CP, % DM	21.1	20.5
Starch, % DM	1.21	1.24
Particles size distribution[3]		
Wet sieving, % DM retained		
19.0 mm	30.6	27.1
9.50 mm	28.4	24.4
4.75 mm	15.5	28.1
2.36 mm	5.81	14.3
1.18 mm	3.24	4.57
0.600 mm	2.19	2.33
0.300 mm	1.09	1.41
0.150 mm	0.57	0.72
0.075 mm	0.34	0.37
0.038 mm	0.36	0.34
Mean particle size[4], mm	15.3	11.3
Penn State Particle Separator[5], % DM retained		
> 19.0 mm	46.1	26.2
19.0 to 8.0 mm	29.0	31.7
< 8.0 mm	24.9	42.1
Fermentation		
pH	4.63	4.69
Acetic acid, % DM	3.36	5.26
Propionic acid, % DM	0.12	0.49
Butyric acid, % DM	<0.01	0.09
Lactic acid, % DM	10.5	10.6
Lactic:Acetic	3.14	2.01
Ethanol, % DM	0.12	0.40
Ammonia, mM	5.09	4.84

[1]iNDF = indigestible NDF.

[2]ADL = acid detergent lignin.

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

Table 14 (cont'd)

[4]Mean particle size calculated from particle size distribution determined by wet sieving.
[5]Silages were dried to constant weight with forced air (no heat) prior to separation using Penn State Particle Separator due to high moisture content.

Table 15. Ingredients and chemical composition of preliminary and treatment diets (as analyzed) containing either long (19 mm) or short cut (10 mm) orchardgrass silage as the sole source of forage

	Preliminary	Long	Short
Ingredients, % DM			
Orchardgrass silage, long cut	26.0	49.8	---
Orchardgrass silage, short cut	26.0	---	49.7
Dry ground corn	36.9	38.4	37.9
Soybean meal (48% CP)	1.99	2.89	3.45
SoyPlus®	3.47	3.39	3.39
Vitamin mineral mix[1]	3.97	3.97	3.97
Limestone	1.60	1.60	1.60
Chemical composition			
DM, %	41.7	40.8	46.5
OM, % DM	89.9	90.0	90.1
NDF, % DM	28.5	28.3	27.4
% forage NDF	23.8	23.3	22.5
% NDF from forage	83.4	82.6	82.1
iNDF[2], % DM	6.25	8.20	7.90
iNDF, % of NDF	21.9	29.0	28.8
CP, % DM	17.1	17.9	17.9
Starch, % DM	29.2	29.8	29.5

[1]Vitamin mineral mix contained (DM basis) 16.5% sodium bicarbonate, 14.2% magnesium sulfate, 7.1% salt, 5.8% dicalcium phosphate, 2.4% trace mineral premix, 0.4% vitamin A, 0.4% vitamin D, 0.2% vitamin E, and 53.1% dry ground corn as a carrier.
[2]iNDF = indigestible NDF.

Table 16. Milk production and composition, feed intake, and BW change of cows fed treatment diets containing either long (19 mm) or short cut (10 mm) orchardgrass silage as the sole source of forage

	Treatment LSM[1]			*P*[2]					
	Long	Short	SE	Trt	Trt x Period	pDMI	Trt x pDMI	pDMI x pDMI	Trt x pDMI x pDMI
Yield, kg/d									
Milk	39.9	40.0	2.3	0.80	NS[3]	<0.001	0.59	0.71	0.15
FCM (3.5 %)	39.8	39.5	1.5	0.62	NS	<0.001	NS	NS	NS
Milk fat	1.40	1.40	0.05	0.99	NS	0.004	NS	NS	NS
Milk protein	1.17	1.18	0.06	0.57	NS	0.006	0.71	0.93	0.18
Milk lactose	1.88	1.89	0.12	0.77	NS	<0.001	0.42	0.68	0.18
SNF	2.26	2.27	0.14	0.73	NS	<0.001	0.41	0.68	0.16
Milk composition, %									
Fat	3.61	3.68	0.12	0.22	NS	0.03	NS	NS	NS
Protein	3.04	3.06	0.06	0.44	NS	0.001	NS	NS	NS
Lactose	4.68	4.67	0.07	0.75	NS	0.11	NS	NS	NS
SNF	5.62	5.61	0.08	0.74	NS	0.16	NS	NS	NS
MUN, mg/dl	12.3	11.2	0.3	<0.001	NS	0.07	NS	NS	NS
DMI, kg/d	21.8	22.7	0.9	0.06	NS	0.02	NS	0.20	NS
3.5% FCM/ DMI	1.64	1.60	0.06	0.39	NS	0.01	0.42	0.21	0.09
BW change, kg/18 d	6.88	2.57	3.91	0.54	NS	0.88	0.02	0.46	0.16
BCS change/18 d	-0.08	-0.06	0.05	0.84	NS	NS	NS	NS	NS

[1]Treatment least squares means.

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

[3]NS = not significant ($P > 0.20$); term removed from statistical model.

Table 17. Nitrogen metabolism of cows fed treatment diets containing either long (19 mm) or short cut (10 mm) orchardgrass silage as the sole source of forage

	Treatment LSM[1]			*P*[2]					
	Long	Short	SE	Trt	Trt x Period	pDMI	Trt x pDMI	pDMI x pDMI	Trt x pDMI x pDMI
N intake, g/d	620	650	25	0.02	NS[3]	0.05	NS	0.15	NS
Ruminal ammonia, mg/dl	8.11	6.47	0.26	<0.001	NS	0.002	NS	0.15	NS
Flow to duodenum									
Ammonia N, g/d	12.5	10.7	0.8	0.004	NS	0.05	NS	0.16	NS
NAN									
g/d	523	514	40	0.64	NS	0.06	NS	0.18	NS
% of N intake	80.3	75.6	3.0	0.14	NS	0.19	NS	NS	NS
NANMN[4]									
g/d	296	269	27	0.18	NS	0.10	0.23	0.09	0.18
% of N intake	45.2	42.3	3.1	0.24	NS	0.20	NS	0.13	NS
% of duodenal NAN	52.0	51.6	1.9	0.83	NS	NS	NS	NS	NS
Microbial N									
g/d	230	230	15	0.98	NS	0.11	NS	NS	NS
% of duodenal NAN	48.0	48.4	1.9	0.83	NS	NS	NS	NS	NS
g/kg TRDOM[5]	19.7	18.8	1.3	0.55	NS	NS	NS	NS	NS
NAN apparent postruminal digestion									
g/d	302	295	29	0.69	NS	0.05	NS	0.11	NS
% of N intake	47.8	45.0	3.2	0.37	NS	0.07	NS	0.10	NS
% of duodenal passage	57.2	56.8	2.1	0.87	NS	0.12	NS	0.05	NS
N apparent total tract digestion									
g/d	399	431	15	0.008	NS	0.03	0.12	0.06	NS
%	63.9	65.8	1.3	0.24	NS	NS	NS	NS	NS

[1]Treatment least squares means.

Table 17 (cont'd)

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

[3]NS = not significant ($P > 0.20$); term removed from statistical model.

[4]NANMN = nonammonia, nonmicrobial nitrogen.

[5]TRDOM = true ruminally digested organic matter.

Table 18. Rumen kinetics of cows fed treatment diets containing either long (19 mm) or short cut (10 mm) orchardgrass silage as the sole source of forage

	Treatment LSM[1]			*P*[2]					
	Long	Short	SE	Trt	Trt x Period	pDMI	Trt x pDMI	pDMI x pDMI	Trt x pDMI x pDMI
Ruminal turnover rate, %/h									
DM	7.55	7.93	0.37	0.29	NS[3]	0.34	0.11	NS	NS
OM	7.62	8.03	0.39	0.27	NS	0.34	0.08	NS	NS
NDF	4.40	4.44	0.22	0.83	NS	0.18	0.07	NS	NS
pdNDF[4]	7.31	7.04	0.36	0.48	NS	0.35	0.11	NS	NS
Starch	32.9	31.0	2.6	0.49	NS	0.16	0.89	0.46	0.09
Ruminal turnover time, h									
DM	13.6	13.0	0.6	0.32	NS	0.29	0.07	NS	NS
OM	13.5	12.9	0.6	0.31	NS	0.28	0.05	NS	NS
NDF	23.6	23.3	1.1	0.83	NS	0.14	0.05	NS	NS
pdNDF	14.1	14.8	0.7	0.37	NS	0.28	0.13	NS	NS
iNDF[5]	48.2	45.2	2.6	0.38	NS	0.08	0.09	NS	NS
Starch	3.12	3.43	0.25	0.21	NS	0.29	0.85	0.48	0.06
Ruminal passage rate, %/h									
pdNDF	0.89	0.75	0.22	0.53	NS	0.12	0.45	0.45	0.19
iNDF	2.23	2.29	0.13	0.70	NS	0.14	NS	NS	NS
Starch	14.3	15.8	1.9	0.41	NS	0.50	NS	0.13	NS
Ruminal digestion rate, %/h									
pdNDF	6.63	6.28	0.29	0.38	NS	0.04	0.08	NS	NS
Starch	19.0	14.9	1.8	0.07	NS	0.01	0.34	0.01	0.02

[1]Treatment least squares means.

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

Table 18 (cont'd)

[3]NS = not significant ($P > 0.20$); term removed from statistical model.
[4]pdNDF = potentially digestible NDF.
[5]iNDF = indigestible NDF.

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

	Treatment LSM[1]			*P*[2]					
	Long	Short	SE	Trt	Trt x Period	pDMI	Trt x pDMI	pDMI x pDMI	Trt x pDMI x pDMI
Starch									
Intake, kg/d	6.49	6.62	0.22	0.41	NS[3]	0.02	NS	NS	NS
Apparent ruminal digestion									
kg/d	3.91	3.31	0.34	0.19	NS	0.80	0.23	0.10	0.15
%	58.9	48.4	4.9	0.09	NS	0.10	0.30	0.02	0.14
True ruminal digestion									
kg/d	4.01	3.40	0.34	0.19	NS	0.75	0.22	0.10	0.14
%	60.3	49.8	4.8	0.09	NS	0.10	0.30	0.02	0.13
Passage to duodenum, kg/d	2.84	3.51	0.35	0.09	NS	0.03	0.34	0.02	0.13
Apparent postruminal digestion									
kg/d	2.53	3.19	0.34	0.11	NS	0.03	0.43	0.02	0.16
% of intake	36.5	47.0	4.8	0.11	NS	0.10	0.40	0.02	0.17
% of duodenal passage	89.2	89.9	1.3	0.63	NS	0.39	NS	0.02	NS
Apparent total tract digestion									
kg/d	6.19	6.32	0.20	0.38	NS	0.02	NS	NS	NS
%	95.4	95.5	0.4	0.76	NS	0.54	0.15	NS	NS

[1]Treatment least squares means.

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

[3]NS = not significant ($P > 0.20$); term removed from statistical model.

Table 20. Rumen pools of cows fed treatment diets containing either long (19 mm) or short cut (10 mm) orchardgrass silage as the sole source of forage

	Treatment LSM[1]			*P*[2]					
	Long	Short	SE	Trt	Trt x Period	pDMI	Trt x pDMI	pDMI x pDMI	Trt x pDMI x pDMI
Wet weight, kg	94.3	92.5	3.4	0.47	0.17	0.18	NS[3]	NS	NS
Volume, L	108	114	5	0.11	0.19	0.09	0.09	0.47	0.07
Density, kg/L	0.87	0.84	0.02	0.30	NS	NS	NS	NS	NS
Rumen pool, kg									
DM	12.4	12.3	0.6	0.89	0.13	0.03	0.09	0.17	NS
OM	11.1	11.0	0.5	0.83	0.14	0.03	0.05	0.17	NS
NDF	5.64	5.67	0.22	0.89	NS	0.004	0.04	NS	NS
pdNDF[4]	2.43	2.58	0.12	0.32	NS	0.02	NS	NS	NS
iNDF[5]	3.22	3.10	0.14	0.48	0.17	0.03	0.12	NS	NS
Starch	0.87	0.97	0.06	0.17	NS	0.008	0.84	0.14	0.11

[1]Treatment least squares means.

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

[3]NS = not significant ($P > 0.20$); term removed from statistical model.

[4]pdNDF = potentially digestible NDF.

[5]iNDF = indigestible NDF.

Table 21. Chewing activity of cows fed treatment diets containing either long (19 mm) or short cut (10 mm) orchardgrass silage as the sole source of forage

	Treatment LSM[1]			*P*[2]					
	Long	Short	SE	Trt	Trt x Period	pDMI	Trt x pDMI	pDMI x pDMI	Trt x pDMI x pDMI
Meals									
Bouts/d	9.28	10.2	0.67	0.13	NS[3]	0.46	0.49	0.61	0.08
Length, min/bout	39.1	33.0	2.3	0.008	NS	0.85	0.007	0.98	0.03
Interval, min	138	123	10	0.14	NS	0.67	0.38	0.95	0.09
Meal size, kg									
DM	2.52	2.28	0.24	0.05	NS	0.52	0.58	0.69	0.01
OM	2.26	2.06	0.21	0.05	NS	0.51	0.58	0.70	0.01
NDF	0.70	0.62	0.06	0.02	NS	0.57	0.50	0.65	0.01
pdNDF[4]	0.50	0.44	0.04	0.02	NS	0.55	0.62	0.64	0.01
iNDF[5]	0.20	0.17	0.02	0.04	NS	0.61	0.38	0.67	0.03
Starch	0.78	0.69	0.07	0.03	NS	0.41	0.75	0.72	0.02
Eating time									
Min/d	342	326	8	0.08	NS	0.08	0.004	NS	NS
Min/kg DMI	16.5	15.1	0.5	0.02	NS	0.47	0.006	NS	NS
Min/kg NDF intake	59.9	55.9	2.0	0.06	NS	0.67	0.006	NS	NS
Min/kg forage NDF intake	70.9	67.2	2.4	0.13	NS	0.39	0.005	NS	NS
Rumination									
Bouts/d	14.5	14.1	0.9	0.44	NS	0.45	0.04	0.51	0.05
Length, min/bout	36.8	34.3	1.4	0.06	NS	NS	NS	NS	NS
Interval, min	55.1	57.3	3.6	0.43	NS	0.19	0.41	0.74	0.13
Ruminating time									
Min/d	525	502	13	0.17	NS	NS	NS	NS	NS
Min/kg DMI	25.4	23.2	0.8	0.05	NS	0.07	NS	NS	NS
Min/kg NDF intake	92.0	86.1	3.1	0.12	NS	0.15	NS	NS	NS
Min/kg forage NDF intake	109	103	4	0.23	NS	0.06	NS	NS	NS

Table 21 (cont'd)

Total chewing time									
Min/d	867	827	16	0.02	NS	0.16	NS	NS	NS
Min/kg DMI	42.0	38.3	1.2	0.02	NS	0.13	NS	NS	NS
Min/kg NDF intake	152	142	5	0.04	NS	NS	NS	NS	NS
Min/kg forage NDF intake	180	170	5	0.12	NS	0.10	NS	NS	NS

[1]Treatment least squares means.

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

[3]NS = not significant ($P > 0.20$); term removed from statistical model.

[4]pdNDF = potentially digestible NDF.

[5]iNDF = indigestible NDF.

Table 22. Ruminal VFA concentrations and pH of cows fed treatment diets containing either long (19 mm) or short cut (10 mm) orchardgrass silage as the sole source of forage

	Treatment LSM[1]			*P*[2]					
	Long	Short	SE	Trt	Trt x Period	pDMI	Trt x pDMI	pDMI x pDMI	Trt x pDMI x pDMI
Total VFA, mM	152	151	2	0.62	NS[3]	NS	NS	NS	NS
Acetate	91.8	91.3	0.9	0.52	NS	0.02	NS	NS	NS
Propionate	35.3	33.7	1.0	0.11	NS	NS	NS	NS	NS
Butyrate	18.5	19.9	0.8	0.002	NS	0.92	NS	0.15	NS
Lactate	0.18	0.34	0.18	0.42	NS	0.10	0.04	0.12	NS
Isobutyrate	1.26	1.18	0.05	0.08	NS	0.55	0.11	0.79	0.18
Valerate	1.87	1.91	0.07	0.32	NS	0.54	NS	0.19	NS
Isovalerate	2.15	1.98	0.09	0.02	NS	0.36	0.78	0.39	0.07
Branch chain VFA	3.42	3.16	0.13	0.01	NS	0.41	0.32	0.51	0.05
Acetate:Propionate	2.62	2.74	0.06	0.09	NS	0.03	0.15	NS	NS
Ruminal pH	5.84	5.84	0.03	0.88	NS	NS	NS	NS	NS

[1]Treatment least squares means.

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

[3]NS = not significant ($P > 0.20$); term removed from statistical model.

Table 23. DM and OM digestion of cows fed treatment diets containing either long (19 mm) or short cut (10 mm) orchardgrass silage as the sole source of forage

	Treatment LSM[1]			*P*[2]					
	Long	Short	SE	Trt	Trt x Period	pDMI	Trt x pDMI	pDMI x pDMI	Trt x pDMI x pDMI
DM									
Intake, kg/d	21.8	22.7	0.9	0.06	NS[3]	0.02	NS	0.20	NS
Apparent total tract digestion									
kg/d	15.5	16.4	0.6	0.02	NS	0.009	0.04	0.07	NS
%	70.6	71.6	0.9	0.42	NS	0.98	0.09	NS	NS
OM									
Intake, kg/d	19.6	20.4	0.8	0.06	NS	0.02	NS	0.20	NS
Apparent ruminal digestion									
kg/d	9.45	10.2	0.41	0.25	NS	0.20	0.16	NS	NS
%	47.4	48.5	2.7	0.64	NS	0.17	0.16	0.06	NS
True ruminal digestion									
kg/d	11.7	12.4	0.4	0.22	NS	0.08	0.15	NS	NS
%	59.2	59.9	2.4	0.71	NS	0.19	0.12	0.04	NS
Passage to duodenum, kg/d	10.4	10.6	0.8	0.74	NS	0.06	0.16	0.08	NS
Apparent postruminal digestion									
kg/d	5.06	5.09	0.55	0.95	NS	0.04	NS	0.02	NS
% of intake	25.4	25.0	2.4	0.87	NS	0.08	NS	0.009	NS
Apparent total tract digestion									
kg/d	14.2	14.9	0.5	0.03	NS	0.009	0.04	0.07	NS
%	72.0	72.6	0.9	0.56	NS	0.87	0.08	NS	NS

[1]Treatment least squares means.

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

Table 23 (cont'd)

[3]NS = not significant ($P > 0.20$); term removed from statistical model.

Table 24. NDF digestion of cows fed treatment diets containing either long (19 mm) or short cut (10 mm) orchardgrass silage as the sole source of forage

	Treatment LSM[1]			*P*[2]					
	Long	Short	SE	Trt	Trt x Period	pDMI	Trt x pDMI	pDMI x pDMI	Trt x pDMI x pDMI
NDF									
Intake, kg/d	6.02	6.12	0.24	0.39	NS[3]	0.03	NS	0.16	NS
Ruminal digestion									
kg/d	3.85	3.91	0.14	0.63	NS	0.18	NS	0.19	NS
%	63.3	64.9	1.8	0.37	NS	0.07	0.16	0.49	0.16
Passage to duodenum, kg/d	2.25	2.14	0.16	0.33	NS	0.03	0.20	0.28	0.08
Postruminal digestion									
kg/d	0.04	-0.04	0.12	0.60	NS	0.11	NS	0.07	NS
% of intake	0.63	-0.23	2.06	0.70	NS	0.10	NS	0.04	NS
Total tract digestion									
kg/d	3.89	3.87	0.13	0.87	NS	0.01	0.14	0.01	NS
%	64.8	63.8	1.5	0.56	NS	0.99	NS	0.04	NS
Potentially digestible NDF									
Intake, kg/d	4.32	4.41	0.16	0.29	NS	0.03	NS	0.14	NS
Ruminal digestion									
kg/d	3.85	3.91	0.14	0.63	NS	0.18	NS	0.19	NS
%	90.7	89.9	1.8	0.69	NS	0.07	NS	NS	NS
Passage to duodenum, kg/d	0.52	0.44	0.12	0.47	NS	0.05	0.23	0.44	0.19
Postruminal digestion									
kg/d	0.04	-0.04	0.12	0.60	NS	0.11	NS	0.07	NS
% of intake	0.91	-0.21	2.89	0.73	NS	0.08	NS	0.04	NS
Total tract digestion									
kg/d	3.89	3.87	0.13	0.87	NS	0.01	0.14	0.01	NS
%	90.6	88.7	2.0	0.44	NS	0.97	NS	0.03	NS
Indigestible NDF									
Intake, kg/d	1.64	1.65	0.06	0.80	NS	0.12	NS	NS	NS

Table 24 (cont'd)

[1]Treatment least squares means.
[2]*P*-values for treatment (Trt), treatment by period interaction (Trt x Period), preliminary DMI (pDMI), treatment by preliminary DMI interaction (Trt x pDMI), quadratic effect of preliminary DMI (pDMI x pDMI), and treatment by quadratic effect of preliminary DMI (Trt x pDMI x pDMI).
[3]NS = not significant ($P > 0.20$); term removed from statistical model.

Figure 9

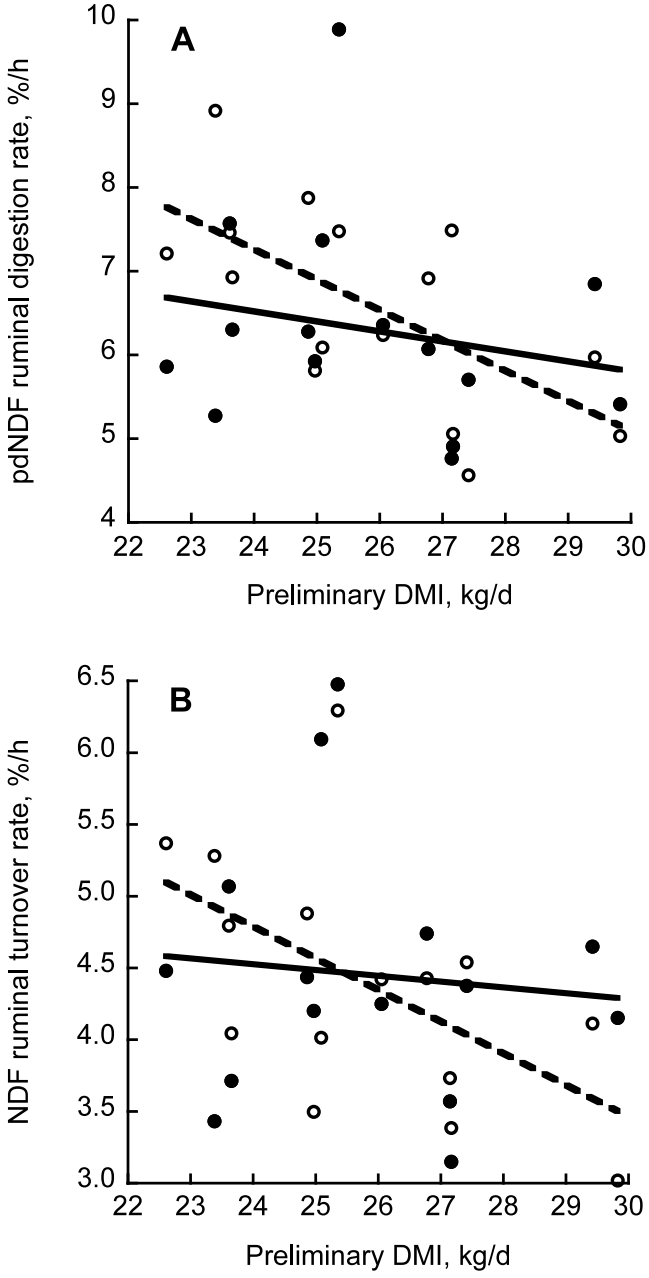

A
pdNDF ruminal digestion rate, %/h
10
9
8
7
6
5
4
22
23
24
25
26
27
28
29
30
Preliminary DMI, kg/d
B
NDF ruminal turnover rate, %/h
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
22
23
24
25
26
27
28
29
30
Preliminary DMI, kg/d

Figure 9 (cont'd)

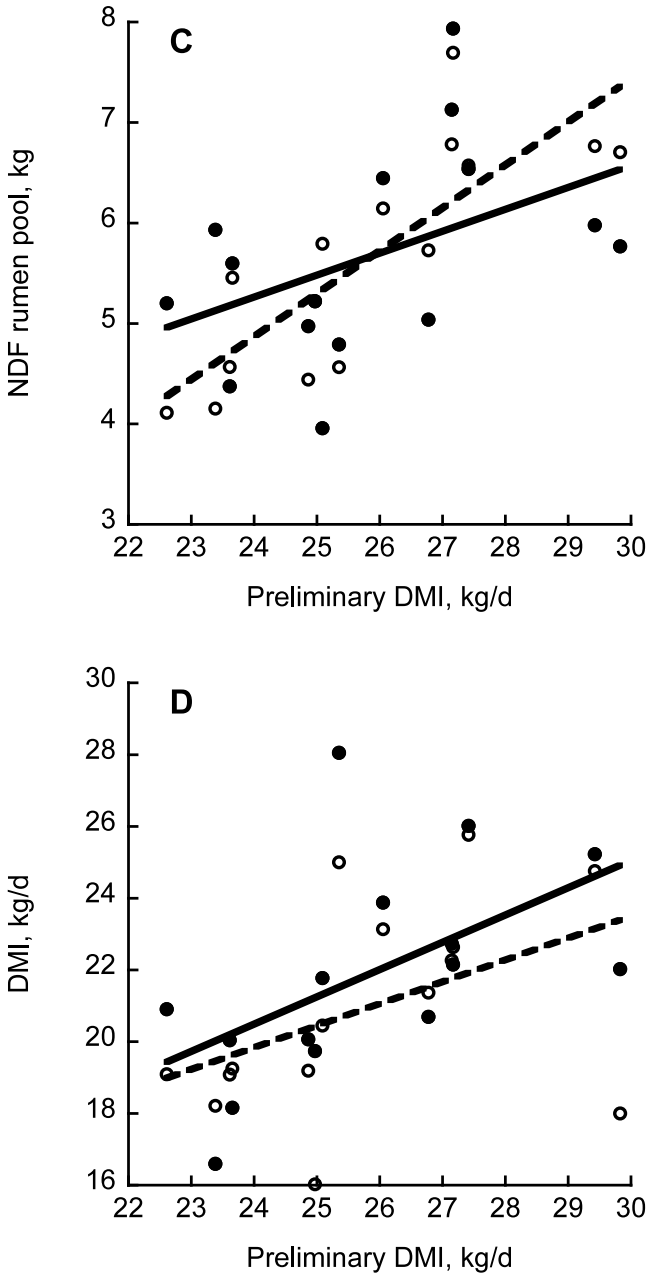


Figure 9 (cont'd)

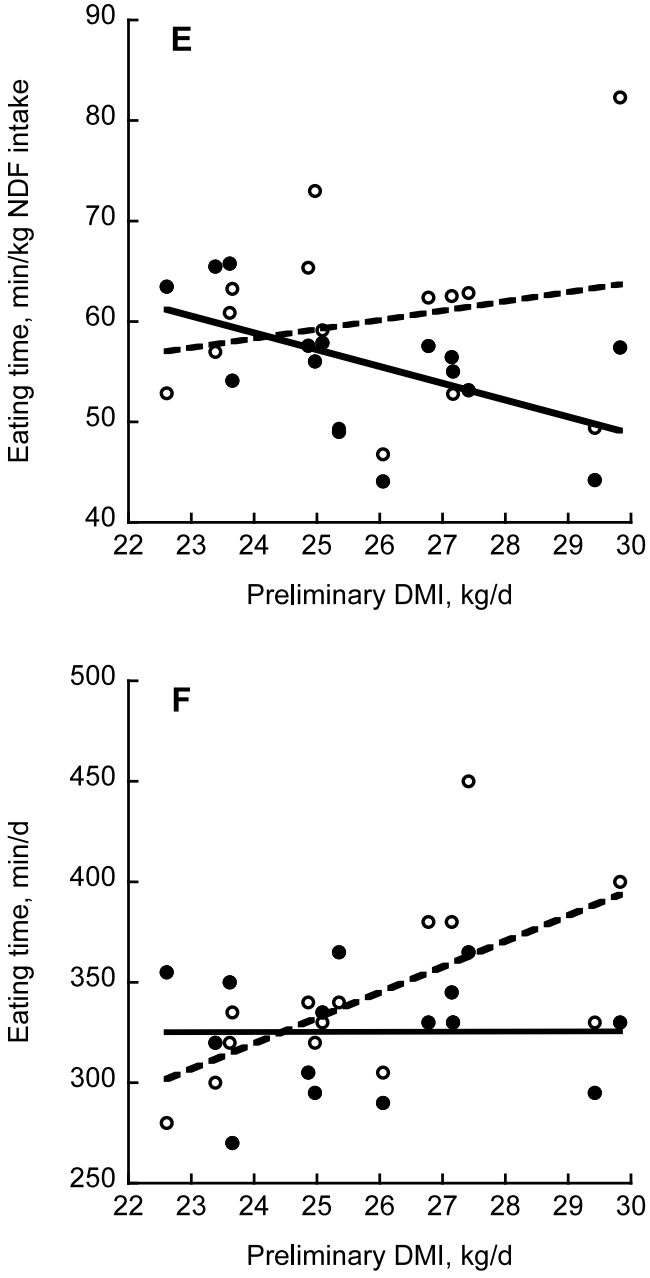


Figure 9. Interaction of long (19 mm; open circles, dashed line) and short (10 mm; closed circles, solid line) orchardgrass particle length with preliminary DMI for: A) potential digestible NDF (pdNDF) ruminal digestion rate ($P = 0.08$), B) NDF ruminal turnover rate ($P = 0.07$), C) NDF rumen pool ($P = 0.04$), D) DMI (interaction not significant), E) eating time, min/kg NDF intake ($P = 0.006$), and F) eating time, min/d ($P = 0.004$). 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.

Figure 10

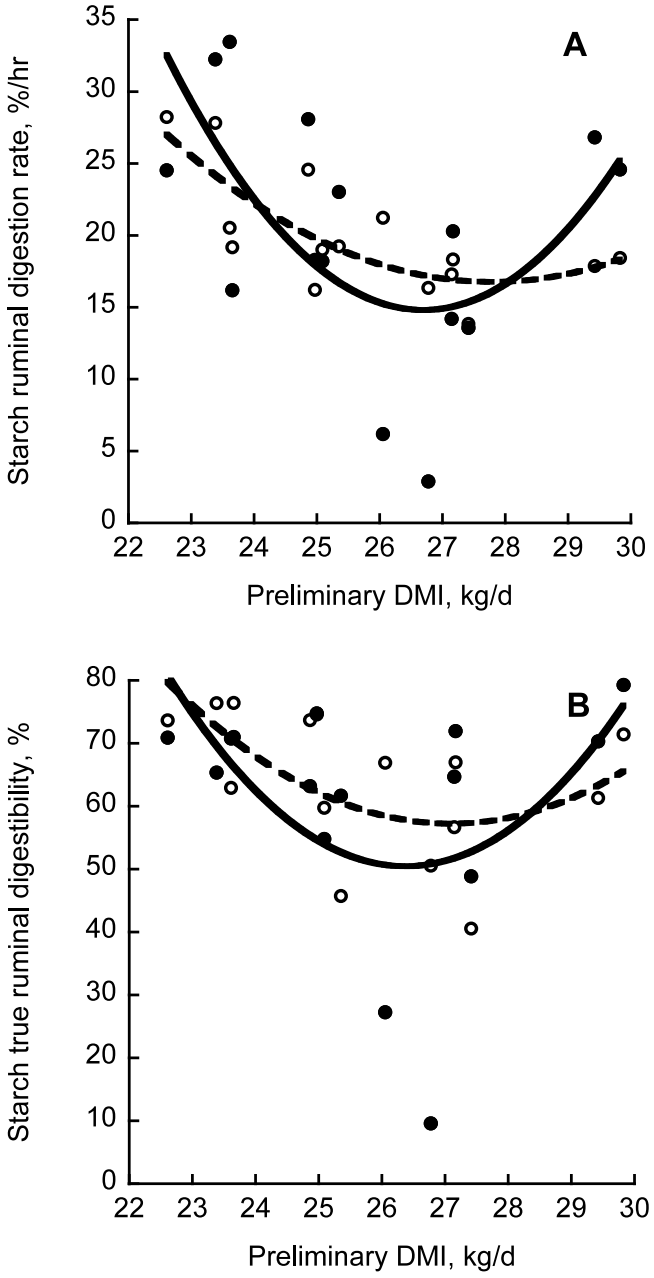
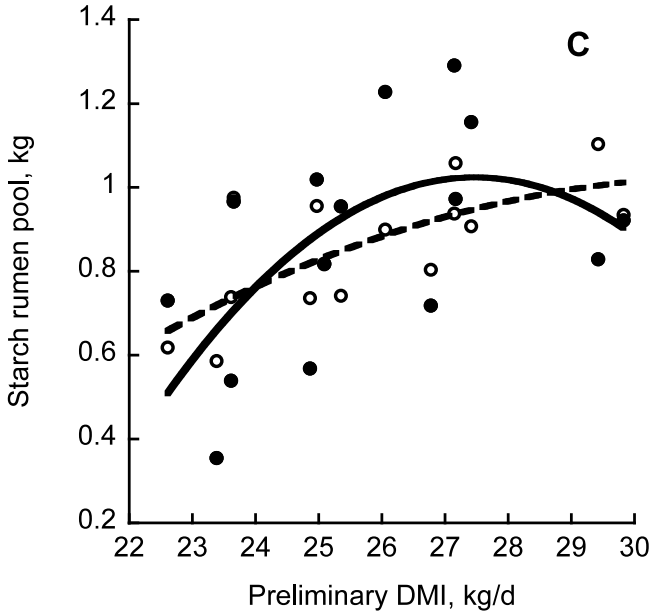

A
Starch ruminal digestion rate, %/hr
35
30
25
20
15
10
5
0
22
23
24
25
26
27
28
29
30
Preliminary DMI, kg/d
B
Starch true ruminal digestibility, %
80
70
60
50
40
30
20
10
0
22
23
24
25
26
27
28
29
30
Preliminary DMI, kg/d

Figure 10 (cont'd)



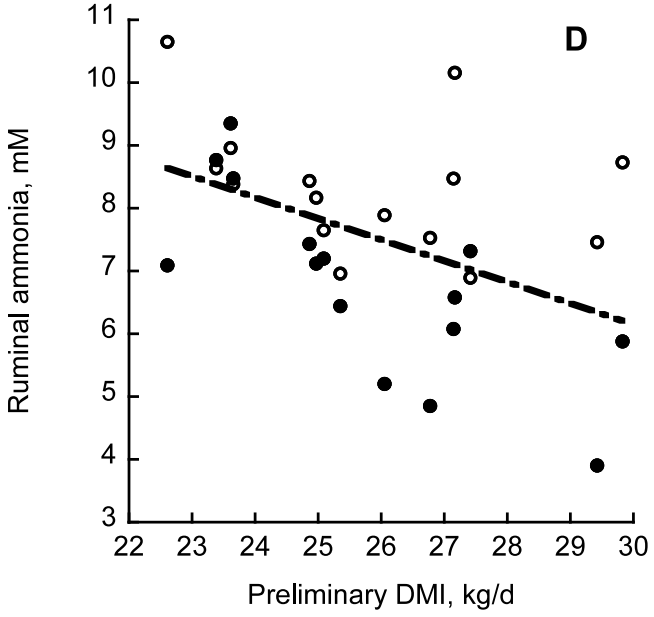


Figure 10 (cont'd)

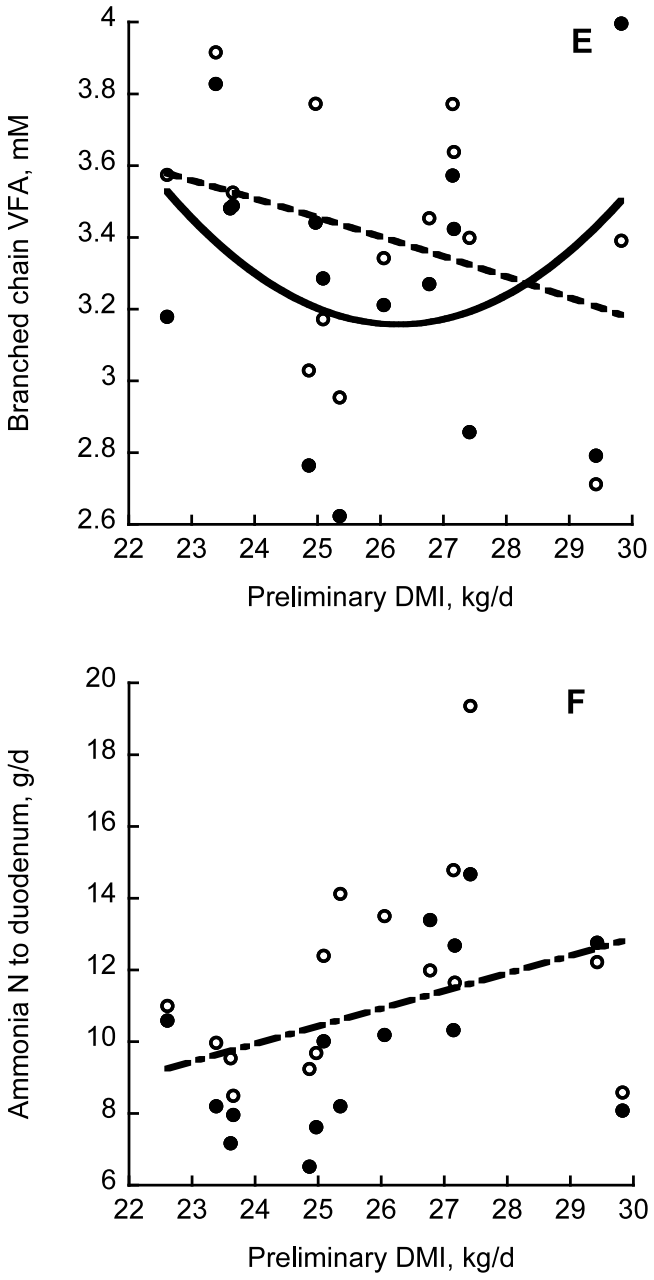


Figure 10. Relationship of long (19 mm; open circles, dashed line) and short (10 mm; closed circles, solid line) orchardgrass particle length with preliminary DMI for: A) starch ruminal digestion rate (interaction $P = 0.02$), B) starch true ruminal digestibility (interaction $P = 0.13$), C) starch rumen pool (interaction $P = 0.11$), D) ruminal ammonia concentration ($P = 0.002$), E) branched chain VFA concentration (interaction $P = 0.05$), and F) ammonia N flow to duodenum ($P = 0.05$). 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.

Figure 11

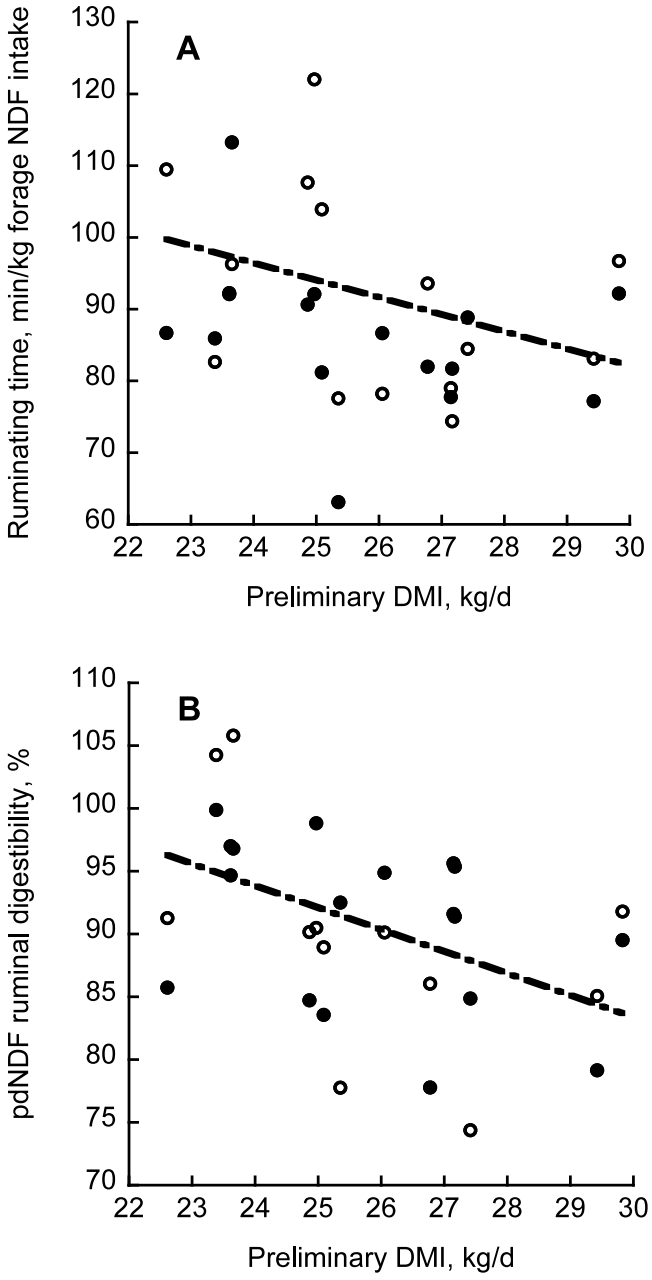

A
Ruminating time, min/kg forage NDF intake
130
120
110
100
90
80
70
60
22
23
24
25
26
27
28
29
30
Preliminary DMI, kg/d
B
pdNDF ruminal digestibility, %
110
105
100
95
90
85
80
75
70
22
23
24
25
26
27
28
29
30
Preliminary DMI, kg/d

Figure 11 (cont'd)

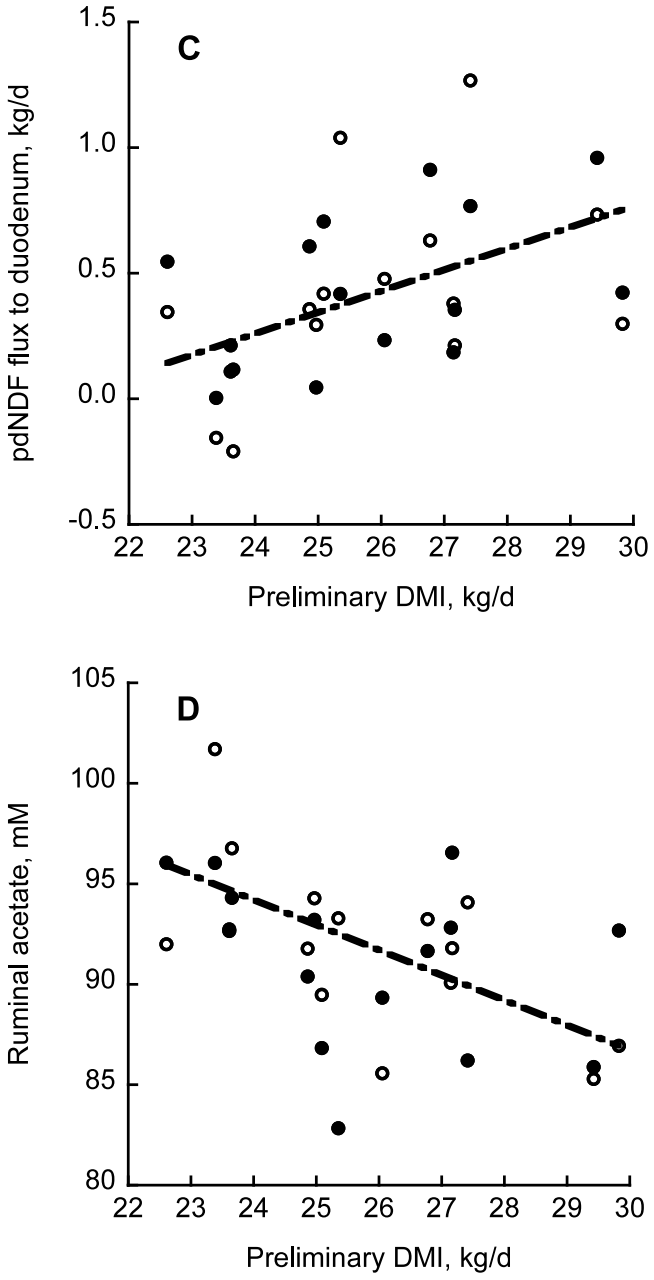


Figure 11. Relationship of long (19 mm; open circles) and short (10 mm; closed circles) orchardgrass particle length with preliminary DMI for: A) ruminating time per unit forage NDF ($P = 0.06$), B) potentially digestible NDF (pdNDF) ruminal digestibility ($P = 0.07$), C) pdNDF flux to duodenum ($P = 0.05$), and D) ruminal acetate concentration ($P = 0.02$). 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.

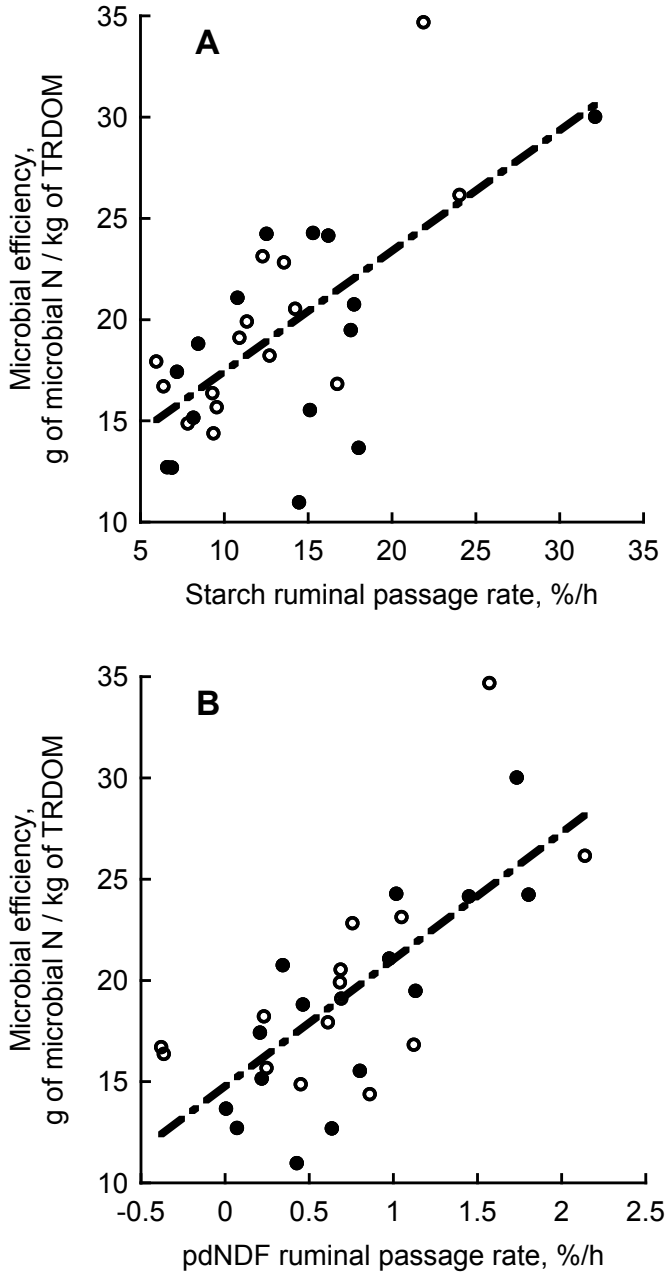


Figure 12. A) Relationship between starch ruminal passage rate and microbial efficiency. Microbial efficiency, g of microbial N/kg of true ruminally digested OM (TRDOM) = 11.5 + 0.595 x starch ruminal passage rate, %/h ($P < 0.001$, $R^2 = 0.44$). B) Relationship between potentially digestible NDF (pdNDF) ruminal passage rate and microbial efficiency. Microbial efficiency, g of microbial N/kg of TRDOM = 14.8 + 6.26 x pdNDF ruminal passage rate, %/h ($P < 0.001$, $R^2 = 0.52$). Open circles denote long (19 mm) and closed circles denote short (10 mm) orchardgrass particle length. Starch and pdNDF ruminal passage rates were also positively correlated ($P < 0.001$, $R^2 = 0.43$, not shown).

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CHAPTER 4

Nutrient Demand Interacts with Legume Maturity to Affect Rumen Pool Sizes in Dairy Cows[1]

ABSTRACT

Effects of legume maturity 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 16 ruminally and duodenally cannulated Holstein cows in a crossover design with a 14-d preliminary period and two 17-d treatment periods. During the preliminary period, pDMI of individual cows ranged from 22.9 to 30.0 kg/d (mean = 25.9 kg/d) and 3.5% fat-corrected milk (FCM) yield ranged from 34.1 to 68.2 kg/d (mean = 43.7 kg/d). Experimental treatments were diets containing alfalfa silage harvested either a) early cut, less mature (EARLY) or b) late cut, more mature (LATE) as the sole forage. Early and late cut alfalfa contained 40.8 and 53.1% neutral detergent fiber (NDF) and 23.7 and 18.1% crude protein, respectively. Forage:concentrate ratios were 53:47 and 42:58 for EARLY and LATE, respectively; both diets contained ~22% forage NDF and 27% total 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 alfalfa maturity and their interaction with pDMI were tested by ANOVA. Alfalfa maturity and its interaction with pDMI did not affect milk yield but EARLY increased DMI compared with LATE; thus, EARLY had lower efficiency of milk production than LATE. EARLY decreased milk fat concentration more per kilogram pDMI increase than LATE, but milk fat yield was not

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affected. The lower concentration and faster passage rate of indigestible NDF (iNDF) for EARLY resulted in lower rumen pools of iNDF, total NDF, and dry matter for EARLY than LATE, which EARLY increased at a slower rate than LATE as pDMI increased. EARLY decreased starch intake and increased ruminal pH compared with LATE. Rate of ruminal starch digestion was related to level of intake, but this did not affect ruminal or postruminal starch digestion. Total tract digestibility of NDF, organic matter, and dry matter was higher for EARLY than LATE. Microbial efficiency tended to decrease for EARLY and increase for LATE as pDMI increased. When alfalfa silage was the only source of forage in the diet, cows supplemented with additional concentrate to account for decreased protein and increased fiber concentrations associated with more mature alfalfa produced similar FCM yields with greater efficiency than cows fed less mature alfalfa.

INTRODUCTION

The supply and utilization of nutrients in dairy cows fed forage-based diets are affected by forage maturity at harvest. Generally, postponing harvest increases DM yield, but nutritive quality declines as the concentration of NDF increases and its digestibility decreases because of greater lignification. Dairy cows fed less mature forage generally produce more milk with less supplemental concentrate than cows fed more mature forage. Thus, harvesting and feeding quality forages may improve animal performance, reduce purchased feed costs, and increase profitability.

Fiber concentration and digestibility affect feed intake by contributing to physical fill in the rumen. As forages mature, the indigestible fraction of fiber increases and the rate of

digestion of the potentially digestible fraction decreases (Smith et al., 1972). Additionally, highly lignified (i.e. more mature) forages increase retention time in the rumen due to a slow rate of digestion (Allen, 2000). This suggests that more mature forages result in slower ruminal turnover and greater ruminal distention. Therefore, the degree to which rumen fill limits feed intake increases when dairy cows are fed more mature forage compared with less mature forage. 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.

In addition to dietary factors affecting ruminal passage, digestion, and distention, the individual cow's appetite will also affect the responses of passage rate and intake to forage maturity. Cows respond differently to treatments depending on their level of intake (Voelker Linton and Allen, 2008) and production (Oba and Allen, 1999). Because legume maturity and level of intake affect ruminal passage and digestion rates and, thus, the physical filling effects in the rumen, the response to effects of legume maturity 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 digesta passage rates to legume maturity are related to level of intake and earlier maturity will permit a greater increase in passage rate than later maturity as feed intake increases.

The objectives of this experiment were to evaluate the relationships between voluntary DMI and effects of legume maturity on DMI, milk production, ruminal fermentation and pool sizes, and digestion and passage kinetics in lactating dairy cows. This study had three distinctive features to improve our understanding of the role of legume maturity and interpret its effect on animal responses. First, it allowed effects of the interaction between legume maturity 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 early and late cut AL as the sole source of forage. 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. Sixteen 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 17-d experimental periods. The first 10 d of each period were allowed for diet adaptation and samples were collected during the final 4 d of the preliminary period and 7 d of each experimental period. Cows were 137±45 (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 22.9 to 30.0 kg/d (mean = 25.9 kg/d) and 3.5% FCM yield ranged from 34.1 to 68.2 kg/d (mean = 43.7 kg/d; Table 25). 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 from the same field harvested either a) early cut, less mature (EARLY) or b) late cut, more mature (LATE) as the sole forage. Alfalfa was produced at the campus farm at Michigan State University (East Lansing), harvested using a New Holland FP230 pull-type forage harvester set according to manufacturer specifications for a theoretical length of cut of 10 mm, and ensiled in Ag-Bags (Ag-Bag Systems Inc., St. Nazianz, WI). During the sample collection periods, early and late cut AL contained 40.8 and 53.1% NDF and 23.7 and 18.1% CP, respectively (DM basis; Table 26). Diets EARLY and LATE were formulated to contain 22% forage NDF, 30% total NDF, and 18% CP. We acknowledge these treatments affect dietary starch concentration but maintaining similar forage and total NDF concentrations for both treatments was of primary interest. The diet fed during the preliminary period was formulated so that early and late cut AL each contributed 50% of forage NDF. Diets also contained dry ground corn, soybean meal (48% CP), SoyPlus® (West Central Soy, Ralston, IA), vitamin-mineral premix, and limestone (Table 27).

Data and Sample Collection

Throughout the experiment, cows were housed in tie-stalls and fed diets as total mixed rations once daily (1130 h) at 110% of expected intake. The amount of feed offered and refused (orts) was 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 11 to 15 during each experimental period. Samples were frozen immediately after collection at −20°C and combined to one composite sample per period prior to analysis.

Cows were moved to an exercise lot twice daily (0230 and 1300 h) prior to 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 11 to 15 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 17 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 11 to 15 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 was 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 five 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.

Ruminal contents were evacuated manually through the ruminal cannula 4.5 h after feeding at the beginning of d 15 (1600 h) and 2 h before feeding at the end of d 17 (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 yield recorded at each milking was 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 one 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 (Tri-Philizer 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 one 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, iNDF, ADF, acid detergent lignin (ADL), CP, and starch. Ash concentration was determined after 5 h 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). Indigestible NDF was estimated as NDF residue after 240 h in vitro fermentation (Goering and Van Soest, 1970); flasks were reinoculated at 120 h to insure 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 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 was blended, strained through nylon mesh, and the liquid portion was centrifuged at 500 x *g* for 15 min. The supernatant was centrifuged at 18,000 x *g* for 15 min, and the pellet was washed with 0.9% NaCl solution and centrifuged again at 18,000 x *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 concentration of major VFA and lactate by HPLC (Waters Corp., Milford, MA) according to Oba and Allen (2003a).

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 ratio of purines to OM (Oba and Allen, 2003a), 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 the following equations:

Turnover rate (%/h) = 100 x (Intake of component / Ruminal pool of component) / 24

Passage rate (%/h) = 100 x (Duodenal flow of component / Ruminal pool of component) / 24

Digestion rate (%/h) = Turnover rate in the rumen (%/h) – Passage rate from the rumen (%/h).

Statistical Analysis

All data were analyzed by using the fit model procedure of JMP (Version 8, SAS Institute, 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: $Y_{ijk} = \mu + C_i + P_j + T_k + PT_{jk} + pDMI + T_k pDMI + pDMI^2 +$

$T_kpDMI^2 + e_{ijk}$ where μ is the overall mean, C_i is the random effect of cow (i = 1 to 16), 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_kpDMI^2 is the interaction of treatment and pDMI (quadratic), and e_{ijk} is the residual error. Statistical significance for T_kpDMI and T_kpDMI^2 indicated 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. Tendencies for treatment effects and their interactions were declared at $P \leq 0.10$ and $P \leq 0.15$, respectively.

RESULTS AND DISCUSSION

Comparison of Forages and Diets

Chemical analyses and physical characteristics of AL silages are listed in Table 26. As expected, delaying harvest of AL decreased the concentration of CP (23.7 vs. 18.1%) and increased concentrations of total NDF (40.8 vs. 53.1%), iNDF (27.7 vs. 39.3%), and ADL (8.41 vs. 11.9%). Indigestible NDF, expressed as a percent of NDF, was lower for early cut AL than for late cut AL (67.8 vs. 74.1% of NDF), but was unusually high for both silages. In vitro NDF digestibility (30 h) was 4.4 percentage units higher for early cut AL than for late cut AL (28.8 vs. 24.4%). Early cut AL had lower DM concentration, higher pH, and contained more lactic and acetic acids than late cut AL. Based on wet sieving, early cut AL had a greater mean particle

size than late cut AL (8.36 vs. 6.92 mm). Additionally, early cut AL contained a larger proportion of particles between 8 and 19 mm (67.3 vs. 35.7%; middle sieve) and a smaller proportion of particles less than 8 mm (25.5 vs. 58.6%; bottom pan) than late cut AL when sieved with the Penn State Particle Separator. Although AL was chopped to the same theoretical length of cut for both silages, the smaller particle size for late cut AL may be due to greater fragility from increased maturity or greater DM concentration or both.

Diet ingredients and chemical composition are shown in Table 27. The preliminary diet contained more early cut AL than late cut AL so each forage supplied similar concentrations of forage NDF. Because treatment diets were formulated to contain similar forage NDF concentrations, forage:concentrate ratios were different between diets with ratios of 53:47 and 42:58 for EARLY and LATE, respectively. Besides forage source, differences in diets included source and concentration of protein supplement and concentration of corn grain, which were both lower for EARLY than LATE, to account for differences between the AL silages. Additionally, limestone was added to LATE to compensate for lower Ca concentration in more mature AL silage. The chemical composition of each ration was mathematically calculated according to the proportion of each feed ingredient in the diet and its respective analytical values, and was similar between rations for OM, total NDF, and CP concentrations. Based on the calculated diet composition, both diets contained lower NDF and higher CP concentrations than the formulated targets but were similar for EARLY and LATE and NDF concentration was above NRC (2001) minimum requirements. Starch concentration was lower for EARLY because of more forage and less concentrate in the diet for EARLY compared with LATE. Differences in DM and iNDF concentrations in diets, which were lower for EARLY than LATE, were reflective of the

concentrations in the forages, which were lower for early cut AL than late cut AL. In both diets, forage NDF provided over 80% of the total diet NDF.

Effects of Legume Maturity and pDMI

EARLY increased DMI (28.6 vs. 26.8 kg/d, $P = 0.003$, Table 28) and total NDF intake (7.63 vs. 7.30 kg/d, $P = 0.03$, Table 29) compared with LATE, but these responses were not related to level of intake ($P \geq 0.53$). We expected LATE to be more filling than EARLY causing greater rumen distention and potentially limiting DMI, particularly in cows with high DMI for which ruminal distention is more likely to limit feed intake (Allen, 1996). Oba and Allen (1999) and Voelker et al. (2002) 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. As fill became more limiting, we expected ruminal passage rate of iNDF to increase more for EARLY than for LATE. However, response of passage rate of iNDF to treatment was not related to pDMI ($P \geq 0.37$), but EARLY increased ruminal passage rate of iNDF compared with LATE (3.86 vs. 3.57%/h, $P = 0.01$, Table 30).

The lower concentration and faster rate of passage of iNDF for EARLY is reflected in the lower rumen pool sizes of iNDF (5.49 vs. 6.24 kg, $P < 0.001$) and NDF (6.23 vs. 6.96 kg, $P < 0.001$) for EARLY compared with LATE (Table 31), which increased at a slower rate for EARLY than LATE as pDMI increased (interaction $P \leq 0.10$, Figure 13). It is also partially responsible for lower rumen pool sizes of OM (10.3 vs. 11.4 kg, $P = 0.006$) and DM (11.3 vs. 12.4, $P = 0.007$) for EARLY compared with LATE (Table 31), which tended to increase at a slower rate for EARLY than LATE as pDMI increased (interaction $P \leq 0.14$). Furthermore, EARLY decreased rumen digesta wet weight (76.9 vs. 80.8 kg, $P = 0.04$) and volume (91.3 vs.

97.8 L, $P = 0.03$) compared with LATE (Table 31). The faster rate of passage of iNDF and lower rumen pools for EARLY are consistent with the faster rates of ruminal turnover of NDF, OM, and DM (5.25 vs. 4.45, 10.8 vs. 9.15, and 10.7 vs. 9.07%/h, respectively, $P < 0.001$) for EARLY compared with LATE (Table 30). The decreased feed intake of LATE was accompanied by increased size of rumen pools suggesting rumen fill as a constraint limiting DMI for cows consuming LATE is possible, but there was no evidence in this experiment that ruminal distention is more likely to limit feed intake for cows with high intake compared to cows with low intake as we were unable to detect a treatment by pDMI interaction for DMI.

Although EARLY increased DMI compared with LATE, forage maturity and its interaction with pDMI did not affect milk, milk component, or FCM yields (Table 28). Therefore, EARLY decreased efficiency of milk production (FCM/DMI) compared with LATE (1.45 vs. 1.51, $P = 0.01$), as cows consuming EARLY ate more feed than LATE to produce similar yields of FCM. Response of milk fat concentration to treatment was related to pDMI (interaction $P = 0.09$, Table 28) such that EARLY decreased milk fat concentration more per kilogram pDMI increase than LATE. However, this effect was small and milk fat yield was not affected. The aforementioned results are likely because of the greater concentration of supplemental concentrate in the diet for LATE.

The different forage:concentrate ratios in the diets were necessary to account for changes in chemical composition of AL with increasing maturity and maintain the same concentration of forage NDF in the diets. As a result, EARLY decreased starch intake (8.16 vs. 8.74 kg/d, $P = 0.007$, Table 32), decreased ruminal propionate (29.5 vs. 31.0 mM, $P = 0.04$) and butyrate (16.1 vs. 18.1 mM, $P < 0.001$) concentrations, and increased ruminal acetate concentration (86.3 vs. 81.9 mM, $P = 0.005$) compared with LATE (Table 33). EARLY increased the acetate to

propionate ratio compared with LATE (2.94 vs. 2.65, $P < 0.001$), and the difference in ruminal acetate concentration between EARLY and LATE was greatest for cows with low pDMI (interaction $P = 0.09$, Figure 14). These results are reflective of the diets.

EARLY increased mean ruminal pH (6.36 vs. 6.29, $P = 0.04$, Table 33) compared with LATE, which was expected because of lower starch intake for EARLY than LATE, but the reason for the difference in pH is not clear based on our results. Apparent ruminal digestion of OM was greater for EARLY than LATE (9.76 vs. 7.93 kg/d, $P < 0.001$, Table 34), which would suggest greater production of VFA and lower pH for EARLY, but total VFA concentration in the rumen was similar for EARLY and LATE ($P = 0.48$, Table 33) while pH was higher for EARLY than LATE. Additionally, rumen content mass was smaller for EARLY than LATE (Table 31) suggesting less buffering capacity of digesta. The ruminal pH difference observed might be due to chewing activity and buffering through saliva secretion but these responses were not measured. In an experiment of similar design evaluating grass maturity in which chewing behavior was measured, cows spent less time ruminating per day when consuming early cut orchardgrass compared with late cut orchardgrass (Kammes and Allen, submitted). However, it is not known if differences in ruminating time because of maturity would be the same for legumes and grasses.

EARLY increased pdNDF intake (2.71 vs. 2.06 kg/d, $P < 0.001$) and decreased iNDF intake (4.92 vs. 5.24 kg/d, $P = 0.008$) compared with LATE (Table 29) because of differences in chemical composition of forages. Despite the differences in the proportion of iNDF and pdNDF within the total NDF of the forage, both treatments had nearly complete digestion of pdNDF in the rumen leading to essentially no passage of pdNDF to the duodenum (Table 29), and therefore, ruminal passage rate of pdNDF was negligible (Table 30). Ruminal digestibility of

pdNDF in the current experiment is unusually high because the pdNDF fraction was abnormally low. EARLY increased ruminal (35.7 vs. 30.2%, $P = 0.02$) and total tract (28.5 vs. 19.5%, $P < 0.001$) digestibility of NDF compared with LATE (Table 29), which is because of a greater proportion of pdNDF and lower proportion of iNDF in NDF for EARLY compared with LATE (Table 26).

Total tract digestibilities of NDF (and pdNDF) are lower than ruminal digestibility because negative postruminal digestibilities were calculated for NDF (and pdNDF) in the present experiment. This is 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), which is 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. While absolute values are not biologically reasonable, relative comparisons between treatments within the same experiment are useful.

EARLY increased true ruminal OM digestibility (52.5 vs. 46.4%, $P = 0.002$) and digestion (13.4 vs. 11.1 kg/d, $P < 0.001$) compared with LATE (Table 34). Because postruminal OM digestibility and digestion were similar for EARLY and LATE, EARLY also increased total tract OM digestibility (62.8 vs. 58.3%, $P < 0.001$) and digestion (16.1 vs. 14.1 kg/d, $P < 0.001$)

compared with LATE. This resulted in greater total tract DM digestibility (62.5 vs. 58.0%, $P < 0.001$) and digestion (17.4 vs. 15.1 kg/d, $P < 0.001$) for EARLY than LATE (Table 34).

Despite less supplementation with soybean products in EARLY compared with LATE, the high CP concentration in the early cut AL resulted in greater N intake for EARLY than LATE (918 vs. 812 g/d, $P < 0.001$, Table 35). EARLY increased ruminal ammonia concentration compared with LATE (22.5 vs. 20.4 mg/dl, $P < 0.001$, Table 35). Early cut AL had higher ammonia concentration than late cut AL (8.09 vs. 6.04 mM, Table 26), which may be one source for the greater ammonia concentration observed for cows consuming EARLY. Another source might be protein degradation in the rumen as indicated by greater concentrations of isovalerate (2.82 vs. 2.51 mM, $P = 0.003$) and branched chain VFA (4.41 vs. 4.03 mM, $P = 0.005$) for EARLY than LATE (Table 33). This is consistent with the faster rate of ruminal starch digestion previously mentioned for EARLY compared with LATE (Table 30); early cut alfalfa might promote greater numbers or activity of starch-digesting bacteria in the rumen of cows consuming EARLY. Because some starch-digesting bacteria in the rumen (e.g. *Streptococcus bovis*) also have high proteolytic activity (Russell et al., 1981) resulting in deamination of amino acids and production of ammonia, this could contribute to the increased ammonia concentration for EARLY.

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 impact of passage on ruminal digestion and microbial growth, quantitative knowledge of rates of nutrient passage from the rumen is 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).

Rate of ruminal passage for iNDF was related to level of intake independent of treatment ($P = 0.07$), which increased quadratically as pDMI increased with a greater increase for cows with lower pDMI (Figure 15). This is in contrast to results in the previously mentioned experiment of similar design evaluating effects of grass maturity in which cows in the middle of the pDMI range had the slowest rate of iNDF passage (Kammes and Allen, submitted). As previously discussed, ruminal digestion of pdNDF was nearly complete for all cows regardless of treatment and varying levels of pDMI; therefore, rate of ruminal passage for pdNDF was negligible and not related to level of intake.

Level of intake had no effect on the rate of starch passage, but it tended to influence rate of ruminal starch digestion (Table 30). As pDMI increased, EARLY slightly increased ruminal starch digestion rate and LATE quadratically affected it (interaction $P = 0.13$, Table 30). The largest difference in starch digestion rate between EARLY and LATE was observed for cows in the middle of the pDMI range, such that EARLY had a greater starch digestion rate than LATE (Figure 16A). However, this did not affect starch ruminal turnover rate (Table 30), digestibility (%), or digestion (kg/d; Table 32), which were similar for EARLY and LATE. EARLY decreased rumen pool of starch compared with LATE (0.964 vs. 1.13 kg, $P = 0.02$, Table 31) because of the lower intake and faster digestion rate of starch for EARLY but was not related to pDMI. The reduction in starch rate of digestion for LATE might be related to increased liquid dilution rate (not measured) associated with greater increases in rumen pool sizes for LATE compared with EARLY as intake increased, decreasing populations of starch-digesting microbes.

Ruminal ammonia concentration tended to decrease with increased pDMI ($P = 0.14$, Figure 16B) and response of isovalerate (interaction $P = 0.002$, quadratic, Table 33) and branched chain VFA (interaction $P = 0.01$, quadratic, Figure 16C) concentrations, which are derived from degradation of branched chain amino acids, followed a pattern similar to starch rate of digestion for LATE, but not EARLY. This is consistent with less proteolytic activity in the rumen for LATE due to greater removal of ruminal microbes through passage or lysis.

Effects of Treatment and pDMI on N Flux and Microbial Efficiency

Results for N metabolism are shown in Table 35. EARLY decreased NANMN flux (32.5 vs. 41.1% of N intake, $P = 0.008$) and NAN flux (79.5 vs. 88.3% of N intake, $P < 0.001$) to the duodenum as a percent of N intake and tended to increase MN flux (58.2 vs. 52.4% of duodenal NAN, $P = 0.09$) to the duodenum as a percent of duodenal NAN compared with LATE, but the amounts (g/d) of NANMN, NAN, and MN that passed from the rumen to the duodenum were not different ($P \geq 0.13$). However, fluxes of NANMN ($P = 0.03$), NAN ($P = 0.001$), and MN ($P = 0.11$) to the duodenum were positively related to pDMI independent of treatment. This is in agreement with the results in a review by Clark et al. (1992) that reported positive linear relationships between OM intake and fluxes of NAN, NANMN, and MN as OM intake increased over a very wide range (3 to 23 kg/d).

Based on studies with continuous culture fermenters, increases in solid and liquid dilution rates, which can be associated with increased intake, resulted in greater microbial efficiency (Crawford et al., 1980; Shriver et al., 1986). In this experiment, microbial efficiency tended to be related to pDMI (interaction $P = 0.14$, Table 35), but the response differed by treatment; EARLY tended to decrease microbial efficiency and LATE tended to increase it as pDMI increased (Figure 17). Microbial efficiency of cows consuming EARLY quadratically decreased

as true ruminally digested OM increased ($P = 0.03$, $R^2 = 0.42$, Figure 18); however, there was no relationship between microbial efficiency and true ruminally digested OM for cows consuming LATE ($P = 0.65$). This is the opposite of that demonstrated in the experiment of similar design previously mentioned that evaluated the effects of grass maturity; there was no relationship between microbial efficiency and true ruminally digested OM for cows consuming diets with early cut grass silage and a linear decrease in microbial efficiency as true ruminally digested OM increased for cows consuming diets with late cut grass silage (Kammes and Allen, submitted). Additionally, microbial efficiency linearly decreased independent of treatment as ruminal digestion rate of starch increased ($P = 0.002$, Figure 19). Similarly, others have reported that efficiency of MN production was inversely related to true ruminally digested OM (kg/d; Oba and Allen, 2003b) and starch digestion rate (Oba and Allen, 2003b; Voelker and Allen, 2003) in experiments evaluating carbohydrate source, concentration, and fermentability of diets in dairy cattle. These negative relationships indicated factors other than availability of energy limited efficiency of MN production and energy from OM fermentation was uncoupled from microbial growth (Russell and Cook, 1995).

CONCLUSIONS

Delaying the harvest of alfalfa resulted in lower CP and higher NDF and iNDF concentrations. Alfalfa maturity and its interaction with pDMI did not affect milk or FCM yields but EARLY increased DMI compared with LATE; thus, EARLY had lower efficiency of milk production than LATE. EARLY decreased milk fat concentration more per kilogram pDMI increase than LATE, but milk fat yield was not affected. The lower concentration and faster passage rate of iNDF for EARLY resulted in lower rumen pools of iNDF, total NDF, and DM

for EARLY compared with LATE, which EARLY increased at a slower rate than LATE as pDMI increased. EARLY decreased starch intake and increased ruminal pH compared with LATE. Rate of ruminal starch digestion was related to level of intake; however, this did not affect ruminal or postruminal starch digestion. Total tract digestibility of NDF, OM, and DM was higher for EARLY than LATE. When alfalfa silage was the only source of forage in the diet, cows supplemented with additional concentrate to account for decreased protein and increased fiber concentrations associated with more mature alfalfa produced similar FCM yields with greater efficiency than cows fed less mature alfalfa.

APPENDIX

Table 25. Characterization of 16 cows during the final 4 d of the 14-d preliminary period, when cows were fed a common diet

Parameter	Median	Mean	Standard deviation	Minimum	Maximum
Parity	3	2.88	0.79	2	4
BW[1], kg	557	569	43	528	692
BCS	2.22	2.24	0.38	1.63	3.06
DIM	155	137	45	56	208
Milk, kg/d	40.0	41.7	9.6	29.6	66.1
3.5% FCM, kg/d	42.4	43.7	8.6	34.1	68.2
DMI, kg/d	26.1	25.9	2.1	22.9	30.0

[1]Empty BW (ruminal digesta removed).

Table 26. Chemical composition, particle size distribution, and fermentation parameters of the early or late cut alfalfa silage included in the treatment diets

	Alfalfa silage	
Item	Early	Late
Chemical composition		
DM, %	30.2	35.6
OM, % DM	89.5	92.1
NDF, % DM	40.8	53.1
iNDF[1], % DM	27.7	39.3
iNDF, % of NDF	67.8	74.1
ADF, % of DM	37.4	44.8
ADL[2], % of DM	8.41	11.9
CP, % DM	23.7	18.1
Starch, % DM	1.16	1.48
IV NDF digestibility[3], %	28.8	24.4
Particles size distribution[4]		
Wet sieving, % DM retained		
19.0 mm	8.61	8.84
9.50 mm	20.3	11.7
4.75 mm	33.4	33.9
2.36 mm	19.2	27.1
1.18 mm	7.32	7.52
0.600 mm	4.34	4.99
0.300 mm	2.81	2.57
0.150 mm	2.14	1.66
0.075 mm	1.10	0.97
0.038 mm	0.86	0.77
Mean particle size[5], mm	8.36	6.92
Penn State Particle Separator, % DM retained		
> 19.0 mm	7.19	5.69
19.0 to 8.0 mm	67.3	35.7
< 8.0 mm	25.5	58.6
Fermentation		
pH	4.61	4.42
Acetic acid, % DM	4.42	2.17
Propionic acid, % DM	0.45	0.12
Butyric acid, % DM	0.26	<0.01
Lactic acid, % DM	9.01	8.40
Lactic:Acetic	2.04	3.87
Ethanol, % DM	0.14	0.11
Ammonia, mM	8.09	6.04

[1]iNDF = indigestible NDF.

[2]ADL = acid detergent lignin.

Table 26 (cont'd)

[3]30 h in vitro NDF digestibility.
[4]Particle size distributions of silages were measured each period (n = 2).
[5]Mean particle size calculated from particle size distribution determined by wet sieving.

Table 27. Ingredients and chemical composition of preliminary and treatment diets (as analyzed) containing either early or late cut alfalfa silage as the sole source of forage

	Preliminary	Early	Late
Ingredients, % DM			
Alfalfa silage, early cut	27.0	53.4	---
Alfalfa silage, late cut	20.2	---	42.3
Dry ground corn	38.1	34.4	39.3
Soybean meal (48% CP)	6.94	---	13.0
SoyPlus®	2.75	7.57	---
Vitamin mineral mix[1]	4.68	4.68	4.68
Limestone	0.39	---	0.78
Chemical composition			
DM, %	44.8	43.4	54.2
OM, % DM	94.1	91.8	92.8
NDF, % DM	26.8	27.1	27.7
% forage NDF	22.0	21.8	22.4
% NDF from forage	82.0	80.4	81.1
iNDF[2], % DM	NA[3]	17.5	19.9
iNDF, % of NDF	NA	64.6	71.8
CP, % DM	18.4	20.2	19.0
Starch, % DM	32.1	28.2	32.1

[1]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.

[2]iNDF = indigestible NDF.

[3]NA = no analysis for preliminary diet.

Table 28. Milk production and composition, feed intake, and BW change of cows fed treatment diets containing either early or late cut alfalfa silage as the sole source of forage

	Treatment LSM[1]			*P*[2]					
	Early	Late	SE	Trt	Trt x Period	pDMI	Trt x pDMI	pDMI x pDMI	Trt x pDMI x pDMI
Yield, kg/d									
Milk	42.5	41.7	2.6	0.14	NS[3]	0.001	NS	0.09	NS
FCM (3.5 %)	44.0	43.0	2.4	0.24	NS	0.003	NS	0.13	NS
Milk fat	1.59	1.55	0.08	0.33	NS	0.01	NS	0.19	NS
Milk protein	1.31	1.30	0.05	0.62	NS	<0.001	NS	0.04	NS
Milk lactose	2.06	2.03	0.15	0.30	NS	0.001	NS	0.08	NS
SNF	3.76	3.71	0.22	0.40	NS	<0.001	NS	0.06	NS
Milk composition, %									
Fat	3.78	3.74	0.12	0.49	NS	<0.001	0.09	0.06	NS
Protein	3.19	3.23	0.07	0.18	NS	0.03	NS	NS	NS
Lactose	4.82	4.85	0.08	0.30	NS	0.002	NS	0.05	NS
SNF	8.76	8.83	0.07	0.06	NS	NS	NS	NS	NS
DMI, kg/d	28.6	26.8	0.7	0.003	0.02	<0.001	NS	0.14	NS
3.5% FCM/ DMI	1.45	1.51	0.05	0.01	0.02	0.03	NS	0.17	NS
BW change, kg/17 d	4.70	4.61	3.03	0.99	NS	0.02	NS	0.03	NS
BCS change/17 d	0.065	0.067	0.044	0.98	0.11	<0.001	0.72	0.001	0.08

[1]Treatment least squares means.

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

[3]NS = not significant ($P > 0.20$); term removed from statistical model.

Table 29. NDF digestion of cows fed treatment diets containing either early or late cut alfalfa silage as the sole source of forage

	Treatment LSM[1]			*P*[2]					
	Early	Late	SE	Trt	Trt x Period	pDMI	Trt x pDMI	pDMI x pDMI	Trt x pDMI x pDMI
NDF									
Intake, kg/d	7.63	7.30	0.20	0.03	0.15	<0.001	NS[3]	0.14	NS
Ruminal digestion									
kg/d	2.63	2.15	0.13	0.003	NS	0.07	NS	NS	NS
%	35.7	30.2	1.5	0.02	NS	NS	NS	NS	NS
Passage to duodenum, kg/d	4.97	5.12	0.17	0.48	0.03	<0.001	NS	0.08	NS
Postruminal digestion									
kg/d	-0.50	-0.74	0.14	0.19	NS	NS	NS	NS	NS
Total tract digestion									
kg/d	2.13	1.40	0.09	<0.001	NS	0.02	NS	NS	NS
%	28.5	19.5	1.0	<0.001	NS	NS	NS	NS	NS
Potentially digestible NDF									
Intake, kg/d	2.71	2.06	0.07	<0.001	NS	<0.001	NS	0.16	NS
Ruminal digestion									
kg/d	2.63	2.15	0.12	0.003	NS	0.07	NS	NS	NS
%	99.9	107.8	5.1	0.29	NS	NS	NS	NS	NS
Passage to duodenum, kg/d	0.01	-0.15	0.11	0.29	NS	NS	NS	NS	NS
Postruminal digestion									
kg/d	-0.50	-0.74	0.14	0.19	NS	NS	NS	NS	NS
Total tract digestion									
kg/d	2.13	1.40	0.09	<0.001	NS	0.02	NS	NS	NS
%	80.0	69.2	3.3	0.007	NS	NS	NS	NS	NS
Indigestible NDF									
Intake, kg/d	4.92	5.24	0.14	0.008	0.12	<0.001	NS	0.14	NS

[1]Treatment least squares means.

Table 29 (cont'd)

[2]*P*-values for treatment (Trt), treatment by period interaction (Trt x Period), preliminary DMI (pDMI), treatment by preliminary DMI interaction (Trt x pDMI), quadratic effect of preliminary DMI (pDMI x pDMI), and treatment by quadratic effect of preliminary DMI (Trt x pDMI x pDMI).
[3]NS = not significant ($P > 0.20$); term removed from statistical model.

Table 30. Rumen kinetics of cows fed treatment diets containing either early or late cut alfalfa silage as the sole source of forage

	Treatment LSM[1]			*P*[2]					
	Early	Late	SE	Trt	Trt x Period	pDMI	Trt x pDMI	pDMI x pDMI	Trt x pDMI x pDMI
Ruminal turnover rate, %/h									
DM	10.7	9.07	0.31	<0.001	0.002	0.07	NS[3]	0.10	NS
OM	10.8	9.15	0.33	<0.001	0.002	0.09	NS	0.11	NS
NDF	5.25	4.45	0.15	<0.001	0.04	0.05	NS	0.04	NS
pdNDF[4]	17.2	14.1	1.5	0.15	NS	0.80	NS	0.19	NS
Starch	36.5	33.1	2.2	0.21	0.02	NS	NS	NS	NS
Ruminal turnover time, h									
DM	9.56	11.1	0.33	<0.001	0.004	0.03	NS	0.08	NS
OM	9.54	11.0	0.34	0.002	0.003	0.04	NS	0.09	NS
NDF	19.3	22.7	0.71	<0.001	0.10	0.03	NS	0.03	NS
pdNDF	6.70	8.90	0.66	0.05	NS	NS	NS	NS	NS
iNDF[5]	26.6	28.2	1.1	0.11	0.09	0.03	NS	0.07	NS
Starch	2.93	3.19	0.15	0.21	0.005	NS	NS	NS	NS
Ruminal passage rate, %/h									
pdNDF	-0.06	-0.91	0.77	0.42	NS	NS	NS	NS	NS
iNDF	3.86	3.57	0.13	0.01	0.05	0.05	NS	0.07	NS
Starch	19.7	18.4	2.2	0.57	NS	NS	NS	NS	NS
Ruminal digestion rate, %/h									
pdNDF	16.1	13.9	1.5	0.37	NS	NS	NS	NS	NS
Starch	17.1	13.0	1.8	0.03	0.01	0.57	0.06	0.58	0.13

[1]Treatment least squares means.

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

[3]NS = not significant ($P > 0.20$); term removed from statistical model.

Table 30 (cont'd)

[4]pdNDF = potentially digestible NDF.
[5]iNDF = indigestible NDF.

Table 31. Rumen pools of cows fed treatment diets containing either early or late cut alfalfa silage as the sole source of forage

	Treatment LSM[1]			*P*[2]					
	Early	Late	SE	Trt	Trt x Period	pDMI	Trt x pDMI	pDMI x pDMI	Trt x pDMI x pDMI
Wet weight, kg	76.9	80.8	2.2	0.04	NS[3]	0.08	NS	NS	NS
Volume, L	91.3	97.8	3.0	0.03	NS	0.07	NS	NS	NS
Density, kg/L	0.84	0.83	0.01	0.29	NS	NS	NS	NS	NS
Rumen pool, kg									
DM	11.3	12.4	0.3	0.007	NS	0.07	0.13	NS	NS
OM	10.3	11.4	0.3	0.006	NS	0.08	0.14	NS	NS
NDF	6.23	6.96	0.17	<0.001	NS	0.06	0.10	NS	NS
pdNDF[4]	0.75	0.73	0.05	0.83	NS	0.007	NS	NS	NS
iNDF[5]	5.49	6.24	0.18	<0.001	NS	0.18	0.08	NS	NS
Starch	0.964	1.13	0.051	0.02	0.06	0.20	NS	NS	NS

[1]Treatment least squares means.

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

[3]NS = not significant ($P > 0.20$); term removed from statistical model.

[4]pdNDF = potentially digestible NDF.

[5]iNDF = indigestible NDF.

Table 32. Starch digestion of cows fed treatment diets containing either early or late cut alfalfa silage as the sole source of forage

	Treatment LSM[1]			*P*[2]					
	Early	Late	SE	Trt	Trt x Period	pDMI	Trt x pDMI	pDMI x pDMI	Trt x pDMI x pDMI
Starch									
Intake, kg/d	8.16	8.74	0.23	0.007	0.04	0.001	NS[3]	0.18	NS
Apparent ruminal digestion									
kg/d	3.87	3.74	0.32	0.67	0.11	NS	NS	NS	NS
%	48.2	44.6	3.8	0.29	NS	NS	NS	NS	NS
True ruminal digestion									
kg/d	4.01	3.90	0.32	0.73	0.12	NS	NS	NS	NS
%	49.9	46.5	3.8	0.31	NS	NS	NS	NS	NS
Passage to duodenum, kg/d	4.07	4.78	0.35	0.04	NS	0.13	NS	NS	NS
Apparent postruminal digestion									
kg/d	3.46	4.01	0.34	0.13	NS	0.20	NS	NS	NS
% of intake	44.2	46.5	3.8	0.53	NS	NS	NS	NS	NS
% of duodenal passage	84.5	82.9	1.3	0.36	NS	NS	NS	NS	NS
Apparent total tract digestion									
kg/d	7.33	7.74	0.15	0.02	0.03	0.002	NS	NS	NS
%	92.3	91.1	0.5	0.05	NS	0.11	NS	NS	NS

[1]Treatment least squares means.

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

[3]NS = not significant ($P > 0.20$); term removed from statistical model.

Table 33. Ruminal VFA concentrations and pH of cows fed treatment diets containing either early or late cut alfalfa silage as the sole source of forage

	Treatment LSM[1]			*P*[2]					
	Early	Late	SE	Trt	Trt x Period	pDMI	Trt x pDMI	pDMI x pDMI	Trt x pDMI x pDMI
Total VFA, mM	141	139	2.0	0.48	NS[3]	NS	NS	NS	NS
Acetate	86.3	81.9	1.0	0.005	NS	0.74	0.09	NS	NS
Propionate	29.5	31.0	0.6	0.04	NS	NS	NS	NS	NS
Butyrate	16.1	18.1	0.4	<0.001	0.06	NS	NS	NS	NS
Lactate	1.79	1.37	0.33	0.37	NS	0.31	NS	0.06	NS
Isobutyrate	1.61	1.54	0.02	0.02	NS	0.11	NS	NS	NS
Valerate	2.68	2.47	0.07	0.05	NS	0.13	0.95	0.60	0.02
Isovalerate	2.82	2.51	0.09	0.003	NS	0.07	0.04	0.02	0.002
Branch chain VFA	4.41	4.03	0.10	0.005	NS	0.02	0.09	0.01	0.01
Acetate:Propionate	2.94	2.65	0.05	<0.001	NS	NS	NS	NS	NS
Ruminal pH	6.36	6.29	0.02	0.04	NS	NS	NS	NS	NS

[1]Treatment least squares means.

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

[3]NS = not significant ($P > 0.20$); term removed from statistical model.

Table 34. DM and OM digestion of cows fed treatment diets containing either early or late cut alfalfa silage as the sole source of forage

	Treatment LSM[1]			*P*[2]					
	Early	Late	SE	Trt	Trt x Period	pDMI	Trt x pDMI	pDMI x pDMI	Trt x pDMI x pDMI
DM									
Intake, kg/d	28.6	26.8	0.7	0.003	0.02	<0.001	NS[3]	0.14	NS
Apparent total tract digestion									
kg/d	17.4	15.1	0.4	<0.001	0.005	0.003	NS	NS	NS
%	62.5	58.0	0.5	<0.001	0.05	0.60	0.12	NS	NS
OM									
Intake, kg/d	26.2	24.8	0.7	0.009	0.02	<0.001	NS	0.14	NS
Apparent ruminal digestion									
kg/d	9.76	7.93	0.41	<0.001	0.009	NS	NS	NS	NS
%	37.9	33.2	1.6	0.007	0.14	NS	NS	NS	NS
True ruminal digestion									
kg/d	13.4	11.1	0.5	<0.001	0.03	NS	NS	NS	NS
%	52.5	46.4	1.7	0.002	NS	NS	NS	NS	NS
Passage to duodenum, kg/d	16.5	17.0	0.7	0.40	NS	0.001	NS	0.10	NS
Apparent postruminal digestion									
kg/d	6.30	6.12	0.44	0.73	NS	0.05	NS	NS	NS
% of intake	24.8	25.1	1.7	0.90	NS	NS	NS	NS	NS
Apparent total tract digestion									
kg/d	16.1	14.1	0.3	<0.001	0.003	0.003	NS	NS	NS
%	62.8	58.3	0.5	<0.001	0.03	NS	NS	NS	NS

[1]Treatment least squares means.

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

Table 34 (cont'd)

[3]NS = not significant ($P > 0.20$); term removed from statistical model.

Table 35. Nitrogen metabolism of cows fed treatment diets containing either early or late cut alfalfa silage as the sole source of forage

	Treatment LSM[1]			*P*[2]					
	Early	Late	SE	Trt	Trt x Period	pDMI	Trt x pDMI	pDMI x pDMI	Trt x pDMI x pDMI
N intake, g/d	918	812	23	<0.001	0.07	<0.001	NS[3]	0.14	NS
Ruminal ammonia, mg/dl	22.5	20.4	0.6	<0.001	NS	0.58	0.14	NS	NS
Flow to duodenum									
Ammonia N, g/d	17.8	16.9	0.9	0.31	NS	0.004	NS	NS	NS
NAN									
g/d	727	715	27	0.49	NS	0.001	NS	0.07	NS
% of N intake	79.5	88.3	2.4	<0.001	NS	0.13	NS	0.17	NS
NANMN[4]									
g/d	292	321	19	0.14	0.16	0.03	0.17	NS	NS
% of N intake	32.5	41.1	2.3	0.008	NS	NS	NS	NS	NS
% of duodenal NAN	41.8	47.6	2.5	0.09	NS	0.31	0.17	NS	NS
Microbial N									
g/d	397	357	19	0.13	NS	0.11	NS	NS	NS
% of duodenal NAN	58.2	52.4	2.5	0.09	NS	0.31	0.17	NS	NS
g/kg TRDOM[5]	30.2	32.2	1.4	0.27	0.03	0.54	0.14	NS	NS
NAN apparent postruminal digestion									
g/d	405	394	22	0.53	0.15	0.01	NS	0.15	NS
% of N intake	42.6	46.8	1.8	0.06	NS	NS	NS	NS	NS
% of duodenal passage	55.1	54.2	1.1	0.56	0.10	NS	NS	NS	NS
N apparent total tract digestion									
g/d	586	480	13	<0.001	0.02	0.005	NS	NS	NS
%	64.7	60.0	0.7	<0.001	0.02	0.05	0.20	0.08	NS

[1]Treatment least squares means.

Table 35 (cont'd)

[2]*P*-values for treatment (Trt), treatment by period interaction (Trt x Period), preliminary DMI (pDMI), treatment by preliminary DMI interaction (Trt x pDMI), quadratic effect of preliminary DMI (pDMI x pDMI), and treatment by quadratic effect of preliminary DMI (Trt x pDMI x pDMI).
[3]NS = not significant ($P > 0.20$); term removed from statistical model.
[4]NANMN = nonammonia, nonmicrobial nitrogen.
[5]TRDOM = true ruminally digested OM.

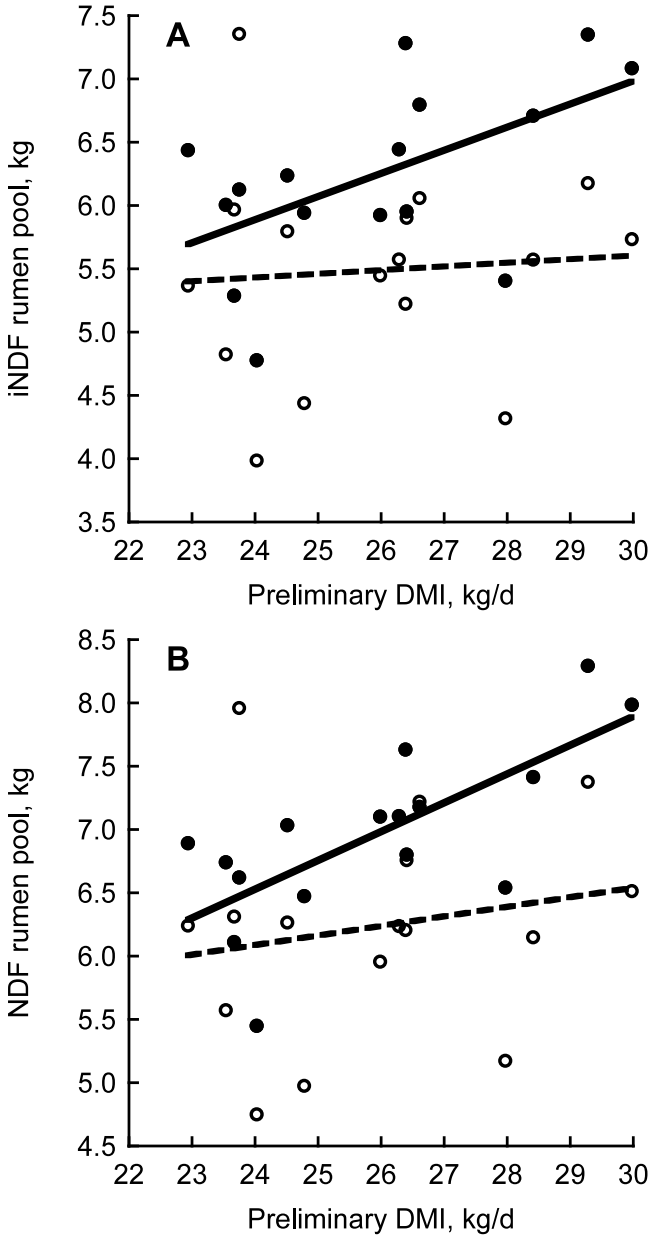

Figure 13. Interaction of early (open circles, dashed line) and late (closed circles, solid line) alfalfa maturity with preliminary DMI for A) indigestible NDF (iNDF) rumen pool ($P = 0.08$) and B) NDF rumen pool ($P = 0.10$). The preliminary DMI on the x-axis are the mean DMI of individual cows during the final 4 d of the preliminary period when all cows were fed a common diet.

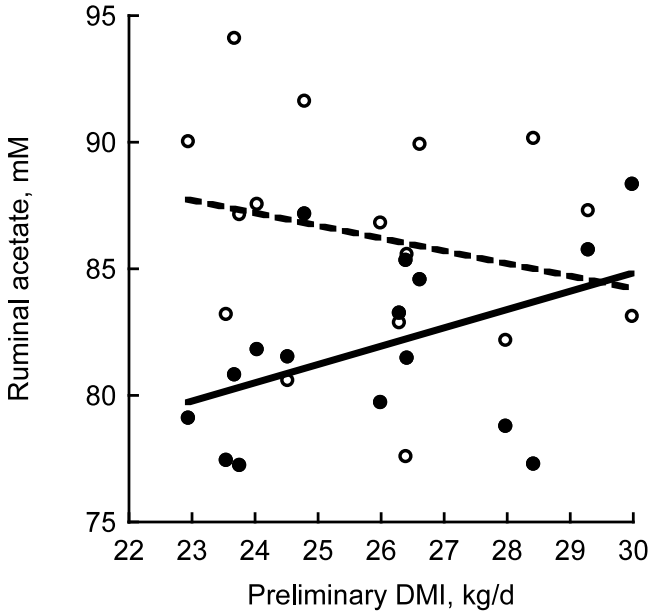

Figure 14. Interaction of early (open circles, dashed line) and late (closed circles, solid line) alfalfa maturity with preliminary DMI for ruminal acetate concentration ($P = 0.09$). The preliminary DMI on the x-axis are the mean DMI of individual cows during the final 4 d of the preliminary period when all cows were fed a common diet.

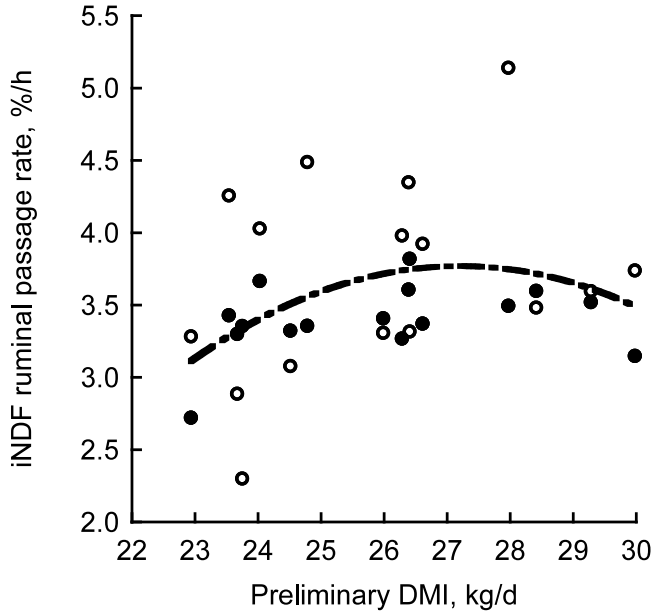

Figure 15. Relationship of early (open circles) and late (closed circles) alfalfa maturity with preliminary DMI for indigestible NDF (iNDF) ruminal passage rate ($P = 0.07$). The preliminary DMI on the x-axis are the mean DMI of individual cows during the final 4 d of the preliminary period when all cows were fed a common diet.

Figure 16

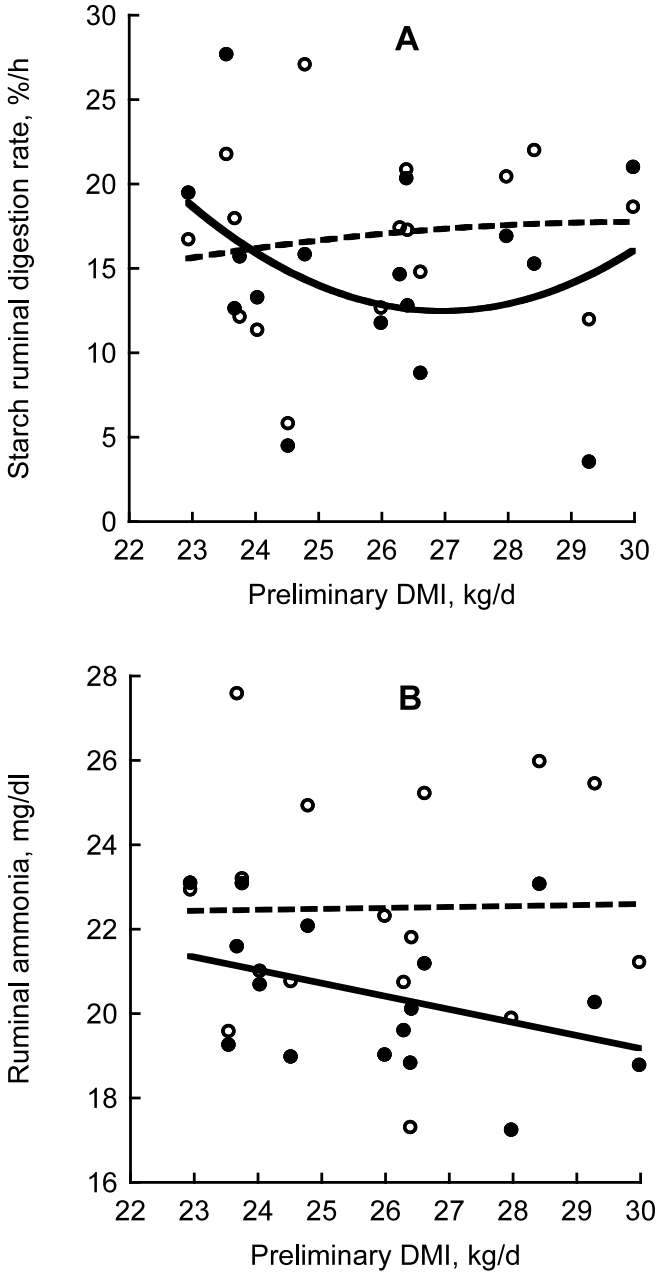
A
Starch ruminal digestion rate, %/h
Preliminary DMI, kg/d
B
Ruminal ammonia, mg/dl
Preliminary DMI, kg/d

Figure 16 (cont'd)

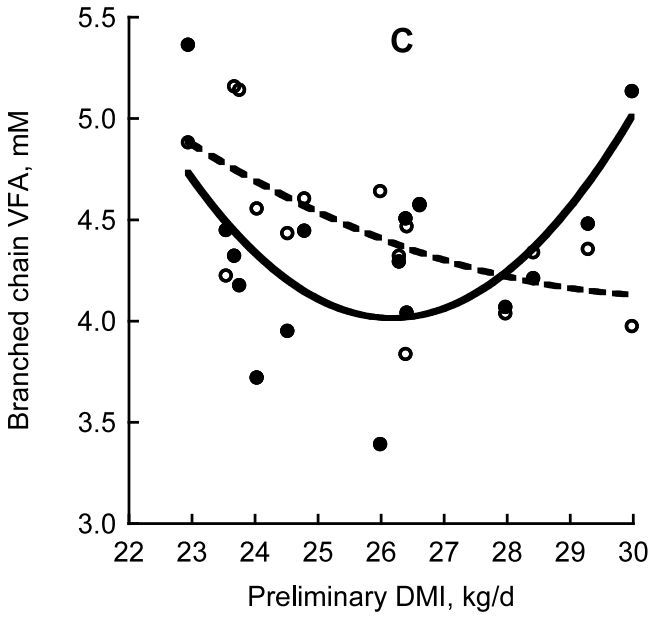

Figure 16. Interaction of early (open circles, dashed line) and late (closed circles, solid line) alfalfa maturity with preliminary DMI for A) starch ruminal digestion rate ($P = 0.13$), B) ruminal ammonia concentration ($P = 0.14$ linear), and C) branched chain VFA concentration ($P = 0.01$). The preliminary 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.

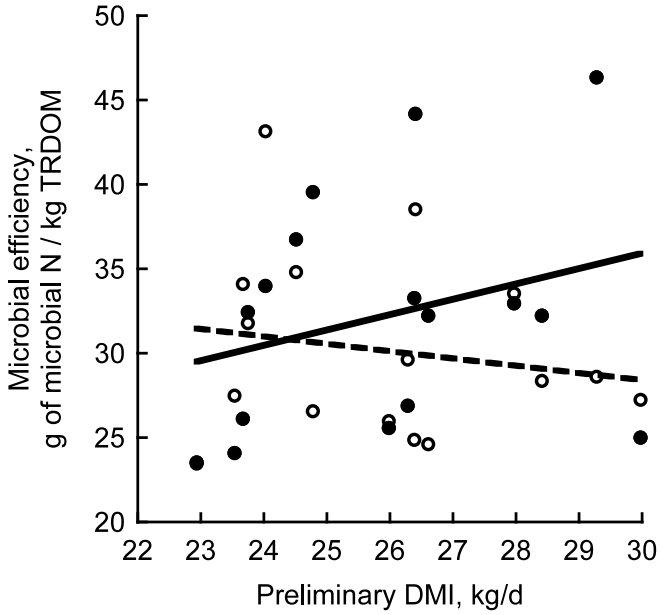

Figure 17. Interaction of early (open circles, dashed line) and late (closed circles, solid line) alfalfa maturity with preliminary DMI for microbial efficiency ($P = 0.14$) expressed as g of microbial N/kg of true ruminally digested OM (TRDOM). The preliminary DMI on the x-axis are the mean DMI of individual cows during the final 4 d of the preliminary period when all cows were fed a common diet.

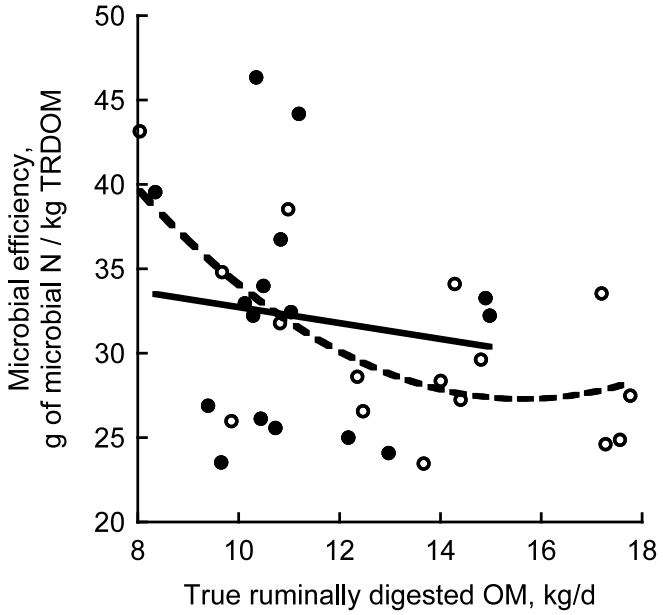

Figure 18. Relationship between true ruminally digested OM (TRDOM) and microbial efficiency for early (open circles, dashed line; microbial efficiency, g of microbial N/kg of TRDOM = [47.3 – (1.43 x TRDOM, kg/d) + (0.213 x (TRDOM, kg/d – 12.3)^2)]; $P = 0.03$, $R^2 = 0.42$) and late (closed circles, solid line; $P = 0.65$, $R^2 = 0.02$) alfalfa maturity.

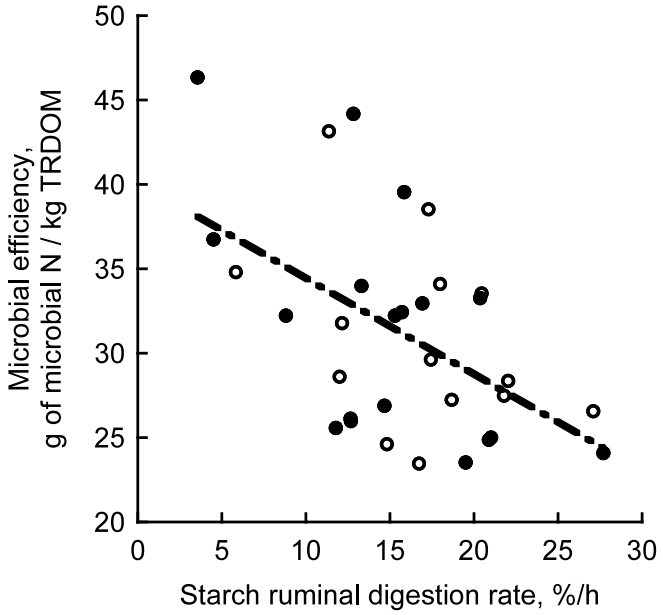

Figure 19. Relationship between starch ruminal digestion rate and microbial efficiency. Microbial efficiency, g of microbial N/kg of true ruminally digested OM (TRDOM) = 40.1 – 0.568 x starch ruminal digestion rate, %/h ($P = 0.002$, $R^2 = 0.27$). Open circles denote early and closed circles denote late alfalfa maturity.

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CHAPTER 5

Nutrient Demand Interacts with Grass Maturity to Affect Milk Fat Concentration and Digestion Responses in Dairy Cows[1]

ABSTRACT

Effects of grass maturity on dry matter intake (DMI), milk production, ruminal fermentation and pool sizes, digestion and passage kinetics, and chewing activity 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 18-d treatment periods. During the preliminary period, pDMI of individual cows ranged from 23.5 to 28.2 kg/d (mean = 26.1 kg/d) and 3.5% fat-corrected milk (FCM) yield ranged from 30.8 to 57.2 kg/d (mean = 43.7 kg/d). Experimental treatments were diets containing orchardgrass silage harvested either a) early cut, less mature (EARLY) or b) late cut, more mature (LATE) as the sole forage. Early and late cut orchardgrass contained 44.9 and 54.4% neutral detergent fiber (NDF) and 19.9 and 14.8% crude protein, respectively. Forage:concentrate ratio was 58:42 and 46:54 for EARLY and LATE, respectively; both diets contained ~25% forage NDF and 30% total 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 grass maturity and their interaction with pDMI were tested by ANOVA. EARLY decreased milk yield and increased milk fat concentration compared with LATE, which was likely due to lower inclusion of concentrate for EARLY than LATE. Grass maturity and its interaction with pDMI did not affect FCM yield,

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DMI, rumen pH, or microbial efficiency. EARLY increased rates of ruminal digestion of potentially digestible NDF and passage of indigestible NDF (iNDF) compared with LATE. The lower concentration and faster passage rate of iNDF for EARLY resulted in lower rumen pools of iNDF, total NDF, organic matter, and dry matter for EARLY than LATE. Ruminal passage rates of potentially digestible NDF and starch were related to level of intake (quadratic interactions) and subsequently affected ruminal digestion of these nutrients. EARLY decreased eating, ruminating, and total chewing time per unit of forage NDF intake compared with LATE. When grass silage was the only source of forage in the diet, cows supplemented with additional concentrate to account for decreasing protein and increasing fiber concentrations associated with more mature grass produced similar FCM yields as cows fed less mature grass.

INTRODUCTION

Forage maturity at harvest affects the supply and utilization of nutrients in dairy cows fed forage-based diets. Maturity affects the yield and quality of forages, and time of harvest is a compromise between these factors. As plants grow and mature, total yield of DM increases but nutritive quality generally decreases due to increasing fiber, and lignification of fiber, and decreasing protein concentrations. Therefore, producing high quality forage is largely dependent on harvesting at the optimum maturity. While dairy cows can be fed supplemental concentrate to improve milk production when cows are fed low quality forage, feeding quality forages can optimize milk production, improve cow health, reduce purchased feed costs, and increase dairy profitability.

Increasing maturity in perennial forages reduces digestibility and intake potential of

forages due to increased concentration of NDF and greater lignification of the NDF. This not only decreases the potentially digestible NDF (pdNDF) concentration but also decreases digestion rate of the remaining pdNDF (Smith et al., 1972), which might allow particulate matter to remain buoyant longer, decreasing specific gravity and rate of passage from the rumen (Jung and Allen, 1995). This suggests that more mature forages will result in slower passage rates from the rumen and greater ruminal distention. However, increased maturity at harvest increased rate of particle size reduction by chewing for grass stems and leaves (Poppi et al., 1981) and for ryegrass (Ulyatt, 1983) because of greater fragility. This more rapid particle size reduction could increase passage rate for more mature forages, acting in opposition to the expected effects of buoyancy. Orchardgrass (OG; *Dactylis glomerata L.*) was selected as a representative cool-season grass for use in this experiment.

In addition to the combination of dietary factors affecting ruminal distention and rate of particle breakdown, the individual cow's appetite will also affect the responses of passage rate and intake to forage maturity. Cows respond differently to treatments depending on their level of intake (Voelker Linton and Allen, 2008) and production (Oba and Allen, 1999). Because grass maturity and level of intake affect ruminal passage and digestion rates and, thus, digesta fill in the rumen, the response to effects of grass maturity 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 digesta passage rates to grass maturity are related to level of intake and less mature grass will permit a greater increase in passage rate than more mature grass as feed intake increases.

The objectives of this experiment were to evaluate the relationships between voluntary DMI and effects of grass maturity on DMI, milk production, ruminal fermentation and pool

sizes, digestion and passage kinetics, and chewing behavior in lactating dairy cows. This study had three unique features to improve our understanding of the role of grass maturity and interpret its effect on animal responses. First, it allowed effects of the interaction between grass maturity 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 early and late cut OG as the sole source of forage. 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 18-d experimental periods. The first 10 d of each period were allowed for diet adaptation and samples were collected during the final 4 d of the preliminary period and 8 d of each experimental period. Cows were 164±85 (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 23.5 to 28.2 kg/d (mean = 26.1 kg/d) and 3.5% FCM yield ranged from 30.8 to 57.2 kg/d (mean = 43.7 kg/d; Table 36). 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 OG silage from one field harvested either a) early cut, less mature (EARLY) or b) late cut, more mature (LATE) as the sole forage. Orchardgrass (Baridana cultivar, Barenbrug USA, Tangent, OR) was produced at the campus farm at Michigan State University (East Lansing), chopped to 10 mm theoretical length of cut, and ensiled in Ag-Bags (Ag-Bag Systems Inc., St. Nazianz, WI). During the sample collection periods, early and late cut OG contained 44.9 and 54.4% NDF and 19.9 and 14.8% CP, respectively (DM basis; Table 37). Diets EARLY and LATE were formulated to contain 25% forage NDF, 30% total NDF, and 18% CP. We acknowledge these treatments affect dietary starch concentration but maintaining similar forage and total NDF concentrations for both treatments was of primary interest. The diet fed during the preliminary period was formulated so that early and late cut OG each contributed 50% of forage NDF. Diets also contained dry ground corn, soybean meal (48% CP), SoyPlus® (West Central Soy, Ralston, IA), vitamin-mineral premix, and limestone (Table 38).

Data and Sample Collection

Throughout the experiment, cows were housed in tie-stalls and fed diets as total mixed rations once daily (1130 h) at 110% of expected intake. The amount of feed offered and refused (orts) was 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 11 to 15 during each experimental period. Samples were frozen immediately after

collection at −20°C and combined to one composite sample per period prior to analysis.

Cows were moved to an exercise lot twice daily (0230 and 1300 h) prior to 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 11 to 15 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 18 of each experimental period. Body condition score was determined on the same days by 3 trained investigators blinded to treatments (Wildman et al., 1982; 5-point scale where 1 = thin and 5 = fat). Chewing activity was monitored and recorded by observation every 5 min for 24 h on d 16 of each experimental period. Activity was noted as eating, ruminating, drinking, or idle for each cow at each time.

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 11 to 15 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 was collected from the reticulum, near the reticular-omasal orifice, transported to the laboratory, and processed. Rumen fluid for pH, VFA, lactate, and ammonia were obtained by combining digesta from five 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.

Ruminal contents were evacuated manually through the ruminal cannula 4 h after feeding at the beginning of d 17 (1530 h) and 2 h before feeding at the end of d 18 (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 yield recorded at both milkings were summed for a daily total, which were averaged for each period. Milk samples were analyzed for fat, true protein, lactose, SNF, and MUN 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. Milk samples used for analysis of fatty acid profile were composited based on milk fat yield and centrifuged at 17,800 × *g* for 30 min at 8°C. Fat cake (300 to 400 mg) was extracted according to Hara and Radin (1978), and methyl esters were formed according to Christie (1982) as modified by Chouinard et al. (1999). Fatty acids were quantified by gas chromatography (model 8500; Perkins-Elmer Corp, Norwalk, CT) according to Kramer et al. (1997) using a SP-2560 capillary column (100 m × 0.20 mm id with 0.02-μm film thickness; Supelco, Bellefonte, PA). Oven temperature was 70°C for 4 min, then increased 13°C/min to 175°C and held for 27 min before being increased again at 4°C/min to 215°C and held for 31 min. Helium flow was 20 cm/s, and the total run time was 80 min.

Forage samples were combined to one 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 (Tri-Philizer 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 one 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 lignin (ADL), CP, and starch. Ash concentration was determined after 5 h 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). Indigestible NDF was estimated as NDF residue after 240 h in vitro fermentation (Goering and Van Soest, 1970); flasks were reinoculated at 120 h to insure a viable microbial population. Ruminal fluid for the in vitro incubations was collected from a nonpregnant dry cow fed dry hay only. Fraction of 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 was blended, strained through nylon mesh, and the liquid portion was centrifuged at 500 x *g* for 15 min. The supernatant was centrifuged at 18,000 x *g* for 15 min, and the pellet was washed with 0.9% NaCl solution and centrifuged again at 18,000 x *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 concentration of major VFA and lactate by HPLC (Waters Corp., Milford, MA) according to Oba and Allen (2003a).

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 ratio of purines to OM (Oba and Allen, 2003a), 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 the following equations:

Turnover rate (%/h) = 100 x (Intake of component / Ruminal pool of component) / 24

Passage rate (%/h) = 100 x (Duodenal flow of component / Ruminal pool of component) / 24

Digestion rate (%/h) = Turnover rate in the rumen (%/h) – Passage rate from the rumen (%/h).

Manually observed chewing activity was summarized by a logic script in Igor Pro (Version 6.12, WaveMetrics Inc., Lake Oswego, OR) to generate meal and rumination bout information according to previously established criteria (Dado and Allen, 1994). Variables determined included frequency of meal bouts per day, interval between meals, frequency of ruminating bouts per day, interval between ruminating bouts, eating time per day, ruminating time per day, and total chewing time per day.

Statistical Analysis

All data were analyzed by using the fit model procedure of JMP (Version 8, SAS Institute, 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: $Y_{ijk} = \mu + C_i + P_j + T_k + PT_{jk} + pDMI + T_kpDMI + pDMI^2 + T_kpDMI^2 + e_{ijk}$ where 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_kpDMI^2 is the interaction of treatment and pDMI (quadratic), and e_{ijk} is the residual error. Statistical

significance for T_kpDMI and T_kpDMI^2 indicated 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. Tendencies 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, one cow was removed from the study due to circumstances unrelated to treatments. Additionally, data from two cows were excluded prior to statistical analysis; one cow had a broken duodenal cannula during the last collection of the second experimental period that was replaced but affected her DMI and subsequently rumen evacuation measurements, and the other cow was considered an outlier based on large Cook's distance values (Cook and Weisberg, 1982) for several response variables of primary interest. Thus, data from 13 cows were statistically analyzed.

RESULTS AND DISCUSSION

Comparison of Forages and Diets

Chemical analyses of OG silages are listed in Table 37. As expected, delaying harvest of OG decreased the concentration of CP (19.9 vs. 14.8%) and increased concentrations of total NDF (44.9 vs. 54.4%) and iNDF (10.8 vs. 14.5%). Indigestible NDF, expressed as a percent of NDF, was slightly lower (24.1 vs. 26.6% of NDF) for early cut OG than for late cut OG. Early cut OG had greater DM concentration, higher pH, and contained less lactic and acetic acids than late cut OG silage. Physical characteristics (Table 37) of early and late cut OG silages were similar for mean particle size and particle size distribution.

Diet ingredients and chemical composition are shown in Table 38. The preliminary diet

contained more early cut OG than late cut OG so each forage supplied similar concentrations of forage NDF. Because treatment diets were formulated to contain similar forage NDF concentrations, forage:concentrate ratios were different between diets with ratios of 58:42 and 46:54 for EARLY and LATE, respectively. Besides forage source, the main differences in diets were the concentrations of corn grain and soybean meal, which were both lower for EARLY than LATE, to account for differences between the grass silages. Both diets had a similar chemical composition, which was mathematically calculated according to the proportion of each feed ingredient in the diet and its respective analytical values, except for starch which was lower for EARLY due to more forage and less concentrate in the diet for EARLY compared with LATE. In both diets, forage NDF provided over 82% of the total diet NDF.

Effects of Grass Maturity and pDMI

Results of grass maturity and its interaction with pDMI on milk yield and composition are shown in Table 39. EARLY decreased milk yield (36.6 vs. 39.2 kg/d, $P < 0.001$) and subsequently yields of protein (1.14 vs. 1.22 kg/d, $P = 0.008$), lactose (1.71 vs. 1.84 kg/d, $P = 0.001$), and SNF (2.06 vs. 2.22 kg/d, $P < 0.001$) compared with LATE, as concentrations of protein, lactose, and SNF were similar. EARLY decreased MUN concentration (7.70 vs. 10.5 mg/dl, $P < 0.001$) and increased milk fat concentration (3.70 vs. 3.38%, $P < 0.001$) compared with LATE. The lower milk yield, higher milk fat concentration for EARLY and higher milk yield, lower milk fat concentration for LATE resulted in similar FCM and milk fat yields. Response of milk fat concentration to treatment was related to pDMI (interaction $P = 0.07$, Figure 20) such that EARLY increased milk fat concentration and LATE decreased it as pDMI increased.

The aforementioned results are related to different concentrate levels in the diets, which

were necessary to account for changes in chemical composition of OG with increasing maturity and maintain the same concentration of forage NDF in the diets. EARLY decreased starch intake (5.48 vs. 5.98 kg/d, $P < 0.001$, Table 40) and ruminal propionate concentration (37.6 vs. 41.5 mM, $P = 0.01$, Table 41) compared with LATE. Because there was no difference between treatments for acetate concentration, EARLY increased acetate to propionate ratio (2.50 vs. 2.30, $P = 0.04$, Table 41) compared with LATE.

Although N intake was similar for EARLY and LATE ($P = 0.89$, Table 42), differences in N sources might have affected N digestion as greater quantities of N were contributed from grass silage for EARLY and soybean meal for LATE (Table 38). Lower MUN concentration for EARLY is consistent with lower ruminal ammonia concentration (11.0 vs. 12.9 mg/dl, $P = 0.001$) and flow of ammonia N to the duodenum (15.0 vs. 17.5 g/d, $P = 0.01$) for EARLY than LATE (Table 42). The increased ammonia for LATE was not because of ammonia concentration in silage, which was higher for early cut OG than late cut OG (Table 37), nor due to excessive degradation of amino acids in the rumen, which would result in the production of ammonia and branched chain VFA, because isovalerate and branched chain VFA were higher for EARLY than LATE (Table 41). The source of increased ruminal ammonia for LATE is not known but might be from greater degradation of protein in soybean meal compared with protein in early cut OG. EARLY decreased total tract N digestion (376 vs. 438 g/d, $P = 0.003$) and digestibility (56.1 vs. 65.1%, $P < 0.001$) compared with LATE (Table 42), which is likely associated with the differences in N sources in diets.

Differences in starch intake (Table 40) and milk fatty acid profile (Table 43) suggest reduction in milk fat concentration exhibited by cows consuming LATE might be related to diet-induced milk fat depression (MFD). Three conjugated linoleic acid (CLA) isomers, *trans*-10,

cis-12 CLA; *trans*-9, *cis*-11 CLA; *cis*-10, *trans*-12 CLA, produced as intermediates in rumen biohydrogenation have been shown to reduce milk fat to date (Bauman et al., 2008). There was no difference in concentrations of *trans*-10, *cis*-12 CLA between treatments ($P = 0.76$), and the latter two isomers were not detected by our method of analysis. Besides CLA isomers, the reduction in milk fat during diet-induced MFD is highly correlated with increases in the milk fat concentration of many *trans*-$C_{18:1}$ isomers (Kadegowda et al., 2008). EARLY decreased concentrations of rumen biohydrogenation intermediates including several milk *trans* $C_{18:1}$ fatty acid isomers and specifically *trans*-10 $C_{18:1}$ (0.24 vs. 0.32%, $P = 0.009$) compared with LATE. Although a relationship between the *trans*-10 $C_{18:1}$ increase in milk fat and the reduction in milk fat concentration has been demonstrated (Loor et al., 2005), abomasal infusions of pure *trans*-10 $C_{18:1}$ in dairy cows showed this isomer did not reduce milk fat synthesis and was not the direct cause of diet-induced MFD (Lock et al., 2007). While *trans*-10 $C_{18:1}$ is not causative, it might be an indicator of other isomers that could play a role in the reduction of milk fat but have not yet been identified.

EARLY increased the concentration of C_4 (3.26 vs. 2.96 g/100 g total fatty acids, $P = 0.002$) in the milk compared with LATE, which is consistent with the higher concentration of ruminal butyrate (22.5 vs. 20.6 mM, $P = 0.02$, Table 41) for EARLY than LATE. Despite the greater concentration of C_4 fatty acid in the milk, EARLY tended to decrease the proportion of de novo synthesized ($< C_{16}$) milk fatty acids (24.3 vs. 25.2 g/100 g total fatty acids, $P = 0.09$) compared with LATE. The proportion of milk fatty acids from preformed ($> C_{16}$) fatty acids was not different ($P = 0.80$) between EARLY and LATE. Therefore, de novo synthesized (short and medium chain) and preformed (long chain) fatty acids were similarly responsible for decreased milk fat observed for LATE.

EARLY increased pdNDF intake (4.88 vs. 4.54 kg/d, $P = 0.002$) and decreased iNDF intake (1.83 vs. 2.00 kg/d, $P = 0.003$) compared with LATE (Table 44) because of differences in chemical composition of forages due to maturity. However, grass maturity and its interaction with pDMI did not affect total NDF intake ($P \geq 0.17$, Table 44) or DMI ($P \geq 0.70$, Table 45). These results are contrary to our expectation that LATE would be more filling than EARLY causing greater rumen distention and potentially limiting DMI, particularly in cows with high DMI for which ruminal distention is more likely to limit feed intake (Allen, 1996). Oba and Allen (1999) and Voelker et al. (2002) found DMI responses 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. Additionally, as DMI became more limited by fill, we expected passage rate of iNDF to increase more for EARLY than for LATE. Although EARLY increased ruminal passage rate of iNDF compared with LATE (2.76 vs. 2.10%/h, $P < 0.001$, Table 46), effect of treatment on passage rate of iNDF was not related to pDMI ($P \geq 0.32$).

The lower concentration and faster rate of passage of iNDF for EARLY are reflected in the lower rumen pool sizes (Table 47) of iNDF (2.76 vs. 4.17 kg, $P < 0.001$) compared with LATE, which also resulted in lower rumen pools of NDF (6.03 vs. 7.30 kg, $P < 0.001$), OM (10.4 vs. 11.8 kg, $P < 0.001$), and DM (11.4 vs. 12.8 kg, $P < 0.001$) for EARLY than LATE. Furthermore, rumen content wet weight tended to be lower for EARLY than LATE ($P = 0.06$, Table 47) and volume increased at a faster rate for EARLY than LATE as pDMI increased (interaction $P = 0.10$, Figure 21) such that cows with high pDMI had similar volumes for EARLY and LATE.

EARLY increased ruminal digestion rate of pdNDF (4.95 vs. 3.98%/h, $P = 0.007$) and

decreased ruminal passage rate of pdNDF (0.66 vs. 1.32%/h, $P = 0.04$) compared with LATE (Table 46). The difference in ruminal passage rate of pdNDF between EARLY and LATE tended to be greatest for cows in the middle of the pDMI range (interaction $P = 0.11$, Figure 22A), and the reason for this is not known. These rates affected fiber digestion such that EARLY had greater pdNDF ruminal digestion (4.27 vs. 3.34 kg/d, $P = 0.01$) and digestibility (88.4 vs. 74.5%, $P = 0.02$) and lower pdNDF flux to the duodenum (0.55 vs. 1.13 kg/d, $P = 0.03$) than LATE (Table 44). The patterns for treatment by pDMI interactions for pdNDF ruminal digestibility (interaction $P = 0.10$, Figure 22B) and pdNDF flux (interaction $P = 0.08$, Figure 22C) correspond to pdNDF ruminal passage rate; such that the faster rate of pdNDF passage yielded lower pdNDF digestibility in the rumen and higher pdNDF flux from the rumen and vice versa. Despite differences in ruminal pdNDF digestion, there was no difference in total tract digestibility of pdNDF between treatments. Similar to pdNDF, EARLY had greater true ruminal digestibility of OM (67.4 vs. 60.2%, $P = 0.01$) than LATE, and the difference was greater for cows in the middle of the pDMI range (interaction $P = 0.05$), but EARLY tended to decrease total tract OM digestibility (62.3 vs. 66.2%, $P = 0.06$) compared with LATE (Table 45). EARLY decreased DM total tract digestibility (61.2 vs. 65.5%, $P = 0.04$) compared with LATE (Table 45), which is likely due to the lower digestibility of forage compared with concentrate and lower inclusion of supplemental concentrate for EARLY than LATE.

As expected, ruminal turnover rate of NDF (4.43 vs. 3.54%/h, $P < 0.001$) was greater for EARLY than LATE due to faster rates of passage of iNDF and digestion of pdNDF and despite slower passage of pdNDF (Table 46). Although rate of particle size reduction was not directly measured in this experiment, the faster passage rate of pdNDF observed for LATE could be the result of greater fragility and breakdown of the more mature grass. Additionally, greater

chewing time for LATE may be responsible for greater breakdown of particles resulting in faster passage rate of pdNDF because EARLY decreased eating time (42.1 vs. 50.9 min/kg forage NDF, $P = 0.03$), ruminating time (88.6 vs. 101 min/kg forage NDF, $P = 0.003$), and thus total chewing time (132 vs. 150 min/kg forage NDF, $P < 0.001$) per unit forage NDF intake compared with LATE (Table 48). There was no difference in eating time per day ($P = 0.31$) for EARLY and LATE, but EARLY decreased ruminating time per day (523 vs. 562 min/d, $P = 0.04$) compared with LATE (Table 48). The lower ruminating time observed for EARLY may be because of the smaller rumen pool size (Table 47) for EARLY compared with LATE.

Grass maturity and its interaction with pDMI did not affect ruminal pH (Table 41) despite lower starch intake for EARLY compared with LATE (Table 40). This is likely because EARLY tended to increase OM truly digested in the rumen (13.6 vs. 12.1 kg/d, $P = 0.07$, Table 45) compared with LATE and because increased salivary buffer secretion through greater chewing time for LATE (Table 48) may have offset the expected reduction in pH because of greater starch intake for LATE (Table 40).

The lack of interactions between DMI, rumen pools, chewing activity and level of intake indicate that rumen fill and chewing time were not constraints limiting DMI for cows with high intake, especially cows consuming EARLY. Based on the results obtained from the data and samples collected during this experiment, the limiting constraint for DMI is not clear. Metabolites or hormones (not measured) or both might have provided additional clues to determine what limited intake.

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 impact of passage on ruminal digestion and microbial growth,

quantitative knowledge of rates of nutrient passage from the rumen is 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).

Rate of ruminal passage for starch was related to level of intake. As pDMI increased, EARLY slightly decreased ruminal passage rate of starch and LATE increased it (interaction $P = 0.03$, Figure 23A). This is consistent with expected effects of passage rate on true rumen digestibility of starch; EARLY slightly increased true ruminal starch digestibility and LATE decreased it as pDMI increased (interaction $P = 0.13$, Figure 23B). The reverse was observed for postruminal starch digestibility (interaction $P = 0.10$, Figure 23C).

Fiber passage rates were also related to level of intake. Rate of ruminal passage for pdNDF tended to be related to pDMI (interaction $P = 0.11$) and influenced pdNDF ruminal digestion as previously discussed (Figure 22). Rate of ruminal passage of iNDF tended to be quadratically related to pDMI ($P = 0.08$, Figure 24) independent of treatment response. Although we expected iNDF passage rate to increase as pDMI increased, we observed cows with low pDMI had faster iNDF passage rates than cows in the middle of the pDMI range. The reason for this is unclear.

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 and the response differed by treatment; EARLY increased NANMN flux and LATE decreased it as pDMI increased (interaction $P = 0.04$, Figure 25). This interaction contributed to the tendency

for a treatment by pDMI interaction for NAN flux (interaction $P = 0.12$, Table 42) as level of intake did not affect MN flux. This is in contrast to the results in a review by Clark et al. (1992) that reported positive linear relationships between OM intake and fluxes of NAN, NANMN, and MN as OM intake increased over a very wide range (3 to 23 kg/d) compared with this study. Based on studies with continuous culture fermenters, increases in solid and liquid dilution rates, which can be associated with increased intake, resulted in greater microbial efficiency (Crawford et al., 1980; Shriver et al., 1986). In this experiment, microbial efficiency was not related to pDMI ($P \geq 0.29$, Table 42) despite the linear increase in true ruminally digested OM with increasing pDMI ($P = 0.009$, Table 45). There was no relationship between microbial efficiency and true ruminally digested OM for cows consuming EARLY ($P = 0.68$, $R^2 = 0.02$); however, microbial efficiency of cows consuming LATE decreased dramatically as true ruminally digested OM increased ($P = 0.03$, $R^2 = 0.37$, Figure 26), indicating that factors other than availability of energy limited efficiency of MN production and energy from OM fermentation was uncoupled from microbial growth (Russell and Cook, 1995). Similarly, Oba and Allen (2003b) reported that efficiency of MN production was inversely related to true ruminally digested OM (kg/d) in an experiment comparing starch concentration and fermentability of diets in dairy cattle.

CONCLUSIONS

Delaying the harvest of grass resulted in lower CP and higher NDF and iNDF concentrations. EARLY decreased milk yield and increased milk fat concentration compared with LATE, which was likely because of a lower inclusion of concentrate for EARLY than LATE. Grass maturity and its interaction with pDMI did not affect FCM yield, DMI, rumen pH, or microbial efficiency. EARLY increased rates of ruminal digestion of pdNDF and passage of

iNDF compared with LATE. The faster passage rate of iNDF for EARLY resulted in lower rumen pools of iNDF, total NDF, OM, and DM for EARLY than LATE. Ruminal passage rates of pdNDF and starch were related to level of intake (quadratic interactions) and subsequently affected ruminal digestion. EARLY decreased eating, ruminating, and total chewing time per unit of forage NDF intake compared with LATE. When grass silage was the only source of forage in the diet, cows supplemented with additional concentrate to account for decreasing protein and increasing fiber concentrations associated with more mature grass produced similar FCM yields as cows fed less mature grass.

APPENDIX

Table 36. 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	Standard deviation	Minimum	Maximum
Parity	3	2.77	1.07	2	6
BW[1], kg	598	588	55	498	665
BCS	2.42	2.48	0.68	1.50	4.00
DIM	126	164	85	73	329
Milk, kg/d	43.2	43.2	7.7	29.1	55.7
3.5% FCM, kg/d	44.9	43.7	7.7	30.8	57.2
DMI, kg/d	26.2	26.1	1.4	23.5	28.2

[1]Empty BW (ruminal digesta removed).

Table 37. Chemical composition, particle size distribution, and fermentation parameters of the early or late cut orchardgrass silage included in the treatment diets

	Orchardgrass silage	
Item	Early	Late
Chemical composition		
DM, %	37.3	31.9
OM, % DM	90.7	92.2
NDF, % DM	44.9	54.4
iNDF[1], % DM	10.8	14.5
iNDF, % of NDF	24.1	26.6
ADF, % of DM	28.3	33.5
ADL[2], % of DM	4.08	4.67
CP, % DM	19.9	14.8
Starch, % DM	2.92	2.10
Particles size distribution[3]		
Wet sieving, % DM retained		
19.0 mm	18.1	20.6
9.50 mm	22.9	24.3
4.75 mm	32.9	24.6
2.36 mm	18.7	21.5
1.18 mm	3.98	5.17
0.600 mm	1.59	2.00
0.300 mm	0.90	0.91
0.150 mm	0.52	0.50
0.075 mm	0.26	0.25
0.038 mm	0.26	0.17
Mean particle size[4], mm	11.4	12.0
Penn State Particle Separator, % DM retained		
> 19.0 mm	37.8	39.7
19.0 to 8.0 mm	38.5	37.6
< 8.0 mm	23.7	22.7
Fermentation		
pH	4.90	4.57
Acetic acid, % DM	1.37	2.10
Propionic acid, % DM	0.38	0.24
Butyric acid, % DM	<0.01	<0.01
Lactic acid, % DM	5.93	6.30
Lactic:Acetic	4.33	3.00
Ethanol, % DM	0.20	0.11
Ammonia, mM	4.31	3.12

[1]iNDF = indigestible NDF.

[2]ADL = acid detergent lignin.

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

Table 37 (cont'd)

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

Table 38. Ingredients and chemical composition of preliminary and treatment diets (as analyzed) containing either early or late cut orchardgrass silage as the sole source of forage

	Preliminary	Early	Late
Ingredients, % DM			
Orchardgrass silage, early cut	27.5	58.2	---
Orchardgrass silage, late cut	23.1	---	45.9
Dry ground corn	33.3	31.1	35.2
Soybean meal (48% CP)	7.49	2.16	10.3
SoyPlus®	3.39	3.40	3.39
Vitamin mineral mix[1]	3.99	4.00	3.99
Limestone	1.20	1.20	1.20
Chemical composition			
DM, %	48.7	49.5	49.2
OM, % DM	91.5	90.8	91.9
NDF, % DM	29.6	30.4	30.3
% forage NDF	25.3	26.1	25.0
% NDF from forage	85.5	85.9	82.4
iNDF[2], % DM	NA[3]	8.18	9.08
iNDF, % of NDF	NA	26.9	30.0
CP, % DM	21.8	18.7	18.7
Starch, % DM	26.9	24.0	26.5

[1]Vitamin mineral mix contained (DM basis) 16.5% sodium bicarbonate, 14.2% magnesium sulfate, 7.1% salt, 5.8% dicalcium phosphate, 2.4% trace mineral premix, 0.4% vitamin A, 0.4% vitamin D, 0.2% vitamin E, and 53.1% dry ground corn as a carrier.

[2]iNDF = indigestible NDF.

[3]NA = no analysis for preliminary diet.

Table 39. Milk production and composition, feed intake, and BW change of cows fed treatment diets containing either early or late cut orchardgrass silage as the sole source of forage

	Treatment LSM[1]			*P*[2]					
	Early	Late	SE	Trt	Trt x Period	pDMI	Trt x pDMI	pDMI x pDMI	Trt x pDMI x pDMI
Yield, kg/d									
Milk	36.6	39.2	1.8	<0.001	0.08	0.19	NS[3]	NS	NS
FCM (3.5 %)	37.6	38.1	1.6	0.49	0.06	0.17	NS	NS	NS
Milk fat	1.35	1.31	0.06	0.34	0.07	0.18	0.18	NS	NS
Milk protein	1.14	1.22	0.04	0.008	0.07	0.19	NS	NS	NS
Milk lactose	1.71	1.84	0.08	0.001	0.05	NS	NS	NS	NS
SNF	2.06	2.22	0.10	<0.001	0.05	NS	NS	NS	NS
Milk composition, %									
Fat	3.70	3.38	0.09	<0.001	NS	0.96	0.07	NS	NS
Protein	3.15	3.14	0.07	0.82	NS	NS	NS	NS	NS
Lactose	4.68	4.68	0.07	0.99	NS	NS	NS	NS	NS
SNF	5.65	5.67	0.09	0.65	NS	NS	NS	NS	NS
MUN, mg/dl	7.70	10.5	0.38	<0.001	NS	0.81	0.45	0.10	0.17
DMI, kg/d	22.5	22.4	0.4	0.70	0.12	0.002	NS	NS	NS
3.5% FCM/ DMI	1.49	1.58	0.06	0.005	0.16	0.83	0.14	NS	NS
BW change, kg/18 d	5.06	9.81	3.11	0.36	0.03	NS	NS	NS	NS
BCS change/18 d	-0.05	0.05	0.05	0.20	0.02	NS	NS	NS	NS

[1]Treatment least squares means.

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

[3]NS = not significant ($P > 0.20$); term removed from statistical model.

Table 40. Starch digestion of cows fed treatment diets containing either early or late cut orchardgrass silage as the sole source of forage

	Treatment LSM[1]			*P*[2]					
	Early	Late	SE	Trt	Trt x Period	pDMI	Trt x pDMI	pDMI x pDMI	Trt x pDMI x pDMI
Starch									
Intake, kg/d	5.48	5.98	0.14	<0.001	NS[3]	0.02	NS	NS	NS
Apparent ruminal digestion									
kg/d	3.85	4.09	0.16	0.21	0.002	0.51	NS	0.01	NS
%	71.5	69.8	2.8	0.56	0.002	0.15	0.16	0.01	NS
True ruminal digestion									
kg/d	4.05	4.30	0.16	0.21	0.004	0.50	NS	0.01	NS
%	75.2	73.3	2.8	0.52	0.002	0.15	0.13	0.007	NS
Passage to duodenum, kg/d	1.52	1.76	0.22	0.20	0.007	0.04	0.17	0.02	NS
Apparent postruminal digestion									
kg/d	1.24	1.52	0.22	0.12	0.01	0.08	0.11	0.03	NS
% of intake	23.4	26.0	2.9	0.35	0.005	0.26	0.10	0.01	NS
% of duodenal passage	84.1	86.5	1.6	0.15	NS	0.57	0.15	NS	NS
Apparent total tract digestion									
kg/d	5.18	5.71	0.14	<0.001	NS	0.03	NS	NS	NS
%	94.7	95.6	0.4	0.17	0.02	0.14	NS	NS	NS

[1]Treatment least squares means.

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

[3]NS = not significant ($P > 0.20$); term removed from statistical model.

Table 41. Ruminal VFA concentrations and pH of cows fed treatment diets containing either early or late cut orchardgrass silage as the sole source of forage

	Treatment LSM[1]			*P*[2]					
	Early	Late	SE	Trt	Trt x Period	pDMI	Trt x pDMI	pDMI x pDMI	Trt x pDMI x pDMI
Total VFA, mM	158	161	3	0.18	NS[3]	NS	NS	NS	NS
Acetate	92.1	92.8	1.0	0.54	NS	NS	NS	NS	NS
Propionate	37.6	41.5	1.8	0.01	NS	0.63	0.15	NS	NS
Butyrate	22.5	20.6	0.8	0.02	NS	0.64	0.99	0.17	0.16
Lactate	0.070	0.028	0.046	0.54	NS	NS	NS	NS	NS
Isobutyrate	1.81	1.62	0.09	0.13	NS	NS	NS	NS	NS
Valerate	2.92	2.81	0.19	0.44	NS	NS	NS	NS	NS
Isovalerate	2.30	1.89	0.12	0.01	NS	NS	NS	NS	NS
Branch chain VFA	4.11	3.51	0.19	0.03	NS	NS	NS	NS	NS
Acetate:Propionate	2.50	2.30	0.10	0.04	NS	0.57	0.12	NS	NS
Ruminal pH	5.66	5.64	0.05	0.51	NS	0.54	0.30	0.14	0.19

[1]Treatment least squares means.

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

[3]NS = not significant ($P > 0.20$); term removed from statistical model.

Table 42. Nitrogen metabolism of cows fed treatment diets containing either early or late cut orchardgrass silage as the sole source of forage

	Treatment LSM[1]			*P*[2]					
	Early	Late	SE	Trt	Trt x Period	pDMI	Trt x pDMI	pDMI x pDMI	Trt x pDMI x pDMI
N intake, g/d	670	672	13	0.89	0.01	0.002	NS[3]	NS	NS
Ruminal ammonia, mg/dl	11.0	12.9	0.4	0.001	NS	0.04	NS	NS	NS
Flow to duodenum									
Ammonia N, g/d	15.0	17.5	1.3	0.01	NS	0.95	0.15	0.07	NS
NAN									
g/d	535	531	22	0.88	0.09	0.53	0.12	NS	NS
% of N intake	80.9	86.6	4.3	0.31	NS	0.14	0.06	0.18	0.16
NANMN[4]									
g/d	135	167	13	0.05	0.18	0.59	0.04	0.07	NS
% of N intake	20.8	26.0	1.9	0.05	NS	0.16	0.04	0.03	NS
% of duodenal NAN	24.5	31.2	2.2	0.07	NS	0.29	NS	0.04	NS
Microbial N									
g/d	418	382	22	0.31	NS	NS	NS	NS	NS
% of duodenal NAN	75.5	68.8	2.2	0.07	NS	0.29	NS	0.04	NS
g/kg TRDOM[5]	31.0	30.4	1.7	0.85	0.02	NS	NS	NS	NS
NAN apparent postruminal digestion									
g/d	250	343	31	0.02	NS	0.54	0.20	0.18	0.15
% of N intake	37.1	52.0	4.9	0.02	NS	0.19	0.18	0.21	0.17
% of duodenal passage	44.5	55.0	2.5	0.002	NS	NS	NS	NS	NS
N apparent total tract digestion									
g/d	376	438	11	0.003	0.10	0.005	NS	NS	NS
%	56.1	65.1	1.2	<0.001	0.15	NS	NS	NS	NS

[1]Treatment least squares means.

Table 42 (cont'd)

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

[3]NS = not significant ($P > 0.20$); term removed from statistical model.

[4]NANMN = non-ammonia non-microbial nitrogen.

[5]TRDOM = true ruminally digested OM.

Table 43. Milk fatty acid profile of cows fed treatment diets containing either early or late cut orchardgrass silage as the sole source of forage

Fatty acid, g/100g total fatty acids	Treatment LSM[1]		SE	*P*[2]					
	Early	Late		Trt	Trt x Period	pDMI	Trt x pDMI	pDMI x pDMI	Trt x pDMI x pDMI
C4:0	3.26	2.96	0.06	0.002	0.01	0.81	0.13	0.55	0.11
C5:0	0.037	0.036	0.003	0.82	NS[3]	0.29	0.03	NS	NS
C6:0	1.74	1.68	0.02	0.06	NS	NS	NS	NS	NS
C7:0	0.026	0.029	0.002	0.12	NS	0.28	0.02	NS	NS
C8:0	0.972	0.975	0.021	0.87	NS	NS	NS	NS	NS
C9:0	0.030	0.037	0.003	0.008	NS	0.29	0.09	NS	NS
C10:0	2.19	2.33	0.07	0.07	0.14	NS	NS	NS	NS
C11:0	0.264	0.282	0.009	0.09	NS	NS	NS	NS	NS
C12:0	2.83	3.10	0.10	0.02	0.08	0.16	NS	NS	NS
C13:0 iso	0.026	0.035	0.001	<0.001	NS	NS	NS	NS	NS
C13:0 anteiso	0.090	0.107	0.004	0.006	NS	NS	NS	NS	NS
C13:0	0.195	0.239	0.009	<0.001	NS	0.83	0.09	NS	NS
C14:0 iso	0.113	0.115	0.01	0.78	NS	0.85	0.06	NS	NS
C14:0	10.0	10.3	0.2	0.15	0.10	0.05	NS	0.02	NS
C15:0 iso	0.216	0.229	0.005	0.004	NS	NS	NS	NS	NS
C14:1n5t	0.008	0.009	0.001	0.63	NS	0.68	0.18	0.14	NS
C15:0 anteiso	0.440	0.485	0.010	<0.001	NS	0.20	0.02	NS	NS
C14:1n5c	0.976	1.09	0.037	0.02	NS	NS	NS	NS	NS
C16:0 iso	0.227	0.237	0.018	0.54	NS	NS	NS	NS	NS
C16:0	29.1	28.8	0.8	0.46	NS	0.79	NS	0.08	NS
C16:1n7t	0.329	0.356	0.007	<0.001	NS	0.40	NS	0.001	NS
C16:1n7c	1.89	2.07	0.10	0.02	0.20	0.22	NS	0.13	NS
C17:0	0.484	0.529	0.016	<0.001	NS	0.52	0.09	NS	NS
C17:1n7c	0.231	0.266	0.021	0.04	NS	0.69	NS	0.17	NS
C18:0	11.3	10.1	0.4	0.007	NS	NS	NS	NS	NS

Table 43 (cont'd)

C18:1 t6-8	0.205	0.230	0.009	<0.001	NS	0.24	0.27	0.05	0.05
C18:1 t9	0.164	0.185	0.004	<0.001	NS	0.14	NS	0.09	NS
C18:1 t10	0.235	0.316	0.032	0.009	NS	NS	NS	NS	NS
C18:1 t11	0.871	1.03	0.062	0.005	NS	NS	NS	NS	NS
C18:1 t12	0.314	0.280	0.010	<0.001	NS	NS	NS	NS	NS
C18:1 c9	22.9	23.2	0.70	0.67	NS	0.80	NS	0.01	NS
C18:1 c11	0.547	0.660	0.052	0.008	NS	0.71	NS	0.15	NS
C18:1 c12	0.305	0.263	0.010	<0.001	NS	0.20	NS	NS	NS
C18:2n6c	2.06	2.41	0.06	<0.001	NS	0.23	0.16	NS	NS
C20:0	0.147	0.137	0.005	0.02	NS	NS	NS	NS	NS
C18:3n6c	0.024	0.035	0.001	<0.001	NS	NS	NS	NS	NS
C18:3n3c	0.978	0.792	0.03	<0.001	NS	NS	NS	NS	NS
C20:1n12c	0.037	0.039	0.003	0.53	NS	0.90	NS	0.18	NS
CLA (c9, t11)	0.448	0.586	0.029	<0.001	NS	NS	NS	NS	NS
CLA (t10, c12)	0.016	0.016	0.002	0.76	NS	NS	NS	NS	NS
C20:2n6c	0.024	0.027	0.001	0.01	NS	NS	NS	NS	NS
C20:3n6c	0.125	0.139	0.008	<0.001	NS	0.84	NS	0.07	NS
C20:3n3c	0.055	0.052	0.002	0.44	0.12	0.14	NS	0.16	NS
C20:4n6c	0.175	0.212	0.011	<0.001	0.12	0.30	0.31	0.42	0.009
C24:0	0.063	0.063	0.003	0.80	0.08	0.16	NS	0.03	NS
C22:5n18c	0.030	0.026	0.002	0.002	NS	NS	NS	NS	NS
C22:5n15c	0.047	0.049	0.003	0.58	NS	0.53	0.05	NS	NS
C22:5n3c	0.079	0.091	0.006	0.02	0.02	0.61	0.35	0.11	0.17
Unidentified	2.08	1.57	0.06	<0.001	NS	NS	NS	NS	NS
C18:1 trans isomers	1.76	2.06	0.08	<0.001	NS	0.41	0.32	0.83	0.11
< C16	24.3	25.2	0.5	0.09	0.19	0.16	NS	0.09	NS
C16	31.5	31.4	0.8	0.80	NS	0.69	NS	0.11	NS
> C16	41.9	41.7	1.0	0.80	0.17	0.29	NS	0.05	NS

Table 43 (cont'd)

[1]Treatment least squares means.
[2]*P*-values for treatment (Trt), treatment by period interaction (Trt x Period), preliminary DMI (pDMI), treatment by preliminary DMI interaction (Trt x pDMI), quadratic effect of preliminary DMI (pDMI x pDMI), and treatment by quadratic effect of preliminary DMI (Trt x pDMI x pDMI).
[3]NS = not significant ($P > 0.20$); term removed from statistical model.

Table 44. NDF digestion of cows fed treatment diets containing either early or late cut orchardgrass silage as the sole source of forage

	Treatment LSM[1]			*P*[2]					
	Early	Late	SE	Trt	Trt x Period	pDMI	Trt x pDMI	pDMI x pDMI	Trt x pDMI x pDMI
NDF									
Intake, kg/d	6.71	6.53	0.11	0.17	0.13	<0.001	NS[3]	NS	NS
Ruminal digestion									
kg/d	4.27	3.34	0.17	0.01	0.08	<0.001	0.61	0.04	0.15
%	64.1	52.1	2.0	0.009	<0.001	0.07	0.51	0.03	0.12
Passage to duodenum, kg/d	2.38	3.08	0.15	0.01	0.001	0.25	0.62	0.23	0.14
Postruminal digestion									
kg/d	-0.91	-0.29	0.18	0.06	NS	0.04	0.99	0.02	0.15
Total tract digestion									
kg/d	3.29	2.97	0.13	0.12	NS	0.06	NS	NS	NS
%	49.1	45.6	1.8	0.23	0.14	NS	NS	NS	NS
Potentially digestible NDF									
Intake, kg/d	4.88	4.54	0.07	0.002	NS	<0.001	NS	NS	NS
Ruminal digestion									
kg/d	4.27	3.34	0.17	0.01	0.08	<0.001	0.61	0.04	0.15
%	88.4	74.5	2.9	0.02	0.003	0.10	0.48	0.03	0.10
Passage to duodenum, kg/d	0.55	1.13	0.13	0.03	0.003	0.38	0.49	0.05	0.08
Postruminal digestion									
kg/d	-0.91	-0.29	0.18	0.06	NS	0.04	0.99	0.02	0.15
Total tract digestion									
kg/d	3.29	2.97	0.13	0.12	NS	0.06	NS	NS	NS
%	67.4	65.4	2.6	0.61	NS	NS	NS	NS	NS
Indigestible NDF									
Intake, kg/d	1.83	2.00	0.04	0.003	0.007	0.002	NS	NS	NS

[1]Treatment least squares means.

Table 44 (cont'd)

[2]*P*-values for treatment (Trt), treatment by period interaction (Trt x Period), preliminary DMI (pDMI), treatment by preliminary DMI interaction (Trt x pDMI), quadratic effect of preliminary DMI (pDMI x pDMI), and treatment by quadratic effect of preliminary DMI (Trt x pDMI x pDMI).
[3]NS = not significant ($P > 0.20$); term removed from statistical model.

Table 45. DM and OM digestion of cows fed treatment diets containing either early or late cut orchardgrass silage as the sole source of forage

	Treatment LSM[1]			*P*[2]					
	Early	Late	SE	Trt	Trt x Period	pDMI	Trt x pDMI	pDMI x pDMI	Trt x pDMI x pDMI
DM									
Intake, kg/d	22.5	22.4	0.4	0.70	0.12	0.002	NS[3]	NS	NS
Apparent total tract digestion									
kg/d	13.8	14.6	0.4	0.09	NS	0.01	NS	NS	NS
%	61.2	65.5	1.2	0.04	0.03	NS	NS	NS	NS
OM									
Intake, kg/d	20.4	20.5	0.4	0.78	0.11	0.002	NS	NS	NS
Apparent ruminal digestion									
kg/d	9.61	8.40	0.51	0.18	0.03	0.01	0.22	0.32	0.10
%	47.5	41.7	2.0	0.14	<0.001	0.67	0.23	0.44	0.09
True ruminal digestion									
kg/d	13.6	12.1	0.5	0.07	0.16	0.009	0.29	0.32	0.14
%	67.4	60.2	1.6	0.01	<0.001	0.49	0.20	0.61	0.05
Passage to duodenum, kg/d	10.5	11.8	0.5	0.13	<0.001	0.02	0.25	0.79	0.09
Apparent postruminal digestion									
kg/d	3.02	5.20	0.42	0.01	0.01	0.43	0.39	0.30	0.04
% of intake	15.0	25.7	2.0	0.02	0.009	0.48	0.40	0.10	0.05
Apparent total tract digestion									
kg/d	12.7	13.6	0.3	0.06	NS	0.01	NS	NS	NS
%	62.3	66.2	1.1	0.06	0.03	NS	NS	NS	NS

[1]Treatment least squares means.

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

Table 45 (cont'd)

[3]NS = not significant ($P > 0.20$); term removed from statistical model.

Table 46. Rumen kinetics of cows fed treatment diets containing either early or late cut orchardgrass silage as the sole source of forage

	Treatment LSM[1]			*P*[2]					
	Early	Late	SE	Trt	Trt x Period	pDMI	Trt x pDMI	pDMI x pDMI	Trt x pDMI x pDMI
Ruminal turnover rate, %/h									
DM	7.85	6.90	0.64	0.002	NS[3]	0.71	NS	0.14	NS
OM	7.83	6.86	0.65	0.002	NS	0.70	NS	0.14	NS
NDF	4.43	3.54	0.35	<0.001	NS	0.73	NS	0.14	NS
pdNDF[4]	6.18	5.52	0.35	0.03	NS	NS	NS	NS	NS
Starch	37.7	37.3	4.5	0.92	NS	0.69	NS	0.18	NS
Ruminal turnover time, h									
DM	12.1	13.9	0.8	0.009	NS	NS	NS	NS	NS
OM	12.2	13.9	0.9	0.009	NS	NS	NS	NS	NS
NDF	21.5	27.0	1.7	<0.001	NS	NS	NS	NS	NS
pdNDF	16.9	19.1	1.2	0.03	NS	NS	NS	NS	NS
iNDF[5]	35.9	51.5	4.4	0.005	NS	0.58	0.55	0.14	0.20
Starch	2.86	3.01	0.29	0.57	NS	0.60	NS	0.12	NS
Ruminal passage rate, %/h									
pdNDF	0.66	1.32	0.16	0.04	0.002	0.05	0.64	0.09	0.11
iNDF	2.76	2.10	0.25	<0.001	NS	0.31	NS	0.08	NS
Starch	10.3	11.0	2.2	0.64	0.06	0.19	0.03	0.01	NS
Ruminal digestion rate, %/h									
pdNDF	4.95	3.98	0.50	0.007	NS	0.92	NS	0.19	NS
Starch	27.2	26.1	2.4	0.71	0.12	NS	NS	NS	NS

[1]Treatment least squares means.

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

Table 46 (cont'd)

[3]NS = not significant ($P > 0.20$); term removed from statistical model.
[4]pdNDF = potentially digestible NDF.
[5]iNDF = indigestible NDF.

Table 47. Rumen pools of cows fed treatment diets containing either early or late cut orchardgrass silage as the sole source of forage

	Treatment LSM[1]			*P*[2]					
	Early	Late	SE	Trt	Trt x Period	pDMI	Trt x pDMI	pDMI x pDMI	Trt x pDMI x pDMI
Wet weight, kg	88.4	92.7	4.6	0.06	NS[3]	NS	NS	NS	NS
Volume, L	104	111	5	0.07	NS	0.36	0.10	NS	NS
Density, kg/L	0.85	0.87	0.02	0.32	NS	0.23	0.10	0.30	0.05
Rumen pool, kg									
DM	11.4	12.8	0.8	<0.001	NS	NS	NS	NS	NS
OM	10.4	11.8	0.7	<0.001	NS	NS	NS	NS	NS
NDF	6.03	7.30	0.44	<0.001	NS	NS	NS	NS	NS
pdNDF[4]	3.46	3.60	0.25	0.27	NS	NS	NS	NS	NS
iNDF[5]	2.76	4.17	0.32	<0.001	NS	0.95	0.62	0.14	0.18
Starch	0.64	0.72	0.06	0.18	NS	0.80	NS	0.12	NS

[1]Treatment least squares means.

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

[3]NS = not significant ($P > 0.20$); term removed from statistical model.

[4]pdNDF = potentially digestible NDF.

[5]iNDF = indigestible NDF.

Table 48. Chewing activity of cows fed treatment diets containing either early or late cut orchardgrass silage as the sole source of forage

	Treatment LSM[1]			*P*[2]					
	Early	Late	SE	Trt	Trt x Period	pDMI	Trt x pDMI	pDMI x pDMI	Trt x pDMI x pDMI
Meals									
Bouts/d	9.07	8.75	0.42	0.64	0.03	NS[3]	NS	NS	NS
Length, min/bout	28.9	31.4	1.5	0.28	0.009	NS	NS	NS	NS
Interval, min	143	150	8	0.58	0.11	NS	NS	NS	NS
Meal size, kg									
DM	2.57	2.60	0.13	0.85	NS	0.05	NS	NS	NS
OM	2.33	2.39	0.12	0.72	NS	0.05	NS	NS	NS
NDF	0.76	0.76	0.04	0.97	NS	0.05	NS	NS	NS
pdNDF[4]	0.56	0.53	0.03	0.56	0.11	0.05	NS	NS	NS
iNDF[5]	0.21	0.23	0.01	0.21	NS	0.05	NS	NS	NS
Starch	0.62	0.69	0.03	0.13	NS	0.09	NS	NS	NS
Eating time									
Min/d	255	268	12	0.31	NS	NS	NS	NS	NS
Min/kg DMI	11.0	12.7	1.0	0.08	NS	0.21	0.88	0.92	0.14
Min/kg NDF intake	36.7	43.4	3.2	0.05	NS	0.18	0.90	0.93	0.13
Min/kg forage NDF intake	42.1	50.9	3.9	0.03	NS	0.20	0.93	0.89	0.14
Rumination									
Bouts/d	14.9	15.3	0.7	0.56	NS	0.57	0.08	0.32	0.04
Length, min/bout	35.4	36.4	2.2	0.58	NS	0.16	0.02	0.26	0.13
Interval, min	57.3	50.2	2.1	0.04	0.14	NS	NS	NS	NS
Ruminating time									
Min/d	523	562	16	0.04	NS	0.18	NS	NS	NS
Min/kg DMI	23.2	25.3	0.8	0.02	NS	NS	NS	NS	NS
Min/kg NDF intake	77.7	86.5	2.7	0.01	NS	NS	NS	NS	NS
Min/kg forage NDF intake	88.6	101.2	3.1	0.003	NS	NS	NS	NS	NS

Table 48 (cont'd)

Total chewing time									
Min/d	778	830	19	0.006	NS	NS	NS	NS	NS
Min/kg DMI	34.6	37.4	1.1	0.006	NS	0.18	NS	NS	NS
Min/kg NDF intake	116	128	4	0.003	NS	0.15	NS	NS	NS
Min/kg forage NDF intake	132	150	4	<0.001	NS	0.17	NS	NS	NS

[1]Treatment least squares means.

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

[3]NS = not significant ($P > 0.20$); term removed from statistical model.

[4]pdNDF = potentially digestible NDF.

[5]iNDF = indigestible NDF.

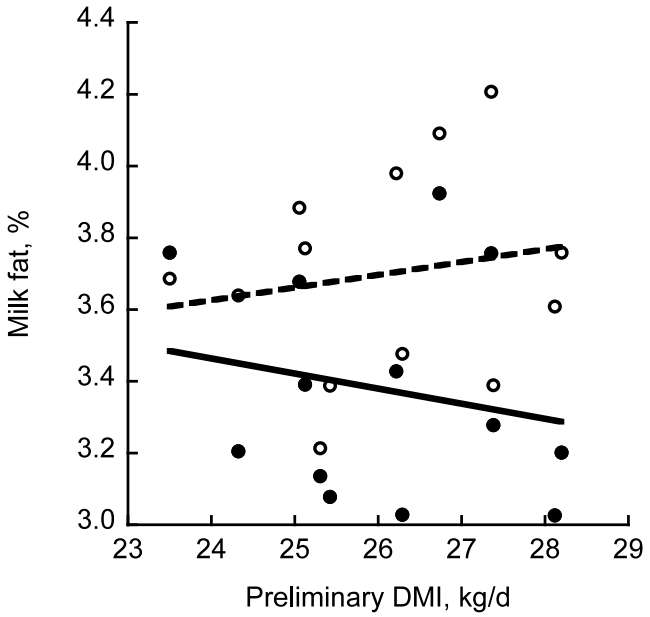

Figure 20. Interaction of early (open circles, dashed line) and late (closed circles, solid line) orchardgrass maturity with preliminary DMI for milk fat concentration ($P = 0.07$). The preliminary DMI on the x-axis are the mean DMI of individual cows during the final 4 d of the preliminary period when all cows were fed a common diet.

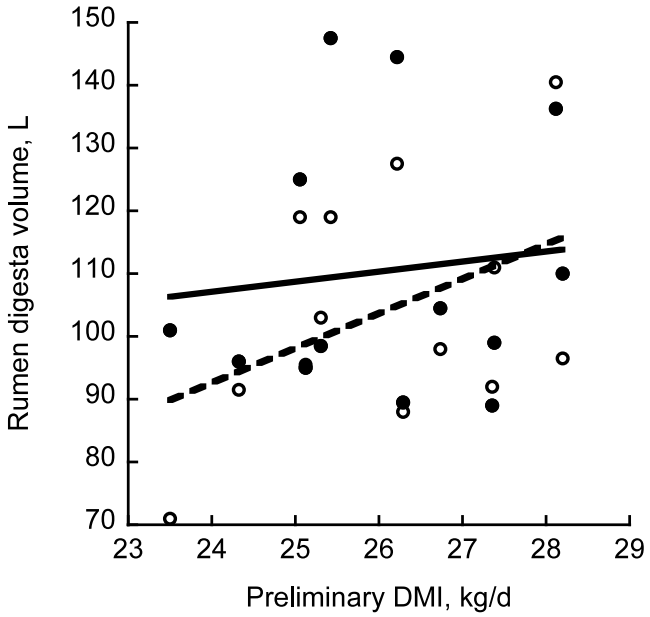

Figure 21. Interaction of early (open circles, dashed line) and late (closed circles, solid line) orchardgrass maturity with preliminary DMI for rumen digesta volume ($P = 0.10$). The preliminary DMI on the x-axis are the mean DMI of individual cows during the final 4 d of the preliminary period when all cows were fed a common diet.

Figure 22

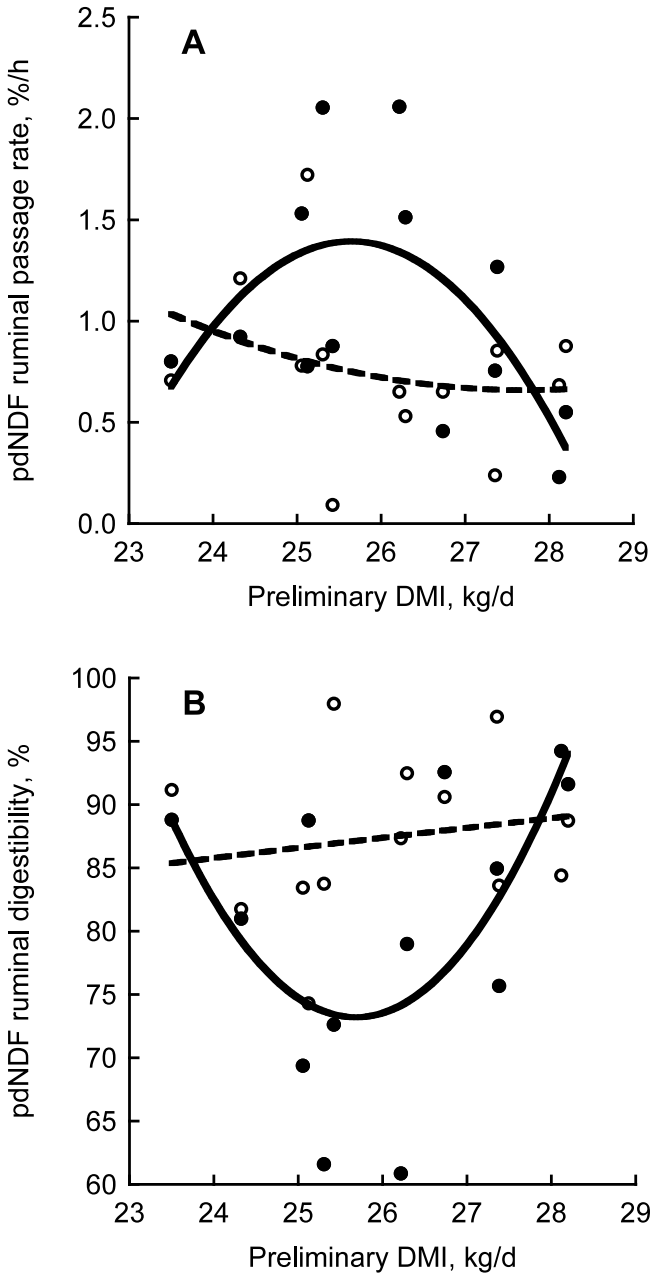
A
pdNDF ruminal passage rate, %/h
2.5
2.0
1.5
1.0
0.5
0.0
23
24
25
26
27
28
29
Preliminary DMI, kg/d
B
pdNDF ruminal digestibility, %
100
95
90
85
80
75
70
65
60
23
24
25
26
27
28
29
Preliminary DMI, kg/d

Figure 22 (cont'd)

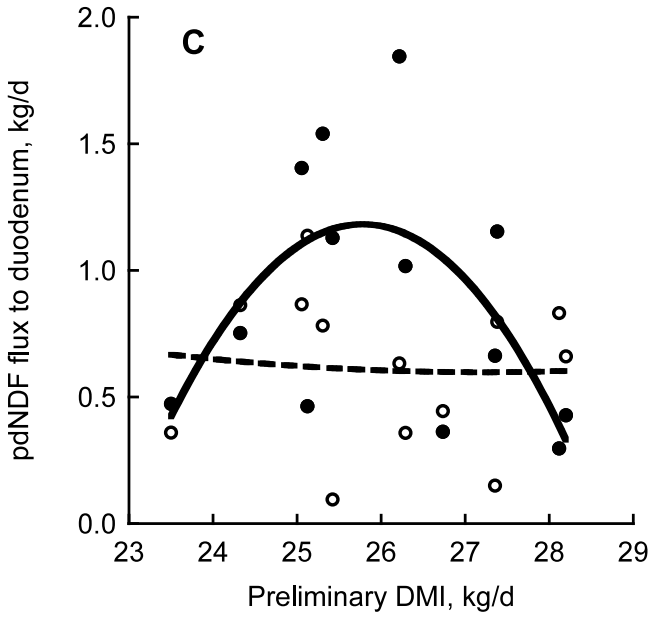

Figure 22. Interaction of early (open circles, dashed line) and late (closed circles, solid line) orchardgrass maturity with preliminary DMI for potentially digestible NDF (pdNDF) A) ruminal passage rate ($P = 0.11$), B) ruminal digestibility ($P = 0.10$), and C) flux to the duodenum ($P = 0.08$). The 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.

Figure 23

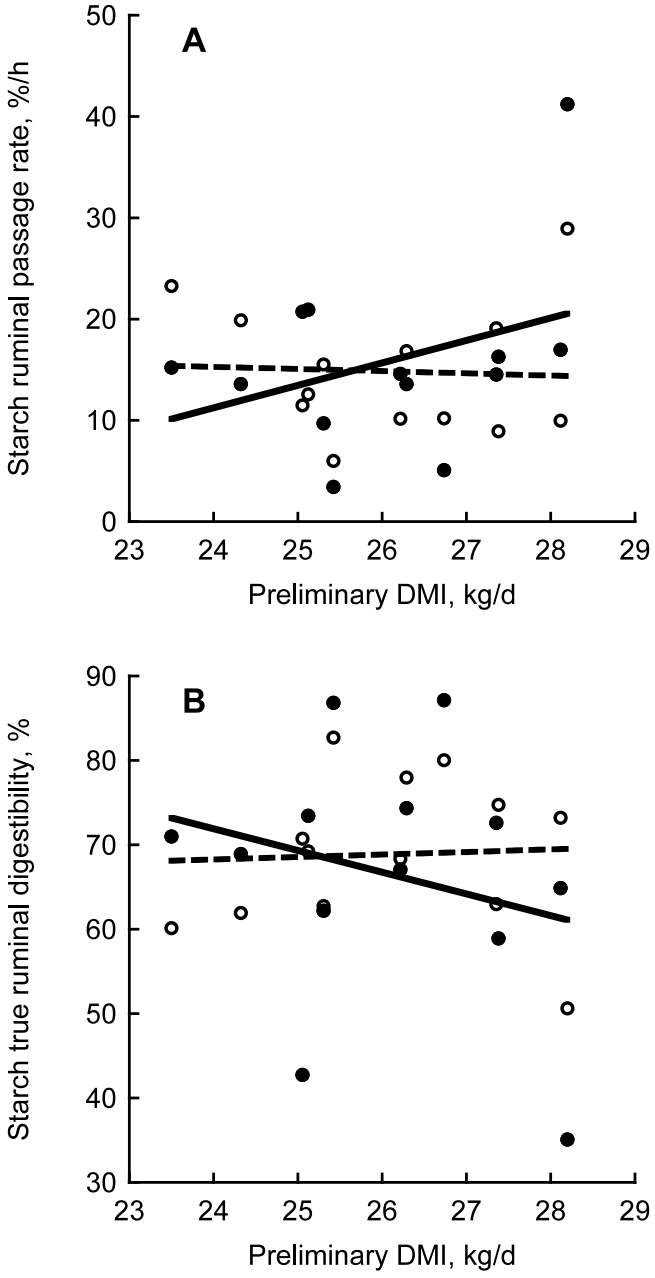
A
Starch ruminal passage rate, %/h
0
10
20
30
40
50
23
24
25
26
27
28
29
Preliminary DMI, kg/d
B
Starch true ruminal digestibility, %
30
40
50
60
70
80
90
23
24
25
26
27
28
29
Preliminary DMI, kg/d

Figure 23 (cont'd)

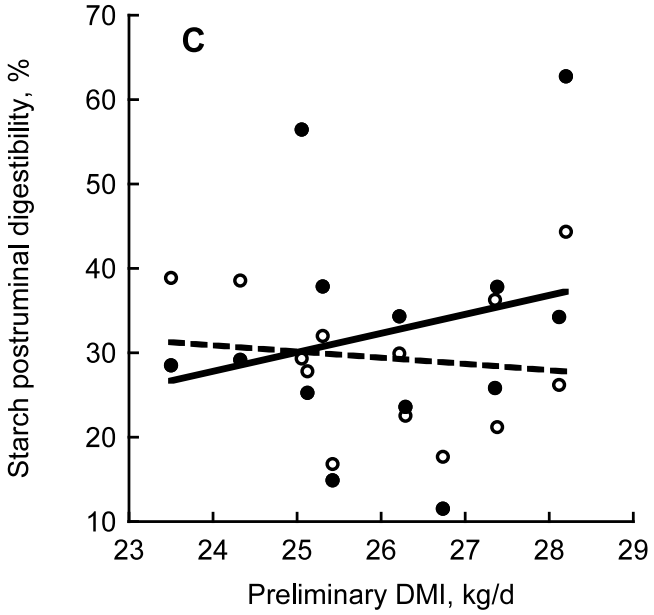

Figure 23. Interaction of early (open circles, dashed line) and late (closed circles, solid line) orchardgrass maturity with preliminary DMI for starch A) ruminal passage rate ($P = 0.03$), B) true ruminal digestibility ($P = 0.13$), and C) postruminal digestibility ($P = 0.11$). The preliminary DMI 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.

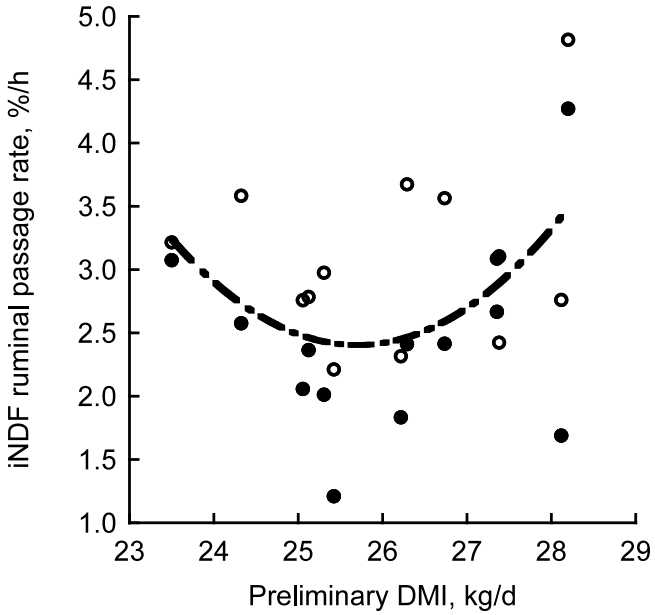

Figure 24. Relationship of early (open circles) and late (closed circles) orchardgrass maturity with preliminary DMI for indigestible (iNDF) ruminal passage rate ($P = 0.08$). The preliminary DMI on the x-axis are the mean DMI of individual cows during the final 4 d of the preliminary period when all cows were fed a common diet.

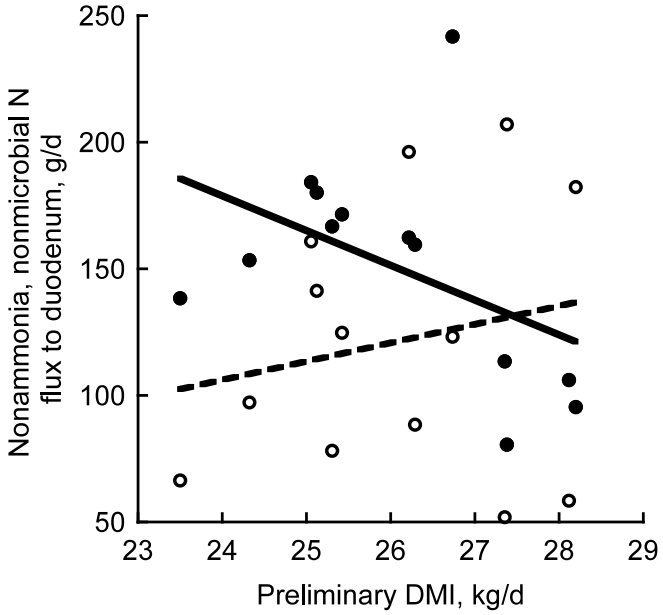

Figure 25. Interaction of early (open circles, dashed line) and late (closed circles, solid line) orchardgrass maturity with preliminary DMI for nonammonia, nonmicrobial N flux to the duodenum ($P = 0.04$). The preliminary DMI on the x-axis are the mean DMI of individual cows during the final 4 d of the preliminary period when all cows were fed a common diet.

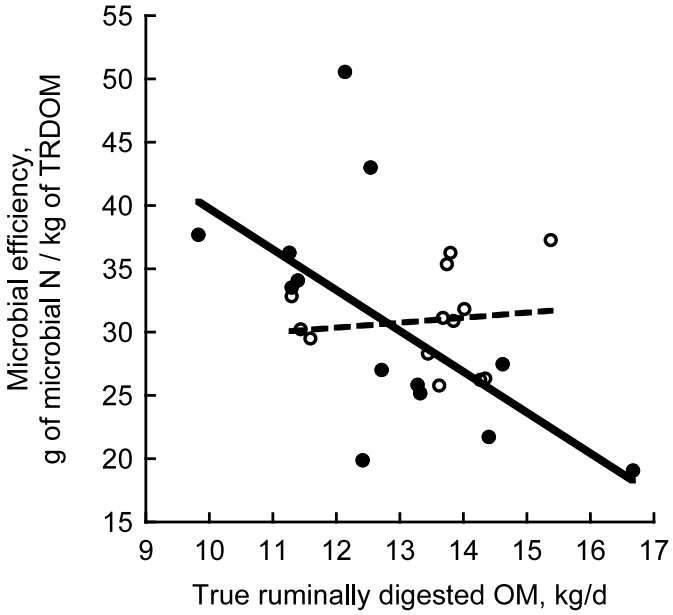

Figure 26. Relationship between true ruminally digested OM (TRDOM) and microbial efficiency for early (open circles, dashed line; $P = 0.68$, $R^2 = 0.02$) and late (closed circles, solid line; microbial efficiency, g of microbial N/kg of TRDOM = 72.0 – 3.22 x TRDOM, kg/d; $P = 0.03$, $R^2 = 0.37$) orchardgrass maturity.

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CHAPTER 6

Nutrient Demand Interacts with Forage Family to Affect Digestion Responses in Dairy Cows[1]

ABSTRACT

Effects of forage family on dry matter intake (DMI), milk production, ruminal pool sizes, digestion and passage kinetics, and chewing activity 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 18-d treatment periods. During the preliminary period, pDMI of individual cows ranged from 19.6 to 29.5 kg/d (mean = 25.9 kg/d) and 3.5% fat-corrected milk yield ranged from 24.3 to 60.3 kg/d (mean = 42.1 kg/d). Experimental treatments were diets containing either a) alfalfa silage (AL) or b) orchardgrass silage (OG) as the sole forage. Alfalfa and orchardgrass contained 42.3 and 58.2% neutral detergent fiber (NDF) and 22.5 and 11.4% crude protein, respectively. Forage:concentrate ratios were 60:40 for AL and 43:57 for OG; both diets contained ~25% forage NDF and ~30% total 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 forage family and their interaction with pDMI were tested by ANOVA. Forage family and its interaction with pDMI did not affect milk yield or composition. Alfalfa decreased efficiency of milk production compared with OG because of numerically greater DMI for AL. Alfalfa increased indigestible NDF (iNDF) intake and decreased potentially digestible NDF (pdNDF) intake compared with OG because of differences in chemical composition of forages. Alfalfa

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increased ruminal pH, digestion rates of pdNDF and starch, and passage rates of pdNDF and iNDF compared with OG, which affected ruminal digestibility. Passage rate of iNDF was related to pDMI; AL increased iNDF passage rate and OG decreased it as pDMI increased. Alfalfa decreased rumen pool sizes of pdNDF, starch, dry matter, and rumen digesta wet weight and volume compared with OG. Alfalfa decreased ruminating time per unit of forage NDF consumed compared with OG indicating that alfalfa provided less effective fiber than orchardgrass. Alfalfa, but not OG, increased ammonia N, nonammonia nonmicrobial N, and nonammonia N fluxes as pDMI increased. Microbial efficiency was positively related to pdNDF passage rate for OG, but not AL. When alfalfa or orchardgrass silage was the only source of forage in diets formulated to contain similar concentrations of forage NDF, forage family did not affect productivity.

INTRODUCTION

Utilization of diets by dairy cows is largely influenced by the nutrient composition and physical characteristics of the forage in the ration. Large differences exist among forage families (grasses and legumes) including chemical composition, anatomical characteristics, and digestion characteristics that affect digestibility (Allen, 1996; Wilson and Kennedy, 1996). The relationship between NDF and its digestibility with feed intake varies across forages. Cool-season grasses and legumes differ in concentration and the rate and extent of digestion of fiber (Van Soest, 1982). Grasses generally contain higher total NDF and potentially digestible NDF (pdNDF) concentrations, which have a slower rate of digestion but greater extent of digestion than legumes. The greater extent of digestion for grasses offers the potential for greater energy availability, but slower digestion rates can result in greater ruminal retention times and

subsequently lower intake, possibly offsetting gains from higher digestibility (Allen, 2000).

Several lactation studies comparing legumes with grasses are reported in the literature; however, many are confounded by the NDF differences between the two species. When diets are formulated to contain an equal amount of forage DM, total and forage NDF concentrations of diets generally will be higher for diets containing grasses compared with legumes. Increasing dietary NDF concentration often has a negative impact on the amount of DM consumed by lactating dairy cows (Allen, 2000). In this experiment, rations were formulated to contain similar forage NDF concentrations in order to specifically measure the effects of forage fiber. Alfalfa (*Medicago sativa*) and orchardgrass (*Dactylis glomerata L.*) were selected as a representative legume and cool-season grass, respectively.

In addition to the combination of dietary factors affecting ruminal digestion and distention, the individual cow's appetite will also affect the responses of passage rate and intake to forage family. Voelker Linton and Allen (2008) found that the response of DMI to forage family depended on the appetite of individual cows as intake was more restricted by orchardgrass than alfalfa as level of intake increased. Because forage family and level of intake affect ruminal passage and digestion rates and, thus, digesta fill in the rumen, the response to effects of forage family and its relationship with intake level was 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 forage family are related to level of intake and legumes will permit a greater increase in passage rate than grasses as feed intake increases.

The objectives of this experiment were to evaluate the relationships between voluntary DMI and effects of forage family on DMI, milk production, ruminal fermentation and pool sizes, digestion and passage kinetics, and chewing behavior in lactating dairy cows. This study had

three unique features to improve our understanding of the role of forage family and interpret its effect on animal responses. First, it allowed effects of the interaction between forage family 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 alfalfa and orchardgrass as the sole source of forage. Third, ruminal passage rates of individual feed fractions, instead of entire feeds, were measured using ruminally and duodenally cannulated cows.

MATERIALS AND METHODS

This article is the first of a set of two from one experiment that evaluated the effects forage family and its interaction with level of feed intake (nutrient demand). This article discusses the effect of pDMI on responses to treatment for production, rumen parameters and kinetics, and chewing activity. The companion article focuses on rates of particle size breakdown in, and particle passage from, the rumen.

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 18-d experimental periods. The first 10 d of each period were allowed for diet adaptation and samples were collected during the final 4 d of the preliminary period and 8 d of each experimental period. Cows were 157±90 (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 19.6 to 29.5 kg/d (mean = 25.9 kg/d) and 3.5% FCM yield ranged from 24.3 to 60.3 kg/d (mean = 42.1 kg/d; Table 49). 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 either a) alfalfa silage (AL) or b) orchardgrass silage (OG) as the sole forage. Alfalfa (Pioneer 54H91, Pioneer Hi-Bred, Johnston, IA) and orchardgrass (Baridana cultivar, Barenbrug USA, Tangent, OR) forages were produced at the campus farm at Michigan State University (East Lansing), chopped to 10 mm theoretical length of cut, and ensiled in Ag-Bags (Ag-Bag Systems Inc., St. Nazianz, WI). During the sample collection periods, alfalfa and orchardgrass contained 42.3 and 58.2% NDF and 22.5 and 11.4% CP, respectively (DM basis; Table 50). Diets AL and OG were formulated to contain 25% forage NDF, 30% total NDF, and 18% CP. We acknowledge these treatments affect dietary starch concentration but maintaining similar forage and total NDF concentrations for both treatments was of primary interest. The diet fed during the preliminary period was formulated so that alfalfa and orchardgrass each contributed 50% of forage NDF. Diets also contained dry ground corn, SoyPlus® (West Central Soy, Ralston, IA), and vitamin-mineral premix (Table 51); soybean meal (48% CP), urea, and limestone were used to compensate for lower CP and Ca concentrations in orchardgrass silage than in alfalfa silage.

Data and Sample Collection

Throughout the experiment, cows were housed in tie-stalls and fed diets as total mixed

rations once daily (1130 h) at 110% of expected intake. The amount of feed offered and refused (orts) was 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 11 to 15 during each experimental period. Samples were frozen immediately after collection at −20°C and combined to one composite sample per period prior to analysis.

Cows were moved to an exercise lot twice daily (0230 and 1300 h) prior to 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 11 to 15 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 18 of each experimental period. Body condition score was determined on the same days by 3 trained investigators blinded to treatments (Wildman et al., 1982; 5-point scale where 1 = thin and 5 = fat). Chewing activity was monitored and recorded by observation every 5 min for 24 h on d 16 of each experimental period. Activity was noted as eating, ruminating, drinking, or idle for each cow at each time.

Duodenal samples (900 mL), fecal samples (500 g), rumen fluid and particulate samples for microbial isolation (400 g), rumen fluid samples for pH, concentrations of VFA, lactate, and ammonia (100 mL), and blood samples for concentrations of glucose, insulin, and glucagon were collected every 15 h from d 11 to 15 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 was collected from the reticulum, near the reticular-omasal orifice, transported to the laboratory, and processed. Rumen fluid for pH, VFA, lactate, and ammonia were obtained by combining digesta from five different

sites in the rumen and straining it through nylon mesh (~1 mm pore size); fluid pH was recorded immediately. Blood was sampled from coccygeal vessels and collected into 2 evacuated tubes, one containing potassium EDTA and the other containing potassium oxalate with sodium fluoride as a glycolytic inhibitor. Both were centrifuged at 2,000 x *g* for 15 min immediately after sample collection and plasma was harvested. Samples containing potassium EDTA were preserved with benzamidine (0.05 M final concentration). Samples were stored at -20°C.

Ruminal contents were evacuated manually through the ruminal cannula 4 h after feeding at the beginning of d 17 (1530 h) and 2 h before feeding at the end of d 18 (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 yield 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, SNF, and MUN 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 one 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 (Tri-Philizer 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 one 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, iNDF, ADF, acid detergent lignin (ADL), CP, and starch. Ash concentration was determined after 5 h 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). Indigestible NDF was estimated as NDF residue after 240 h in vitro fermentation (Goering and Van Soest, 1970); flasks were reinoculated at 120 h to insure 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 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 x *g* for 15 min. The supernatant was centrifuged at 18,000 x *g* for 15 min, and the pellet was washed with 0.9% NaCl solution and centrifuged again at 18,000 x *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 concentration of major VFA and lactate by HPLC (Waters Corp., Milford, MA) according to Oba and Allen (2003a).

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 ratio of purines to OM (Oba and

Allen, 2003a), 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 the following equations:

Turnover rate (%/h) = 100 x (Intake of component / Ruminal pool of component) / 24

Passage rate (%/h) = 100 x (Duodenal flow of component / Ruminal pool of component) / 24

Digestion rate (%/h) = Turnover rate in the rumen (%/h) – Passage rate from the rumen (%/h).

Plasma samples were composited into one sample per cow per period and analyzed using commercial kits to determine concentrations of insulin (Coat-A-Count, Siemens Healthcare Diagnostics, Deerfield, IL), and glucagon (kit #GL-32K, Millipore, Billerica, MA). Plasma glucose concentration was analyzed using a glucose oxidase method (Sigma Chemical Co., St. Louis, MO).

Manually observed chewing activity was summarized by a logic script in Igor Pro (Version 6.12, WaveMetrics Inc., Lake Oswego, OR) to generate meal and rumination bout information according to previously established criteria (Dado and Allen, 1994). Variables determined included frequency of meal bouts per day, interval between meals, frequency of ruminating bouts per day, interval between ruminating bouts, eating time per day, ruminating time per day, and total chewing time per day.

Statistical Analysis

All data were analyzed by using the fit model procedure of JMP (Version 8, SAS Institute, 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: $Y_{ijk} = \mu + C_i + P_j + T_k + PT_{jk} + pDMI + T_kpDMI + pDMI^2 + T_kpDMI^2 + e_{ijk}$ where 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_kpDMI^2 is the interaction of treatment and pDMI (quadratic), and e_{ijk} is the residual error. Statistical significance for T_kpDMI and T_kpDMI^2 indicated 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. Tendencies 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, two cows experienced high fevers and had depressed intake after the first experimental period and were removed. Additionally, data from one cow was excluded prior to statistical analysis because the calculated value for duodenal flow was extremely high for the second experimental period and resulted in unrealistically low ruminal digestibility. Thus, data from 13 cows were statistically analyzed.

RESULTS AND DISCUSSION

Comparison of Forages and Diets

Chemical analyses and physical characteristics of ensiled forages are listed in Table 50. As expected, alfalfa had lower concentration of total NDF (42.3 vs. 58.2%) but higher concentrations of iNDF (23.0 vs. 16.1%), ADL (7.56 vs. 6.03%), and CP (22.5 vs. 11.4%) than orchardgrass. Indigestible NDF for alfalfa expressed as a percent of NDF was nearly twice that for orchardgrass (54.5 vs. 27.7% of NDF). In vitro NDF digestibility (30 h) was 15 percentage units lower for alfalfa than for orchardgrass (38.3 vs. 53.3%). Alfalfa had higher DM concentration than orchardgrass and was drier than expected. Both silages had similar pH and underwent lactic acid fermentation, but alfalfa had a lower lactic:acetic acid ratio than orchardgrass. Based on wet sieving, alfalfa had greater mean particle size (11.6 vs. 9.66 mm) than orchardgrass. Additionally, alfalfa contained a larger proportion of particles > 19 mm (29.3 vs. 17.1%; top sieve) and smaller proportion of particles < 8 mm (22.2 vs. 32.7%; bottom pan) than orchardgrass when sieved with the Penn State Particle Separator. Although forages were chopped to the same theoretical length of cut for both silages, the differences in particle size are likely compared with the differences in physical characteristics between the forage species and orientation of stems in the field.

Diet ingredients and chemical composition are shown in Table 51. The preliminary diet contained more alfalfa silage than orchardgrass silage so each forage supplied similar concentrations of forage NDF. Because treatment diets were formulated to contain similar forage NDF, forage:concentrate ratios were different between diets with ratios of 60:40 and 43:57 for AL and OG, respectively. Besides forage source, differences in diets included sources and concentrations of protein supplements and concentrations of limestone and corn grain, which were lower for AL than OG, to account for differences in chemical composition between alfalfa

and orchardgrass silages. The chemical composition of each diet was mathematically calculated according to the proportion of each feed ingredient in the diet and its respective analytical values, and was similar for forage NDF and total NDF concentrations. Despite increasing the concentration of soybean meal and adding SoyPlus® and urea to increase CP in OG, AL still contained slightly higher concentrations of CP than OG because the changes in CP concentration of forages were greater than expected. Starch concentration was lower for AL because of more forage and less concentrate in the diet for AL compared with OG. Indigestible NDF was higher for AL than OG and is reflective of the iNDF concentration in the forages, which was higher for alfalfa than orchardgrass. In both diets, forage NDF provided over 82% of the total diet NDF.

Effects of Forage Family and pDMI

Forage family and its interaction with pDMI did not affect yields of milk or milk components or milk composition (Table 52). Mean DMI was numerically, but not statistically, greater for AL than OG (24.2 vs. 23.2 kg/d, $P = 0.13$, Table 52). Alfalfa decreased efficiency of milk production compared with OG (FCM/DMI, 1.40 vs. 1.47, $P = 0.005$) because AL numerically increased DMI compared with OG to produce similar yields of FCM, and the difference was greatest for cows with high pDMI (interaction $P = 0.006$, Table 52). Differences in efficiency between AL and OG might be associated with changes in body weight as AL increased and OG decreased body weight (6.05 vs. -3.78 kg over 18 d period, $P = 0.03$, Table 52) or different concentrations of concentrate in the diets.

Our results are not consistent with lower DMI and milk production for lactating dairy cows fed grass-based diets compared with cows fed legume-based diets (Oba and Allen, 1999; Steinshamn, 2010), but they are consistent with Voelker Linton and Allen (2008) who reported no treatment differences for mean milk yield and DMI for cows fed alfalfa or orchardgrass diets.

However, Voelker Linton and Allen (2008) found that testing overall means masked important intake differences; response of DMI to treatment varied for cows with different nutrient demands. Cows with low nutrient demand responded more positively to grass than legume, and cows with high nutrient demand responded more positively to legume than grass. These differences likely depended on the extent to which rumen fill limited feed intake of individual cows. We expected OG to be more filling than AL causing greater rumen distention and potentially limiting DMI, particularly in cows with high DMI for which ruminal distention is more likely to limit feed intake (Allen, 1996; Voelker Linton and Allen, 2008). Although the response of DMI to forage family was not related to pDMI in this experiment ($P \geq 0.21$), a visual examination of a graph with pDMI and DMI (Figure 27) illustrates that the greatest difference in DMI was for cows with high pDMI, which responded more positively to alfalfa than orchardgrass-based diets.

Alfalfa decreased pdNDF intake (3.43 vs. 4.89 kg/d) and increased iNDF intake (3.53 vs. 1.80 kg/d) compared with OG ($P < 0.001$, Table 53) because of differences in chemical composition of forages. Intake of iNDF was related to pDMI such that AL increased intake of iNDF at a faster rate than OG as pDMI increased (interaction $P = 0.05$, Figure 28A). Alfalfa increased rate of ruminal digestion of pdNDF (7.27 vs. 4.74%/h), rate of ruminal passage of pdNDF (2.29 vs. 1.32 %/h), and rate of ruminal passage of iNDF (3.27 vs. 2.52%/h) compared with OG ($P < 0.001$, Table 54). The faster passage rates for AL compared with OG were associated with greater rate of particle size reduction for AL compared with OG (7.16 vs. 4.67%/h, $P < 0.001$, Kammes and Allen, submitted). These ruminal kinetics resulted in shorter ruminal turnover times of pdNDF (10.9 vs. 17.4 h), iNDF (32.0 vs. 41.6 h), and DM (10.5 vs. 12.8 h) for AL than OG ($P < 0.001$, Table 54). Additionally, responses of iNDF ruminal passage

rate and turnover time to treatment were related to pDMI; as pDMI increased, AL increased rate of iNDF passage and OG decreased it (interaction $P = 0.09$, Figure 28B) and AL decreased iNDF turnover time and OG increased it (interaction $P = 0.06$, Figure 28C). The increased turnover time for iNDF for OG as feed intake increased is consistent with Voelker Linton and Allen (2008).

The aforementioned results contributed to the rumen pool sizes listed in Table 55. Despite the faster passage rate and turnover time of iNDF for AL, AL increased the rumen pool size of iNDF compared with OG (4.62 vs. 3.11 kg, $P < 0.001$) because of the greater intake of iNDF. Alfalfa decreased rumen pools of pdNDF (1.55 vs. 3.54 kg, $P < 0.001$) and DM (10.6 vs. 12.4 kg, $P = 0.001$) compared with OG because of lower pdNDF intake and faster rates of ruminal passage and digestion of pdNDF for AL than OG. Furthermore, AL decreased rumen digesta wet weight (82.7 vs. 92.4 kg, $P = 0.008$) and volume (98.5 and 108 L, $P = 0.01$) compared with OG. These ruminal pool sizes indicated that OG had greater filling effects than AL. The numerically lower feed intake for OG was accompanied by greater rumen pools suggesting rumen fill as a constraint limiting DMI for cows consuming OG is possible, but there was not statistically significant evidence in this experiment that ruminal distention is more likely to limit feed intake for cows with high intake compared with cows with low intake because we were unable to detect a treatment by pDMI interaction for DMI.

Effects of treatment on ruminal kinetics affected fiber digestion in the rumen (Table 53). Although rate of ruminal digestion of pdNDF was faster for AL than OG, AL decreased pdNDF digestion in the rumen compared with OG (2.47 vs. 3.70 kg/d, $P < 0.001$) because of lower concentration of pdNDF and shorter retention time in the rumen for AL than OG. As expected, AL decreased ruminal digestibility of NDF (37.7 vs. 57.2%, $P < 0.001$) compared with OG. As

pDMI increased, AL maintained relatively constant ruminal fiber digestibility but OG tended to increase or increased ruminal digestibilities of pdNDF (interaction $P = 0.13$, Figure 29A) and NDF (interaction $P = 0.08$, Figure 29B), respectively. Alfalfa decreased pdNDF flux to the duodenum (0.84 vs. 1.07 kg/d, $P = 0.02$) but increased NDF flux to the duodenum (4.26 vs. 2.89 kg/d, $P < 0.001$) compared with OG. The higher NDF flux for AL than OG is because of the greater concentration of iNDF and lower ruminal digestibility for AL than OG. As a result of increasing pdNDF ruminal digestibility for OG with greater feed intake, pdNDF flux to the duodenum decreased for OG as pDMI increased (interaction $P = 0.07$, Figure 29C) with the greatest difference between AL and OG for cows with low pDMI. Flux of NDF to the duodenum increased for AL as pDMI increased (interaction $P = 0.001$, Figure 29D) with the largest difference between treatments for cows with high pDMI, which is related to the greater increase in iNDF intake for AL as pDMI increased (Figure 28A).

Similar to pdNDF digestion in the rumen, AL decreased total tract digestion of pdNDF compared with OG (2.47 vs. 3.14 kg/d, $P < 0.001$). Despite greater ruminal digestion of pdNDF for OG, AL increased total tract digestibility of pdNDF compared with OG (72.0 vs. 64.7%, $P = 0.02$). Alfalfa decreased total tract digestibility of NDF compared with OG (35.5 vs. 47.1%, $P < 0.001$) because of the higher concentration of iNDF for AL than OG. Total tract digestibilities of NDF (and pdNDF) are lower than ruminal digestibility because negative post ruminal digestibilities were calculated for NDF (and pdNDF) in the present experiment. This is because of 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), which is the type used in this study, and the closed T-type duodenal cannula (Stensig and Robinson, 1997). 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. While absolute values are not biologically reasonable, relative comparisons between treatments within the same experiment are useful.

Different concentrate levels in the diets were necessary to account for changes in chemical composition of forages and maintain the same concentration of forage NDF in the diets. Alfalfa decreased starch intake (6.82 vs. 7.16 kg/d, $P = 0.05$, Table 56) and increased starch ruminal digestion rate (45.7 vs. 29.4%, $P = 0.001$, Table 54) compared with OG. This is consistent with the greater rate of ruminal turnover of starch (59.3 vs. 42.1%/h, $P = 0.005$, Table 54) and smaller rumen pool of starch (0.52 vs. 0.78 kg, $P = 0.002$, Table 55) for AL than OG. Although there was no difference in the amount of starch digested in the rumen per day, AL increased true ruminal starch digestibility (80.4 vs. 74.7%, $P = 0.03$) and decreased starch flux to the duodenum (1.55 vs. 2.02 kg/d, $P = 0.04$, Table 56) compared with OG because of lower intake and faster digestion rate of starch for AL than OG. Alfalfa tended to decrease postruminal starch digestibility (19.6 vs. 24.2%, $P = 0.07$) and decreased starch postruminally digested (1.33 vs. 1.74 kg/d, $P = 0.05$) compared with OG (Table 56). In the total tract, AL increased starch digestibility (97.0 vs. 95.6%, $P = 0.04$) but tended to decrease starch digestion (6.60 vs. 6.89 kg/d, $P = 0.08$) compared with OG (Table 56).

The mechanism by which AL increased ruminal starch digestion is unclear. It is possible that alfalfa promotes greater numbers or activity of starch-digesting bacteria in the rumen than orchardgrass. Because some starch-digesting bacteria (e.g. *Streptococcus bovis*) also have high

proteolytic activity (Russell et al., 1981), resulting in deamination of amino acids and production of ammonia, they might have contributed to the higher ruminal concentrations of isobutyrate (1.71 vs. 1.17 mM, $P < 0.001$), isovalerate (2.32 vs. 1.81 mM, $P = 0.01$), branch chained VFA (4.03 vs. 2.97, $P = 0.001$, Table 57), and ammonia (20.0 vs. 13.5 mg/dl, $P < 0.001$, Table 58) for AL compared with OG. Alfalfa silage, which had higher ammonia concentration than orchardgrass silage (Table 50), is another possible source for the increased ruminal ammonia observed in cows fed AL.

Alfalfa increased ruminal pH (6.07 vs. 5.90, $P = 0.001$, Table 57) compared with OG. Although we expected AL to have higher pH than OG because of lower starch intake, there was no difference in ruminal digestion of starch or OM (kg/d; Tables 56 and 59, respectively), and AL tended to increase total VFA concentration (149 vs. 146 mM, $P = 0.09$, Table 57). Additionally, rumen digesta mass was smaller for AL than OG (Table 55) potentially decreasing buffer capacity, and ruminating time per day was not different between AL and OG (Table 60) suggesting similar buffering through saliva secretion. The pH difference observed was likely because the buffering capacity of the rumen contents were greater for AL than OG; buffer capacity of legumes is greater than grasses (Jasaitis et al., 1987).

Although there was no effect of treatment on ruminating time per day, forage family affected eating time per day (Table 60). Alfalfa tended to increase eating time per day (295 vs. 271 min/d, $P = 0.10$) by increasing the number of meal bouts per day (10.3 vs. 8.96 meals/d, $P = 0.03$), with the greatest difference for cows with high pDMI (interaction $P = 0.10$, Figure 30A), and tending to decrease the interval between meals (131 vs. 152 min, $P = 0.09$). As pDMI increased, AL tended to increase the number of rumination bouts per day (interaction $P = 0.13$, Figure 30B) and decrease the interval between rumination bouts (interaction $P = 0.14$, Figure

30C), whereas the reverse was observed for OG. Alfalfa decreased ruminating time per unit of forage NDF consumed (78.4 vs. 84.7 min/kg forage NDF, $P = 0.02$, Table 60) compared with OG. This indicated that AL provided less effective fiber than orchardgrass.

As previously mentioned, AL increased and OG decreased body weight (Table 52). These body weight changes are consistent with numerically higher DMI for AL but similar FCM yield as OG; however, this occurred despite the tendency for lower plasma insulin concentrations for AL compared with OG (10.4 vs. 12.3 μIU, $P = 0.10$, Table 61). As pDMI increased, plasma concentrations of glucose ($P = 0.004$, Figure 31A), glucagon ($P = 0.02$, Figure 31B), and insulin ($P = 0.02$, Figure 31C) decreased independent of treatment.

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 impact 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. The passage rate of iNDF was related to pDMI as previously discussed, but passage rates of pdNDF and starch were not related to pDMI either independent of or dependent upon treatment (Table 54).

Effects of Treatment and pDMI on N Flux and Microbial Efficiency

Results for N metabolism are shown in Table 58. Alfalfa increased N intake compared with OG (711 vs. 635 g/d, $P = 0.001$) with alfalfa silage as the primary source of N for AL. As previously mentioned, AL increased ruminal ammonia concentration and tended to decrease NANMN flux expressed as percent of N intake (16.6 vs. 22.2% of N intake, $P = 0.10$) compared with OG indicating that protein was more rapidly degraded in the rumen for AL than OG. Ammonia N, NANMN, and NAN fluxes from the rumen to the duodenum were related to pDMI, but the response differed by treatment. As pDMI increased, AL increased flux of ammonia N (interaction $P = 0.05$, Figure 32A), NANMN (interaction $P = 0.09$, Figure 32B), and NAN (interaction $P = 0.02$, Figure 32C), whereas OG decreased ammonia N and NANMN fluxes and maintained relatively constant NAN flux across the range of pDMI. The NANMN interaction contributed to the treatment by pDMI interaction for NAN flux because level of intake did not have an effect on MN flux. These results are in contrast to those in a review by Clark et al. (1992) in which positive linear relationships between OM intake and fluxes of NAN, NANMN, and MN were reported as OM intake increased over a very wide range (3 to 23 kg/d) compared with this experiment.

Based on studies with continuous culture fermenters, increases in solid and liquid dilution rates, which can be associated with increased intake, resulted in greater microbial efficiency (Crawford et al., 1980; Shriver et al., 1986). In this experiment, microbial efficiency tended to be related to pDMI (interaction $P = 0.15$), but the response varied by treatment. Alfalfa slightly increased and OG quadratically affected microbial efficiency as pDMI increased (Figure 33). There was no relationship between microbial efficiency and true ruminally digested OM, which increased linearly with increasing levels of intake (Table 59) for cows consuming AL or OG (not

shown), indicating that factors other than availability of energy limited efficiency of MN production and energy from OM fermentation was uncoupled from microbial growth (Russell and Cook, 1995).

Microbial N flux from the rumen to the duodenum increased independent of treatment as pdNDF ruminal passage rate increased ($P = 0.02$, Figure 34A). Taylor and Allen (2005) reported a tendency for a positive correlation between MN flux and iNDF passage rate, but this relationship was not detected in the present experiment. Microbial efficiency increased as pdNDF ruminal passage rate increased for OG ($P = 0.02$, $R^2 = 0.42$) but not AL ($P = 0.15$, $R^2 = 0.18$; Figure 34B). This response indicates that energy from ruminal fermentation of OG was more efficiently utilized for microbial growth as passage rates for pdNDF increased. Others have reported that microbial efficiency was positively related to passage rates of particulate matter from the rumen including pdNDF (Voelker and Allen, 2003) and starch (Oba and Allen, 2003b; Voelker and Allen, 2003; Taylor and Allen, 2005) in experiments evaluating carbohydrate source, concentration, and fermentability of diets in dairy cattle. The greater passage rates of particulate digesta likely decrease microbial lysis and turnover in the rumen because microbial organisms flow from the rumen primarily attached to feed particles, resulting in improved efficiency.

CONCLUSIONS

Concentration of total NDF was lower but concentrations of iNDF, ADL, and CP were higher for alfalfa compared with orchardgrass. Forage family and its interaction with pDMI did not affect milk yield, milk composition, or DMI. Alfalfa decreased efficiency of milk production compared with OG because of numerically greater DMI for AL. Alfalfa increased ruminal pH,

digestion rates of pdNDF and starch, and passage rates of pdNDF and iNDF compared with OG, affecting ruminal digestibility. Passage rate of iNDF was related to pDMI such that AL increased iNDF passage rate and OG decreased it as pDMI increased. Alfalfa decreased rumen pools of pdNDF, starch, DM, and rumen digesta wet weight and volume compared with OG. Alfalfa decreased ruminating time per unit of forage NDF consumed compared with OG suggesting that alfalfa provided less effective fiber than orchardgrass. Ammonia N, NANMN, and NAN fluxes were increased by AL, but not OG, as pDMI increased. Microbial efficiency was positively related to pdNDF passage rate for OG, but not AL. When alfalfa or orchardgrass silage was the only source of forage in diets formulated to contain similar concentrations of forage NDF, forage family did not adversely affect productivity.

APPENDIX

Table 49. 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	Standard deviation	Minimum	Maximum
Parity	3	3.31	1.16	2	5
BW[1], kg	591	587	51	489	710
BCS	2.00	2.35	0.69	1.58	4.00
DIM	132	157	90	64	337
Milk, kg/d	41.4	41.5	10.8	22.6	57.1
3.5% FCM, kg/d	43.1	42.1	11.9	24.3	60.3
DMI, kg/d	26.7	25.9	3.0	19.6	29.5

[1]Empty BW (ruminal digesta removed).

Table 50. Chemical composition, particle size distribution, and fermentation parameters of alfalfa silage and orchardgrass silage included in the treatment diets

Item	Alfalfa	Orchardgrass
Chemical composition		
DM, %	43.5	33.7
OM, % DM	91.9	90.3
NDF, % DM	42.3	58.2
iNDF[1], % DM	23.0	16.1
iNDF, % of NDF	54.5	27.7
ADF, % of DM	35.0	36.4
ADL[2], % of DM	7.56	6.03
CP, % DM	22.5	11.4
Starch, % DM	1.87	1.37
NDF digestibility[3], %	38.3	53.3
Particles size distribution[4]		
Wet sieving, % DM retained		
19.0 mm	21.4	12.3
9.50 mm	18.0	18.4
4.75 mm	30.8	37.2
2.36 mm	17.0	21.2
1.18 mm	5.72	6.15
0.600 mm	3.09	2.08
0.300 mm	1.97	1.02
0.150 mm	1.16	0.94
0.075 mm	0.40	0.37
0.038 mm	0.50	0.37
Mean particle size[5], mm	11.6	9.66
Penn State Particle Separator, % DM retained		
> 19.0 mm	29.3	17.1
19.0 to 8.0 mm	48.5	50.2
< 8.0 mm	22.2	32.7
Fermentation		
pH	4.58	4.59
Acetic acid, % DM	2.38	0.90
Propionic acid, % DM	0.35	0.07
Butyric acid, % DM	<0.01	0.26
Lactic acid, % DM	5.94	6.10
Lactic:Acetic	2.49	6.78
Ethanol, % DM	0.33	<0.01
Ammonia, mM	4.65	2.86

[1]iNDF = indigestible NDF.

[2]ADL = acid detergent lignin.

[3]30 h in vitro NDF digestibility.

Table 50 (cont'd)

[4]Particle size distributions of silages were measured each period (n = 2).
[5]Mean particle size calculated from particle size distribution determined by wet sieving.

Table 51. Ingredients and chemical composition of preliminary and treatment diets (as analyzed) containing either alfalfa (AL) or orchardgrass (OG) silage as the sole source of forage

	Preliminary	AL	OG
Ingredients, % DM			
Alfalfa silage	30.0	59.9	---
Orchardgrass silage	21.5	---	42.7
Dry ground corn	36.2	33.6	36.6
Soybean meal (48% CP)	5.81	---	11.8
SoyPlus®	1.82	2.50	3.39
Vitamin mineral mix[1]	3.99	3.99	3.99
Urea	0.15	---	0.30
Limestone	0.60	---	1.20
Chemical composition			
DM, %	51.6	54.5	52.3
OM, % DM	92.4	92.7	91.1
NDF, % DM	29.1	29.2	30.2
% forage NDF	24.7	25.3	24.9
% NDF from forage	84.8	86.8	82.3
iNDF[2], % DM	NA[3]	14.8	8.24
iNDF, % of NDF	NA	50.7	27.3
CP, % DM	17.5	18.4	17.0
Starch, % DM	33.5	27.3	29.6

[1]Vitamin mineral mix contained (DM basis) 16.5% sodium bicarbonate, 14.2% magnesium sulfate, 7.1% salt, 5.8% dicalcium phosphate, 2.4% trace mineral premix, 0.4% vitamin A, 0.4% vitamin D, 0.2% vitamin E, and 53.1% dry ground corn as a carrier.

[2]iNDF = indigestible NDF.

[3]NA = no analysis for preliminary diet.

Table 52. Milk production and composition, feed intake, and BW change of cows fed treatment diets containing either alfalfa (AL) or orchardgrass (OG) silage as the sole source of forage

	Treatment LSM[1]			*P*[2]					
	AL	OG	SE	Trt	Trt x Period	pDMI	Trt x pDMI	pDMI x pDMI	Trt x pDMI x pDMI
Yield, kg/d									
Milk	35.1	35.2	2.3	0.92	0.14	0.06	NS[3]	NS	NS
FCM (3.5 %)	36.7	36.5	2.1	0.84	NS	0.02	NS	NS	NS
Milk fat	1.33	1.31	0.07	0.72	NS	0.007	NS	NS	NS
Milk protein	1.08	1.05	0.04	0.29	0.07	0.007	NS	NS	NS
Milk lactose	1.65	1.65	0.12	0.97	NS	0.16	NS	NS	NS
SNF	1.99	1.98	0.15	0.93	NS	0.16	NS	NS	NS
Milk composition, %									
Fat	3.79	3.77	0.09	0.63	0.04	0.16	NS	NS	NS
Protein	3.14	3.10	0.12	0.20	NS	NS	NS	NS	NS
Lactose	4.83	4.81	0.14	0.68	NS	0.28	NS	0.10	NS
SNF	5.82	5.80	0.17	0.63	NS	0.25	NS	0.11	NS
MUN, mg/dl	13.4	12.7	0.4	0.22	NS	NS	NS	NS	NS
DMI, kg/d	24.2	23.2	0.63	0.13	0.09	0.02	NS	NS	NS
3.5% FCM/ DMI	1.40	1.47	0.07	0.005	NS	0.06	0.006	NS	NS
BW change, kg/18 d	6.05	-3.78	3.29	0.03	NS	NS	NS	NS	NS
BCS change/18 d	-0.06	-0.13	0.04	0.28	NS	NS	NS	NS	NS

[1]Treatment least squares means.

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

[3]NS = not significant ($P > 0.20$); term removed from statistical model.

Table 53. NDF digestion of cows fed treatment diets containing either alfalfa (AL) or orchardgrass (OG) silage as the sole source of forage

	Treatment LSM[1]			*P*[2]					
	AL	OG	SE	Trt	Trt x Period	pDMI	Trt x pDMI	pDMI x pDMI	Trt x pDMI x pDMI
NDF									
Intake, kg/d	6.96	6.69	0.19	0.17	0.14	0.02	NS[3]	NS	NS
Ruminal digestion									
kg/d	2.47	3.70	0.15	<0.001	0.005	0.003	0.27	0.16	NS
%	37.7	57.2	1.7	<0.001	NS	0.44	0.08	NS	NS
Passage to duodenum, kg/d	4.26	2.89	0.26	<0.001	NS	0.44	0.001	0.84	0.13
Postruminal digestion									
kg/d	-0.08	-0.42	0.21	0.15	NS	0.10	0.02	0.26	0.19
Total tract digestion									
kg/d	2.47	3.14	0.10	<0.001	0.001	0.08	NS	NS	NS
%	35.5	47.1	1.43	<0.001	0.007	NS	NS	NS	NS
Potentially digestible NDF									
Intake, kg/d	3.43	4.89	0.11	<0.001	0.003	0.02	NS	NS	NS
Ruminal digestion									
kg/d	2.47	3.70	0.15	<0.001	0.005	0.003	NS	0.16	NS
%	76.2	78.2	2.6	0.40	NS	0.33	0.13	NS	NS
Passage to duodenum, kg/d	0.84	1.07	0.13	0.02	NS	0.64	0.07	NS	NS
Postruminal digestion									
kg/d	-0.08	-0.42	0.21	0.15	NS	0.10	0.02	0.26	0.19
Total tract digestion									
kg/d	2.47	3.14	0.10	<0.001	0.001	0.08	NS	NS	NS
%	72.0	64.7	2.1	0.02	NS	0.60	0.19	NS	NS
Indigestible NDF									
Intake, kg/d	3.53	1.80	0.08	<0.001	0.11	0.02	0.05	NS	NS

[1]Treatment least squares means.

Table 53 (cont'd)

[2]*P*-values for treatment (Trt), treatment by period interaction (Trt x Period), preliminary DMI (pDMI), treatment by preliminary DMI interaction (Trt x pDMI), quadratic effect of preliminary DMI (pDMI x pDMI), and treatment by quadratic effect of preliminary DMI (Trt x pDMI x pDMI).
[3]NS = not significant ($P > 0.20$); term removed from statistical model.

Table 54. Rumen kinetics of cows fed treatment diets containing either alfalfa (AL) or orchardgrass (OG) silage as the sole source of forage

	Treatment LSM[1]			*P*[2]					
	AL	OG	SE	Trt	Trt x Period	pDMI	Trt x pDMI	pDMI x pDMI	Trt x pDMI x pDMI
Ruminal turnover rate, %/h									
DM	9.76	8.06	0.41	<0.001	NS[3]	NS	NS	NS	NS
OM	10.0	8.15	0.43	<0.001	NS	NS	NS	NS	NS
NDF	4.80	4.35	0.23	0.02	NS	NS	NS	NS	NS
pdNDF[4]	9.56	6.06	0.51	<0.001	NS	NS	NS	NS	NS
Starch	59.3	42.1	3.7	0.005	NS	NS	NS	NS	NS
Ruminal turnover time, h									
DM	10.5	12.8	0.6	<0.001	NS	NS	NS	NS	NS
OM	10.2	12.7	0.6	<0.001	NS	NS	NS	NS	NS
NDF	21.4	23.9	1.2	0.01	NS	NS	NS	NS	NS
pdNDF	10.9	17.4	0.9	<0.001	NS	NS	NS	NS	NS
iNDF[5]	32.0	41.6	2.2	<0.001	NS	NS	0.06	NS	NS
Starch	1.82	2.59	0.16	0.003	NS	NS	NS	NS	NS
Ruminal passage rate, %/h									
pdNDF	2.29	1.32	0.27	<0.001	NS	NS	NS	NS	NS
iNDF	3.27	2.52	0.15	<0.001	0.17	0.48	0.09	NS	NS
Starch	13.6	12.7	2.0	0.60	NS	NS	NS	NS	NS
Ruminal digestion rate, %/h									
pdNDF	7.27	4.74	0.43	<0.001	NS	NS	NS	NS	NS
Starch	45.7	29.4	2.5	0.001	NS	NS	NS	NS	NS

[1]Treatment least squares means.

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

Table 54 (cont'd)

[3]NS = not significant ($P > 0.20$); term removed from statistical model.
[4]pdNDF = potentially digestible NDF.
[5]iNDF = indigestible NDF.

Table 55. Rumen pools of cows fed treatment diets containing either alfalfa (AL) or orchardgrass (OG) silage as the sole source of forage

	Treatment LSM[1]			*P*[2]					
	AL	OG	SE	Trt	Trt x Period	pDMI	Trt x pDMI	pDMI x pDMI	Trt x pDMI x pDMI
Wet weight, kg	82.7	92.4	3.6	0.008	0.14	0.16	NS[3]	NS	NS
Volume, L	98.5	108	3.6	0.01	NS	0.50	0.16	NS	NS
Density, kg/L	0.84	0.86	0.01	0.44	0.04	0.008	0.11	NS	NS
Rumen pool, kg									
DM	10.6	12.4	0.6	0.001	NS	NS	NS	NS	NS
OM	9.58	11.2	0.59	0.002	NS	NS	NS	NS	NS
NDF	6.19	6.67	0.38	0.06	NS	NS	NS	NS	NS
pdNDF[4]	1.55	3.54	0.20	<0.001	0.14	NS	NS	NS	NS
iNDF[5]	4.62	3.11	0.21	<0.001	NS	0.12	NS	NS	NS
Starch	0.52	0.78	0.06	0.002	NS	NS	NS	NS	NS

[1]Treatment least squares means.

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

[3]NS = not significant ($P > 0.20$); term removed from statistical model.

[4]pdNDF = potentially digestible NDF.

[5]iNDF = indigestible NDF.

Table 56. Starch digestion of cows fed treatment diets containing either alfalfa (AL) or orchardgrass (OG) silage as the sole source of forage

	Treatment LSM[1]			*P*[2]					
	AL	OG	SE	Trt	Trt x Period	pDMI	Trt x pDMI	pDMI x pDMI	Trt x pDMI x pDMI
Starch									
Intake, kg/d	6.82	7.16	0.17	0.05	0.08	0.02	0.13	NS[3]	NS
Apparent ruminal digestion									
kg/d	5.27	5.14	0.22	0.54	0.12	0.06	NS	NS	NS
%	77.3	72.0	2.8	0.05	NS	NS	NS	NS	NS
True ruminal digestion									
kg/d	5.48	5.33	0.23	0.50	0.13	0.06	NS	NS	NS
%	80.4	74.7	2.8	0.03	NS	NS	NS	NS	NS
Passage to duodenum, kg/d	1.55	2.02	0.21	0.04	NS	NS	NS	NS	NS
Apparent postruminal digestion									
kg/d	1.33	1.74	0.20	0.05	NS	NS	NS	NS	NS
% of intake	19.6	24.2	2.7	0.07	NS	NS	NS	NS	NS
% of duodenal passage	84.9	84.2	1.9	0.67	NS	NS	NS	NS	NS
Apparent total tract digestion									
kg/d	6.60	6.89	0.16	0.08	0.10	0.02	0.14	NS	NS
%	97.0	95.6	0.52	0.04	NS	0.93	0.14	0.55	0.17

[1]Treatment least squares means.

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

[3]NS = not significant ($P > 0.20$); term removed from statistical model.

Table 57. Ruminal VFA concentrations and pH of cows fed treatment diets containing either alfalfa (AL) or orchardgrass (OG) silage as the sole source of forage

	Treatment LSM[1]			*P*[2]					
	AL	OG	SE	Trt	Trt x Period	pDMI	Trt x pDMI	pDMI x pDMI	Trt x pDMI x pDMI
Total VFA, mM	149	146	3	0.09	NS[3]	NS	NS	NS	NS
Acetate	90.8	91.5	1.2	0.59	NS	NS	NS	NS	NS
Propionate	31.5	29.0	2.2	0.05	NS	0.93	0.15	0.22	0.03
Butyrate	18.6	17.9	1.0	0.20	NS	0.78	0.58	0.96	0.16
Lactate	0.125	<0.001	0.110	0.30	NS	0.17	NS	0.04	NS
Isobutyrate	1.71	1.17	0.06	<0.001	NS	NS	NS	NS	NS
Valerate	2.52	1.65	0.14	<0.001	NS	0.68	NS	0.18	NS
Isovalerate	2.32	1.81	0.11	0.01	NS	NS	NS	NS	NS
Branch chain VFA	4.03	2.97	0.15	0.001	NS	NS	NS	NS	NS
Acetate:Propionate	2.92	3.19	0.16	0.03	NS	0.92	0.25	0.19	0.07
Ruminal pH	6.07	5.90	0.05	0.001	NS	NS	NS	NS	NS

[1]Treatment least squares means.

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

[3]NS = not significant ($P > 0.20$); term removed from statistical model.

Table 58. Nitrogen metabolism of cows fed treatment diets containing either alfalfa (AL) or orchardgrass (OG) silage as the sole source of forage

	Treatment LSM[1]			*P*[2]					
	AL	OG	SE	Trt	Trt x Period	pDMI	Trt x pDMI	pDMI x pDMI	Trt x pDMI x pDMI
N intake, g/d	711	635	18	0.001	0.16	0.02	0.17	NS[3]	NS
Ruminal ammonia, mg/dl	20.0	13.5	0.7	<0.001	NS	0.18	NS	NS	NS
Flow to duodenum									
Ammonia N, g/d	18.4	14.9	1.3	0.03	NS	0.24	0.05	NS	NS
NAN									
g/d	556	591	45	0.26	NS	0.55	0.02	0.85	0.14
% of N intake	79.1	89.5	3.6	0.01	NS	NS	NS	NS	NS
NANMN[4]									
g/d	122	139	21	0.41	NS	0.17	0.09	0.08	NS
% of N intake	16.6	22.2	2.8	0.10	NS	0.30	0.15	0.04	NS
% of duodenal NAN	21.1	24.5	3.1	0.32	NS	0.18	0.19	0.03	NS
Microbial N									
g/d	433	454	41	0.54	NS	0.96	0.25	0.26	0.16
% of duodenal NAN	78.9	75.5	3	0.32	NS	0.18	0.19	0.03	NS
g/kg TRDOM[5]	30.7	34.7	3.0	0.22	0.16	0.21	0.20	0.14	0.15
NAN apparent postruminal digestion									
g/d	319	361	40	0.22	NS	0.76	0.008	0.53	0.04
% of N intake	44.0	56.4	5.2	0.03	NS	0.31	0.02	0.47	0.04
% of duodenal passage	55.0	60.4	2.8	0.08	NS	0.10	0.002	0.31	0.004
N apparent total tract digestion									
g/d	455	396	17	0.02	0.02	0.12	0.02	0.84	0.06
%	65.6	63.0	1.2	0.18	0.02	0.02	0.009	0.15	0.01

[1]Treatment least squares means.

Table 58 (cont'd)

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

[3]NS = not significant ($P > 0.20$); term removed from statistical model.

[4]NANMN = nonammonia, nonmicrobial nitrogen.

[5]TRDOM = true ruminally digested OM.

Table 59. DM and OM digestion of cows fed treatment diets containing either alfalfa (AL) or orchardgrass (OG) silage as the sole source of forage

	Treatment LSM[1]			*P*[2]					
	AL	OG	SE	Trt	Trt x Period	pDMI	Trt x pDMI	pDMI x pDMI	Trt x pDMI x pDMI
DM									
Intake, kg/d	24.2	23.2	0.6	0.13	0.09	0.02	NS[3]	NS	NS
Apparent total tract digestion									
kg/d	15.7	15.0	0.4	0.20	0.003	0.03	0.13	NS	NS
%	64.5	66.8	1.1	0.18	0.005	0.05	0.05	0.16	0.07
OM									
Intake, kg/d	22.5	21.2	0.6	0.04	0.08	0.02	NS	NS	NS
Apparent ruminal digestion									
kg/d	9.49	9.19	0.6	0.64	0.006	0.01	NS	0.09	NS
%	44.9	42.6	2.8	0.44	0.09	0.27	0.06	0.28	0.12
True ruminal digestion									
kg/d	14.4	13.8	0.5	0.35	0.01	0.009	NS	NS	NS
%	64.1	65.1	1.5	0.55	0.15	0.43	0.09	NS	NS
Passage to duodenum, kg/d	12.1	12.0	0.8	0.85	NS	0.51	0.01	0.74	0.06
Apparent postruminal digestion									
kg/d	4.56	5.33	0.60	0.20	NS	0.35	0.005	0.19	0.01
% of intake	20.1	25.0	2.6	0.12	NS	0.10	0.01	0.14	0.03
Apparent total tract digestion									
kg/d	14.8	14.0	0.4	0.12	0.004	0.03	0.12	NS	NS
%	65.5	68.2	1.1	0.10	0.009	0.06	0.05	0.16	0.08

[1]Treatment least squares means.

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

Table 59 (cont'd)

[3]NS = not significant ($P > 0.20$); term removed from statistical model.

Table 60. Chewing activity of cows fed treatment diets containing either alfalfa (AL) or orchardgrass (OG) silage as the sole source of forage

	Treatment LSM[1]			*P*[2]					
	AL	OG	SE	Trt	Trt x Period	pDMI	Trt x pDMI	pDMI x pDMI	Trt x pDMI x pDMI
Meals									
Bouts/d	10.3	8.96	0.49	0.03	NS[3]	0.11	0.10	NS	NS
Length, min/bout	29.1	30.7	1.6	0.50	NS	0.02	NS	NS	NS
Interval, min	131	152	10	0.09	NS	NS	NS	NS	NS
Meal size, kg									
DM	2.44	2.63	0.13	0.35	0.03	NS	NS	NS	NS
OM	2.26	2.40	0.12	0.46	0.03	NS	NS	NS	NS
NDF	0.70	0.76	0.04	0.34	0.03	NS	NS	NS	NS
pdNDF[4]	0.35	0.55	0.02	<0.001	0.003	NS	NS	NS	NS
iNDF[5]	0.35	0.20	0.02	<0.001	NS	NS	NS	NS	NS
Starch	0.69	0.81	0.04	0.05	0.04	NS	NS	NS	NS
Eating time									
Min/d	295	271	14	0.10	0.20	NS	NS	NS	NS
Min/kg DMI	12.4	11.9	0.6	0.39	0.05	0.04	NS	NS	NS
Min/kg NDF intake	43.0	41.3	2.1	0.40	0.06	0.04	NS	NS	NS
Min/kg forage NDF intake	48.7	47.8	2.5	0.69	0.06	0.04	NS	NS	NS
Rumination									
Bouts/d	13.8	13.6	0.8	0.71	NS	0.51	0.13	0.14	NS
Length, min/bout	33.3	34.3	1.5	0.33	NS	NS	NS	NS	NS
Interval, min	66.1	60.7	3.9	0.25	NS	0.60	0.14	0.11	NS
Ruminating time									
Min/d	477	484	14	0.67	NS	NS	NS	NS	NS
Min/kg DMI	19.8	21.0	0.8	0.07	NS	0.10	NS	NS	NS
Min/kg NDF intake	69.2	73.3	2.7	0.09	NS	0.08	NS	NS	NS
Min/kg forage NDF intake	78.4	84.7	3.0	0.02	NS	0.09	NS	NS	NS

Table 60 (cont'd)

Total chewing time									
Min/d	771	755	21	0.51	NS	0.54	0.19	NS	NS
Min/kg DMI	32.3	33.0	1.1	0.44	0.05	0.02	NS	NS	NS
Min/kg NDF intake	112	115	4	0.47	0.08	0.02	NS	NS	NS
Min/kg forage NDF intake	127	133	4	0.15	0.07	0.02	NS	NS	NS

[1]Treatment least squares means.

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

[3]NS = not significant ($P > 0.20$); term removed from statistical model.

[4]pdNDF = potentially digestible NDF.

[5]iNDF = indigestible NDF.

Table 61. Plasma metabolites of cows fed treatment diets containing either alfalfa (AL) or orchardgrass (OG) silage as the sole source of forage

	Treatment LSM[1]			*P*[2]					
	AL	OG	SE	Trt	Trt x Period	pDMI	Trt x pDMI	pDMI x pDMI	Trt x pDMI x pDMI
Glucose, mg/dl	58.2	60.5	0.6	0.004	NS[3]	0.004	NS	NS	NS
Glucagon, pg/ml	144	164	3	<0.001	NS	0.02	NS	NS	NS
Insulin, uIU/ml	10.4	12.3	1.0	0.10	0.10	0.02	NS	NS	NS

[1]Treatment least squares means.

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

[3]NS = not significant ($P > 0.20$); term removed from statistical model.

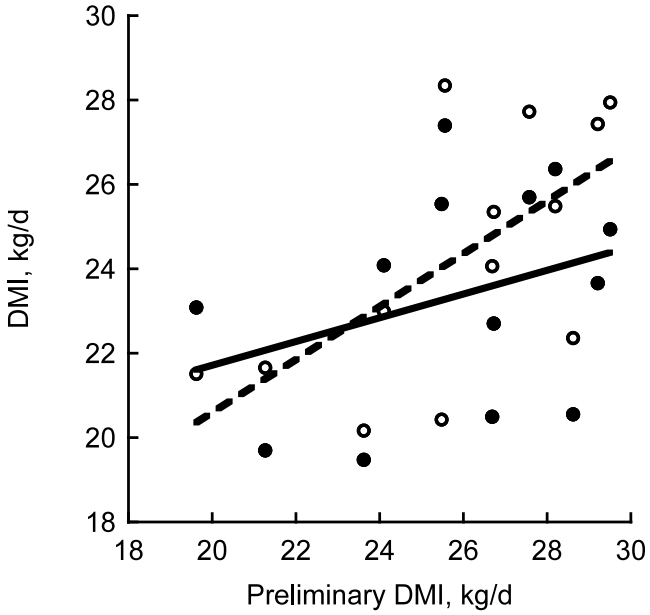

Figure 27. Interaction of alfalfa (open circles, dashed line) and orchardgrass (closed circles, solid line) with preliminary DMI for DMI (interaction not significant). The preliminary DMI on the x-axis are the mean DMI of individual cows during the final 4 d of the preliminary period when all cows were fed a common diet.

Figure 28

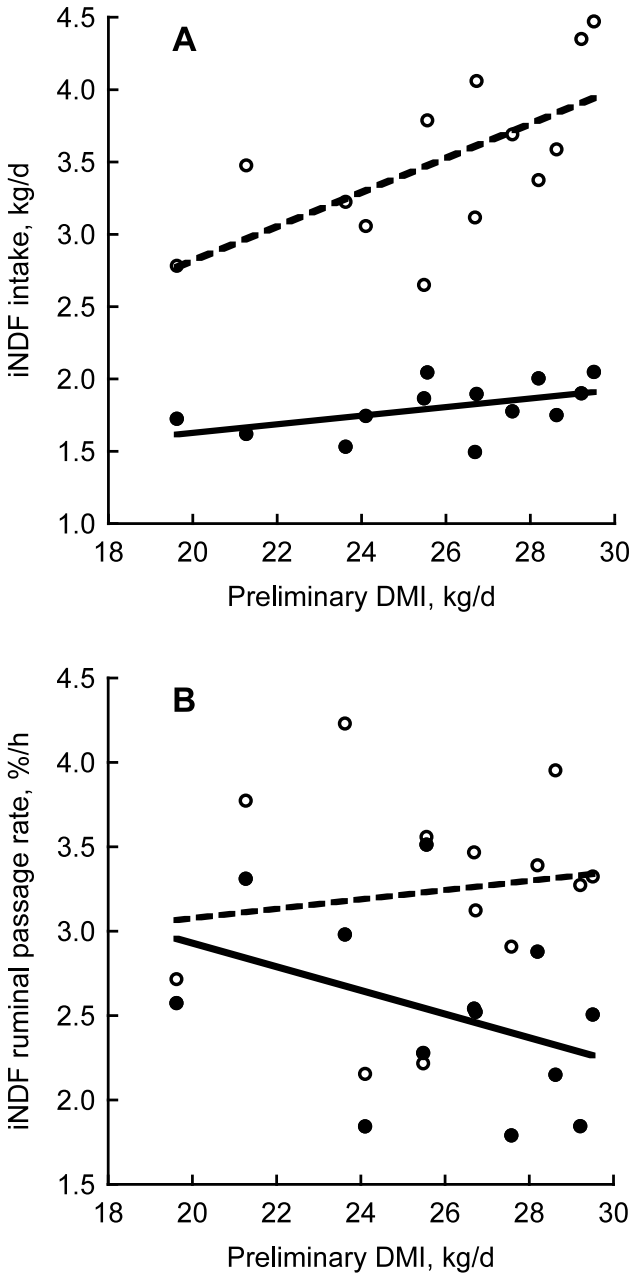
A
iNDF intake, kg/d
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
18
20
22
24
26
28
30
Preliminary DMI, kg/d
B
iNDF ruminal passage rate, %/h
4.5
4.0
3.5
3.0
2.5
2.0
1.5
18
20
22
24
26
28
30
Preliminary DMI, kg/d

Figure 28 (cont'd)

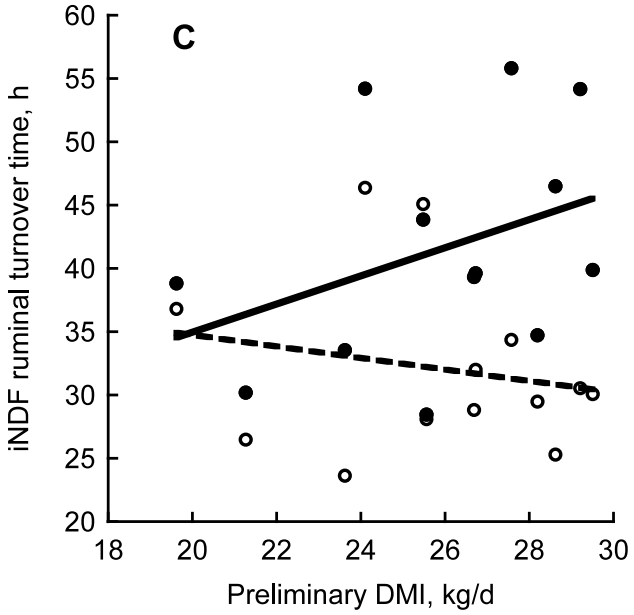

Figure 28. Interaction of alfalfa (open circles, dashed line) and orchardgrass (closed circles, solid line) with preliminary DMI for indigestible NDF (iNDF) A) intake ($P = 0.05$), B) ruminal passage rate ($P = 0.09$), and C) ruminal turnover time ($P = 0.06$). The preliminary DMI on the x-axis are the mean DMI of individual cows during the final 4 d of the preliminary period when all cows were fed a common diet.

Figure 29

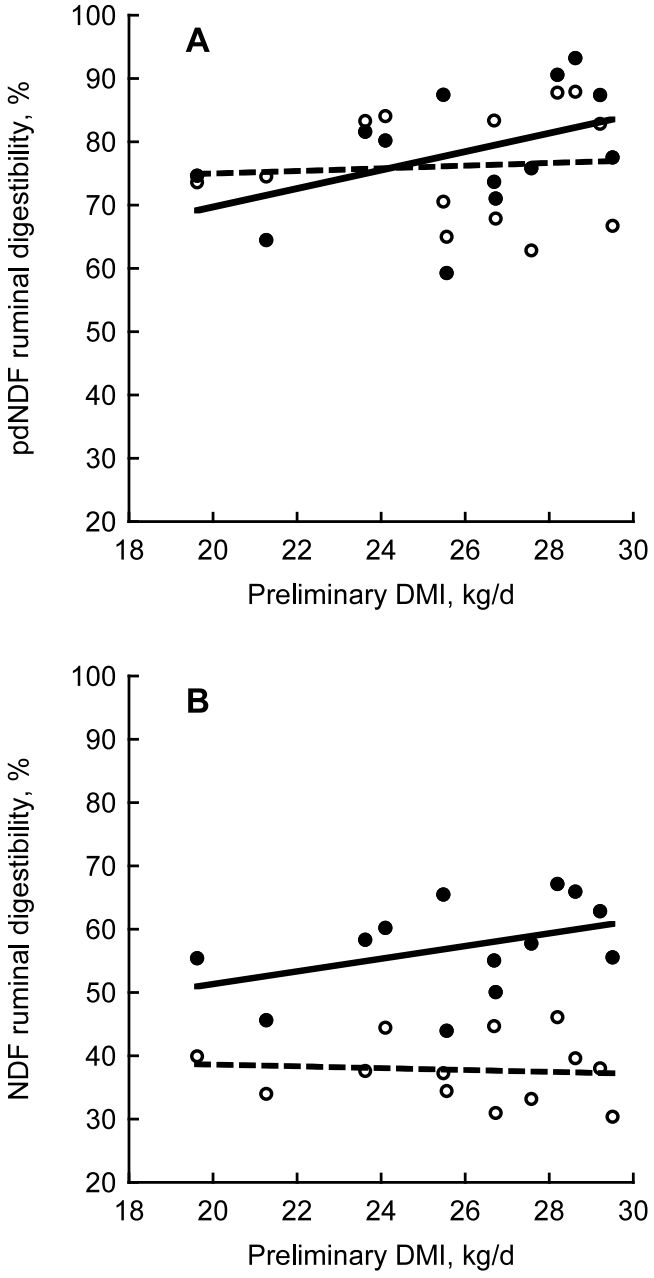
A
pdNDF ruminal digestibility, %
100
90
80
70
60
50
40
30
20
18
20
22
24
26
28
30
Preliminary DMI, kg/d
B
NDF ruminal digestibility, %
100
90
80
70
60
50
40
30
20
18
20
22
24
26
28
30
Preliminary DMI, kg/d

Figure 29 (cont'd)

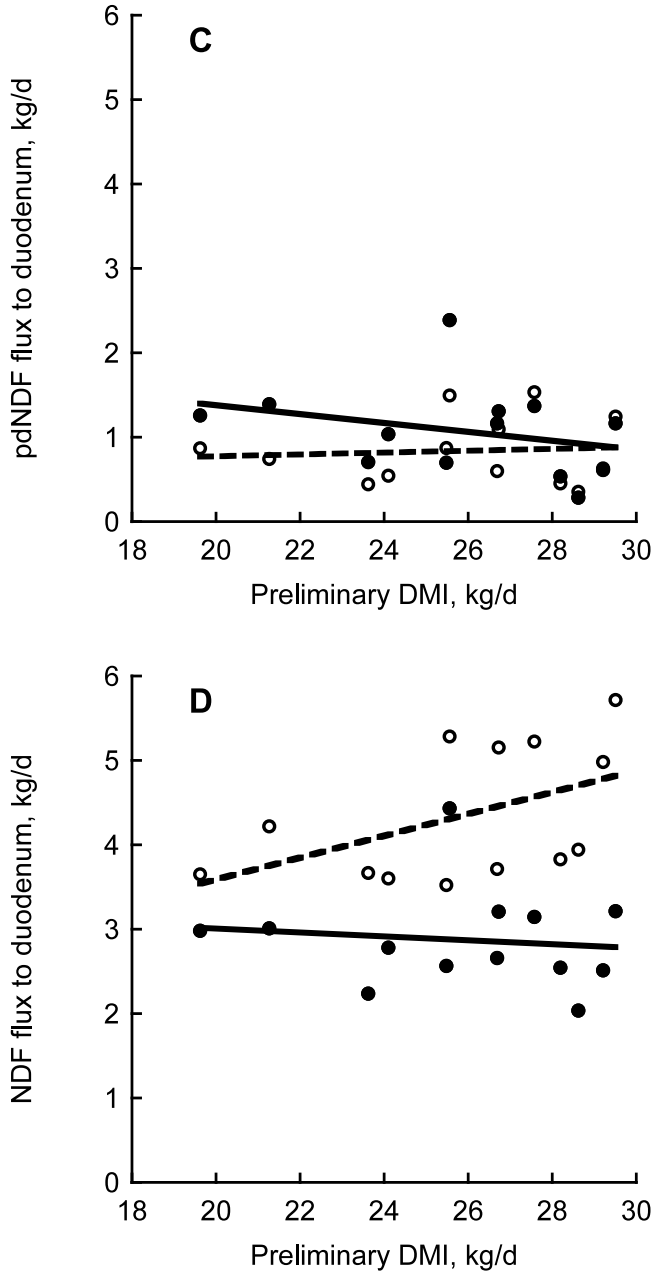

Figure 29. Interaction of alfalfa (open circles, dashed line) and orchardgrass (closed circles, solid line) with preliminary DMI for A) ruminal digestibility of potentially digestible NDF (pdNDF; $P = 0.13$), B) ruminal digestibility of NDF ($P = 0.08$), C) pdNDF flux to duodenum ($P = 0.07$), and D) NDF flux to duodenum ($P = 0.001$). 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.

Figure 30

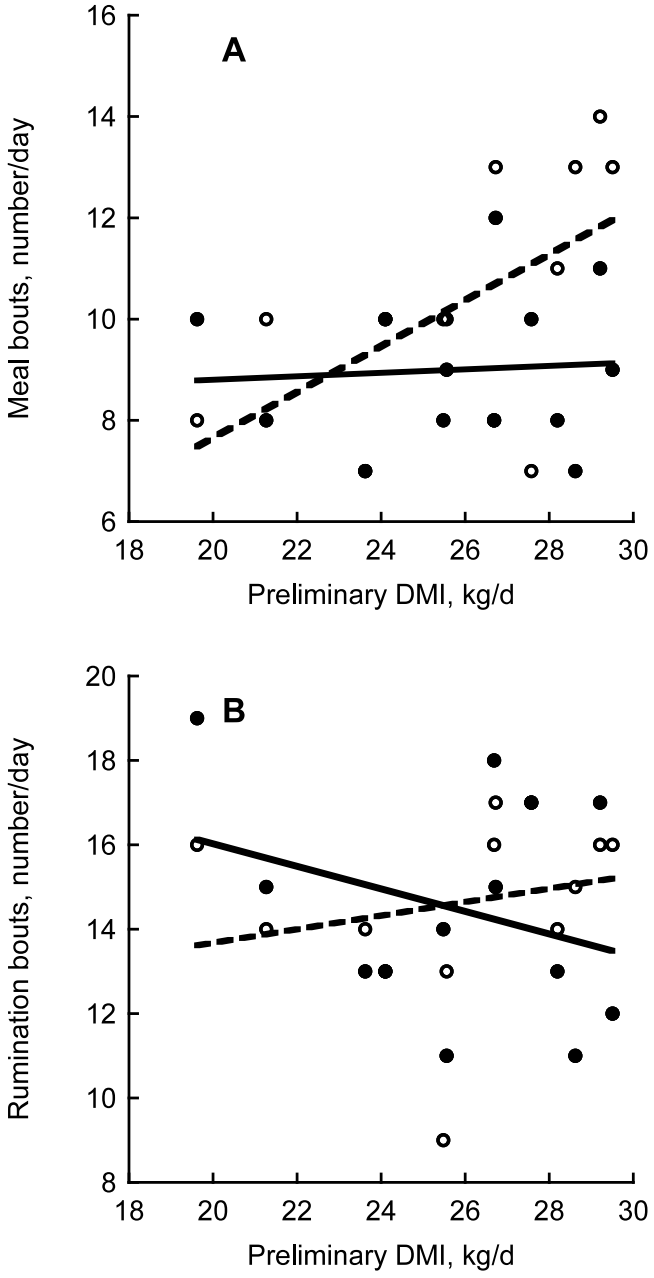
A
Meal bouts, number/day
16
14
12
10
8
6
18
20
22
24
26
28
30
Preliminary DMI, kg/d
B
Rumination bouts, number/day
20
18
16
14
12
10
8
18
20
22
24
26
28
30
Preliminary DMI, kg/d

Figure 30 (cont'd)

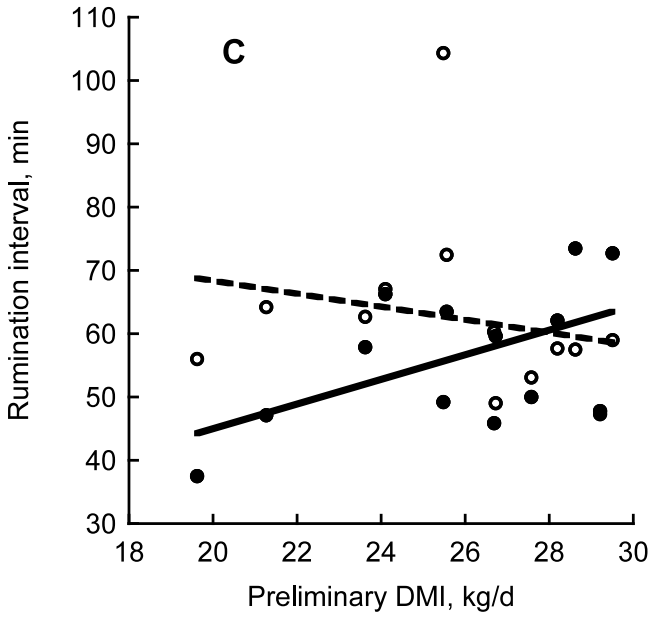

Figure 30. Interaction of alfalfa (open circles, dashed line) and orchardgrass (closed circles, solid line) with preliminary DMI for A) meal bouts ($P = 0.10$), B) rumination bouts ($P = 0.13$), and C) rumination interval ($P = 0.14$). The preliminary DMI on the x-axis are the mean DMI of individual cows during the final 4 d of the preliminary period when all cows were fed a common diet.

Figure 31

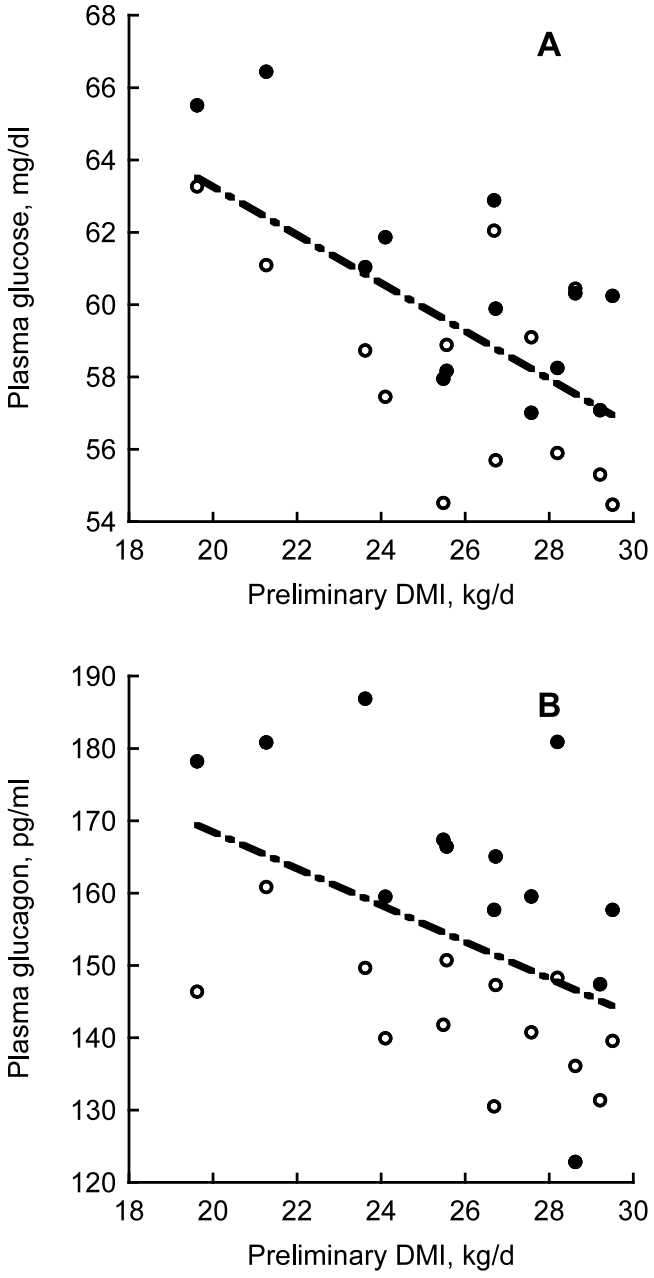
A
Plasma glucose, mg/dl
68
66
64
62
60
58
56
54
18
20
22
24
26
28
30
Preliminary DMI, kg/d
B
Plasma glucagon, pg/ml
190
180
170
160
150
140
130
120
18
20
22
24
26
28
30
Preliminary DMI, kg/d

Figure 31 (cont'd)

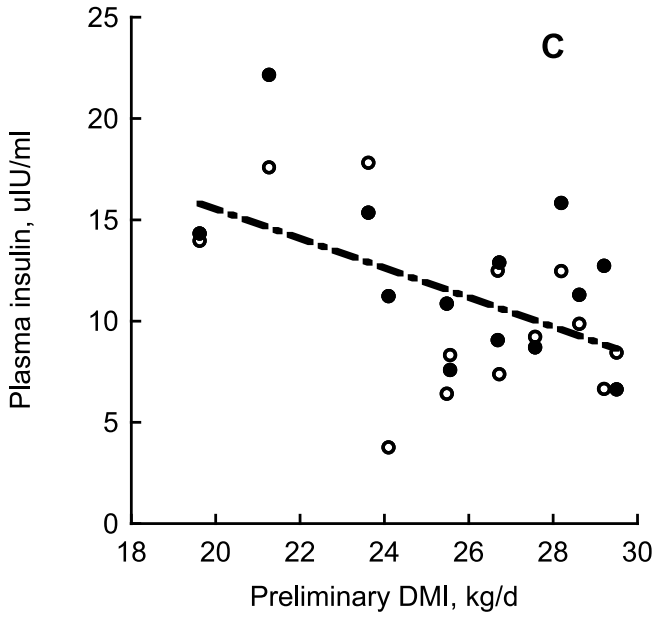

Figure 31. Relationship of alfalfa (open circles) and orchardgrass (closed circles) with preliminary DMI for concentrations of plasma A) glucose ($P = 0.004$), B) glucagon ($P = 0.02$), and C) insulin ($P = 0.02$). The preliminary DMI on the x-axis are the mean DMI of individual cows during the final 4 d of the preliminary period when all cows were fed a common diet.

Figure 32

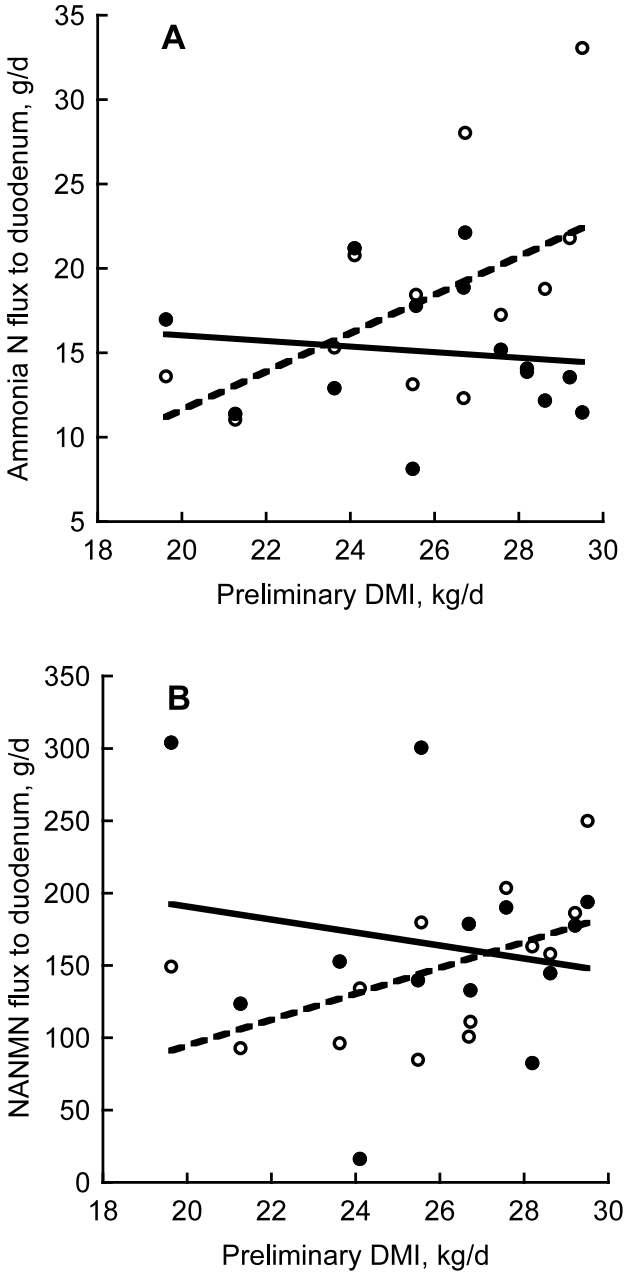
A
Ammonia N flux to duodenum, g/d
35
30
25
20
15
10
5
18
20
22
24
26
28
30
Preliminary DMI, kg/d
B
NANMN flux to duodenum, g/d
350
300
250
200
150
100
50
0
18
20
22
24
26
28
30
Preliminary DMI, kg/d

Figure 32 (cont'd)

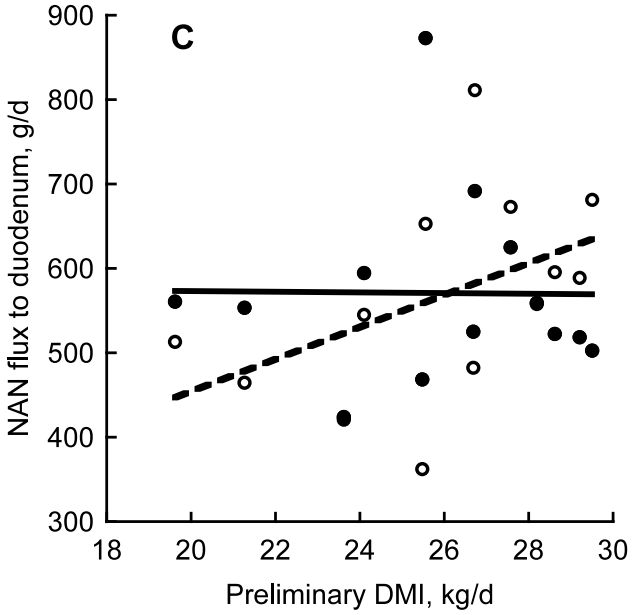

Figure 32. Interaction of alfalfa (open circles, dashed line) and orchardgrass (closed circles, solid line) with preliminary DMI for A) ammonia N ($P = 0.05$), B) nonammonia, nonmicrobial N (NANMN; $P = 0.09$), and C) NAN ($P = 0.02$) flux to the duodenum. The preliminary DMI on the x-axis are the mean DMI of individual cows during the final 4 d of the preliminary period when all cows were fed a common diet.

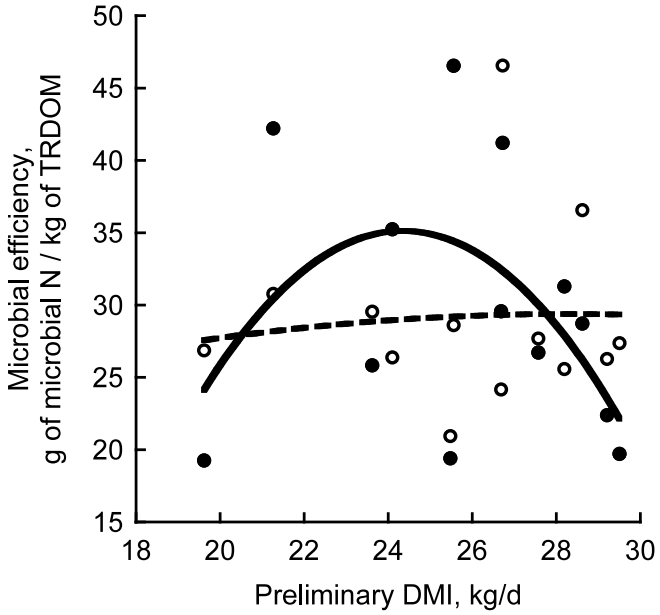

Figure 33. Interaction of alfalfa (open circles, dashed line) and orchardgrass (closed circles, solid line) with preliminary DMI for microbial efficiency ($P = 0.15$) expressed as gram of microbial N produced per kilogram of true ruminally digested OM (TRDOM). The preliminary DMI on the x-axis are the mean DMI of individual cows during the final 4 d of the preliminary period when all cows were fed a common diet.

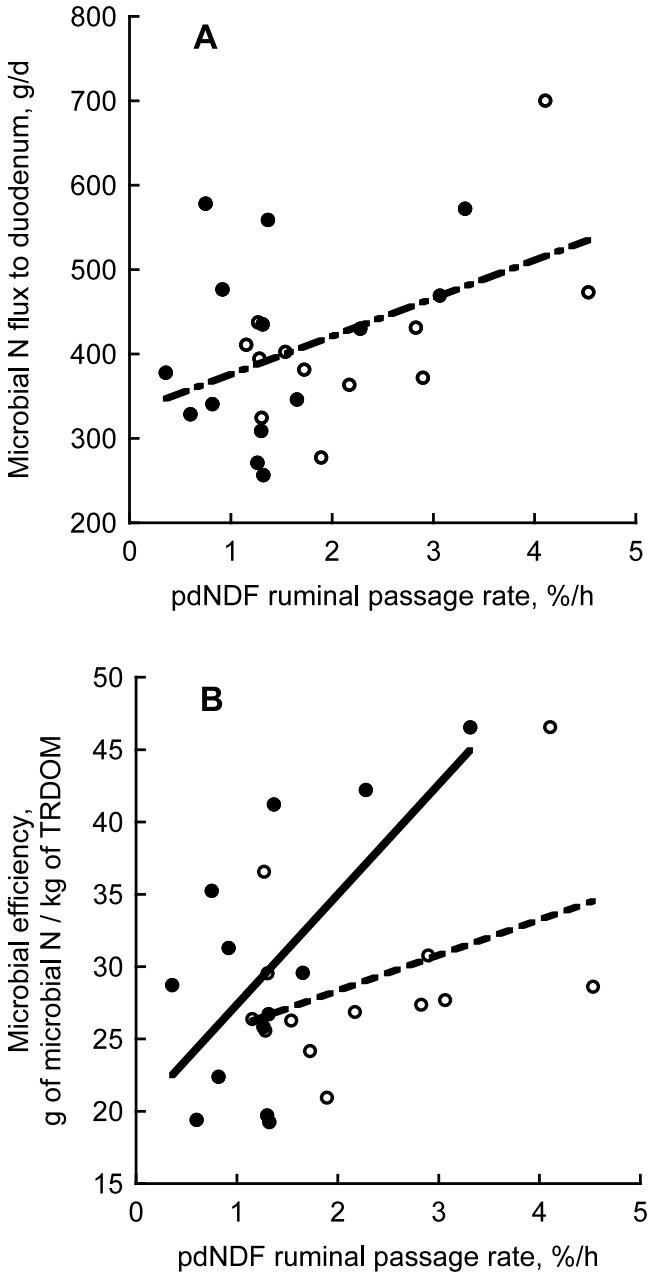

Figure 34. A) Relationship between potentially digestible NDF (pdNDF) ruminal passage rate and microbial N flux to duodenum ($P = 0.02$, $R^2 = 0.21$). B) Relationship between pdNDF ruminal passage rate and microbial efficiency for alfalfa ($P = 0.15$, $R^2 = 0.18$) and orchardgrass (microbial efficiency, g of microbial N/kg of true ruminally digested OM (TRDOM) = 19.8 + 7.60 x pdNDF ruminal passage rate, %/h; $P = 0.02$, $R^2 = 0.42$). Open circles and dashed line denote alfalfa, and closed circles and solid line denote orchardgrass.

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analysis of particle sizes of forage and total mixed rations. J. Dairy Sci. 79:922-928.

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CHAPTER 7

Rates of Particle Size Reduction and Passage are Faster for Legume Compared to Cool-Season Grass Resulting in Lower Rumen Fill and Less Effective Fiber[1]

ABSTRACT

Effects of forage family on rates of particle size reduction in, and passage from, the rumen 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 18-d treatment periods. During the preliminary period, pDMI of individual cows ranged from 19.6 to 29.5 kg/d (mean = 25.9 kg/d) and 3.5% fat-corrected milk yield ranged from 24.3 to 60.3 kg/d (mean = 42.1 kg/d). Experimental treatments were diets containing either a) alfalfa silage (AL) or b) orchardgrass silage (OG) as the sole forage. Silages were chopped to 10 mm theoretical length of cut and contained 42.3 and 58.2% neutral detergent fiber (NDF) for alfalfa and orchardgrass, respectively. Both diets contained ~25% forage NDF and ~30% total NDF. Feed, orts, rumen, and duodenal samples were wet sieved to fractionate particles above (large, L) and below (small, S) 2.36 mm. Indigestible NDF (iNDF) was used as a flow marker. 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 forage family and their interaction with pDMI were tested by ANOVA. Cows consumed ~75% NDF L and 25% NDF S for both treatments, but AL consumed more iNDF and less potentially digestible NDF (pdNDF) than OG. Alfalfa increased the rate of reduction (L to S) compared with OG despite less rumination per unit of forage NDF for AL than OG suggesting

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alfalfa NDF was more fragile than orchardgrass NDF. Over 55% of particles in the rumen were below 2.36 mm (S) for AL and OG indicating that particle size was not a limiting constraint to passage. Passage rates (k_p) of iNDF L and pdNDF L were similar for AL and OG, but AL increased k_p of pdNDF L and OG decreased it as pDMI increased. Alfalfa increased k_p of iNDF S and pdNDF S compared with OG resulting in lower rumen fill for AL than OG. The k_p of iNDF S and pdNDF S were similar within forage family suggesting buoyancy was not limiting passage. Orchardgrass increased rumen pool size of NDF L compared with AL, which likely retained NDF S contributing to the slower k_p of iNDF S and pdNDF S observed for OG. Particle size reduction was a prerequisite to ruminal passage but not a constraint. Selective retention of S particles was less for alfalfa than orchardgrass resulting in lower rumen fill and less effective fiber.

INTRODUCTION

Passage of digesta from the rumen is a complicated, dynamic process and is inversely related to the extent of digestion within the rumen. It involves the selective retention of undigested fiber, which allows ruminants to increase ruminal fiber digestion but extended ruminal retention times of the retained fiber can reduce DMI from ruminal distention. Most models used to predict feed intake and digestion do not include selective retention because it is not fully understood and difficult to measure. This implies that all particles have equal probability of escape from the rumen. However, escapable (i.e., small) and nonescapable (i.e., large) particles have different passage rates (Allen and Mertens, 1988; Voelker Linton and Allen, 2008). Additionally, heterogeneous feed fractions such as NDF, which includes indigestible

NDF (iNDF) and potentially digestible NDF (pdNDF), have different ruminal kinetics (Stensig and Robinson, 1997).

Several factors contribute to the decreased probability of escape of digesta from the rumen. Particle size influences passage from the rumen but is not always a constraint because a large proportion of the particles retained in the rumen are smaller than the maximum particle size in the feces (Allen, 1996). Furthermore, particle density and buoyancy (Jung and Allen, 1995) and sequestration of small particles within the fibrous rumen mat (Sutherland, 1988) affect passage of particles from the rumen. Passage rate of fibrous particles depends on the reduction of particle size and the increase in particle specific gravity to permit particles to escape the rumen mat, sink to the ventral rumen, and exit the rumen via the reticular-omasal orifice (Sutherland, 1988).

Digesta passage from the rumen is impacted by numerous feed and animal factors. Legumes and grasses have different ruminal kinetic parameters (Voelker Linton and Allen, 2008; Bayat et al., 2010; Krizsan et al. 2010) and increases in DMI result in a decrease in the percentage of small particles in the rumen (Okine and Mathison, 1991). Therefore, the effects of forage family and level of feed intake on ruminal passage rates are of interest in this study. Alfalfa (*Medicago sativa*) and orchardgrass (*Dactylis glomerata L.*) were selected as a representative legume and cool-season grass, respectively, and the use of preliminary DMI (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. Additionally, ruminal passage rates of individual digesta fractions, instead of entire feeds, were measured using ruminally and duodenally cannulated cows and the pool and flux method (Robinson et al., 1987).

We hypothesized that rates of particle size reduction in, and particle passage from, the rumen are faster for legumes than grasses and the rate of particle passage from the rumen increases for legumes and grasses as DMI increases. The objective of this experiment was to evaluate the relationships between voluntary DMI and effects of forage family on rates of particle size reduction in, and particle passage from, the rumen. This was accomplished using a model that fractionated the pool of NDF in the rumen into iNDF and pdNDF as well as large and small particle size pools. The rate of reduction for iNDF from large particles to small particles and individual rates of passage for each of the 4 fractions were calculated.

MATERIALS AND METHODS

This article is the second of a set of two from one experiment that evaluated the effects of forage family and its interaction with level of feed intake (nutrient demand). This article focuses on rates of particle size breakdown in, and particle passage from, the rumen. The companion article discusses the effect of pDMI on responses to treatment for production, rumen parameters, digestion kinetics, and chewing activity.

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 18-d experimental periods. The first 10 d of each period were allowed for diet adaptation and samples were collected during the final 4 d of the preliminary period and 8 d of each experimental period. Cows were 157±90 (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 19.6 to 29.5 kg/d (mean = 25.9 kg/d) and 3.5% FCM yield ranged from 24.3 to 60.3 kg/d (mean = 42.1 kg/d; Table 62). 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 either a) alfalfa silage (AL) or b) orchardgrass silage (OG) as the sole forage. Alfalfa (Pioneer 54H91, Pioneer Hi-Bred, Johnston, IA) and orchardgrass (Baridana cultivar, Barenbrug USA, Tangent, OR) forages were produced at the campus farm at Michigan State University (East Lansing), chopped to 10 mm theoretical length of cut, and ensiled in Ag-Bags (Ag-Bag Systems Inc., St. Nazianz, WI). During the sample collection periods, alfalfa and orchardgrass contained 42.3 and 58.2% NDF and 22.5 and 11.4% CP, respectively (DM basis; Table 63). Diets AL and OG were formulated to contain 25% forage NDF, 30% total NDF, and 18% CP. We acknowledge these treatments affect dietary starch concentration but maintaining similar forage and total NDF concentrations for both treatments was of primary interest. The diet fed during the preliminary period was formulated so that alfalfa and orchardgrass each contributed 50% of forage NDF. Diets also contained dry ground corn, SoyPlus® (West Central Soy, Ralston, IA), and vitamin-mineral premix (Table 64); soybean meal (48% CP), urea, and limestone were used to compensate for lower CP and Ca concentrations in orchardgrass silage than in alfalfa silage.

Data and Sample Collection

Throughout the experiment, cows were housed in tie-stalls, milked in a parlor twice daily

(0400 and 1430 h), and fed diets as total mixed rations once daily (1130 h) at 110% of expected intake. The amount of feed offered and refused (orts) was 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 and TMR (0.5 kg) and orts (12.5%) were collected daily from d 11 to 14 during the preliminary period and d 11 to 15 during each experimental period. Samples were frozen immediately after collection at −20°C and combined to one composite sample per period prior to analysis.

Duodenal samples (900 mL) were collected every 15 h from d 11 to 15 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. Ruminal contents were evacuated manually through the ruminal cannula 4 h after feeding at the beginning of d 17 (1530 h) and 2 h before feeding at the end of d 18 (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

Analyses of diet ingredients for chemical composition of forages and treatment diets were described in detail by Kammes and Allen (submitted). Forage samples were combined to one 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

(W. S. Tyler Inc., Gastonia, NC). The fraction of DM retained on the screens from wet sieving was used to calculate mean particle size.

Rates of particle size reduction in, and particle passage from, the rumen were determined by using iNDF as a marker (Figure 35). Quadruplicate 25-g TMR and duplicate 25-g orts samples were sieved. Thawed subsamples of ruminal solid and liquid phases from each of 2 rumen evacuations per period were recombined into duplicate 30-g samples based on the original ratio of solid and liquid phases. Duodenal samples were thawed and combined (8 per cow per period), separated into liquid and solid phases, and recombined in duplicate 350-g samples based on the original ratio of solid and liquid phases. Duodenal samples were sieved first to determine threshold size for passage by individually wet sieving samples sequentially through 4.75, 2.36, and 0.038 mm screens. Because the 2.36 mm screen was the screen with the largest aperture size that retained duodenal digesta for all cows, 2.36 mm was selected as the threshold for passage. Particles retained on the 2.36 and 4.75 mm screens were combined and the resulting fractions were designated as ≥ 2.36 (large particles (L) less likely to escape the rumen) and < 2.36 (small particles (S) more likely to escape the rumen). Orts, TMR, and rumen samples (kept separate for the two sampling times) were wet sieved sequentially through the 2.36 and 0.038 mm screens. Particles retained on each screen were removed, dried at 55°C and then weighed. Materials retained on each screen from replicate sievings were combined. The 2 fractions were ground with a Wiley mill (1-mm screen; Arthur H. Thomas, Philadelphia, PA) and analyzed for DM, iNDF, and pdNDF. Concentrations of NDF were determined according to Mertens (2002). Indigestible NDF was estimated as NDF residue after 240 h in vitro fermentation (Goering and Van Soest, 1970); flasks were reinoculated at 120 h to insure a viable microbial population. Ruminal fluid for the in vitro incubations was collected from a nonpregnant dry cow fed dry hay

only. Fraction of pdNDF was calculated by difference (1.00 – iNDF). 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.

Total intakes, ruminal pool sizes, and duodenal fluxes were discussed in detail by Kammes and Allen (submitted). In brief, nutrient intakes were calculated using the composition of feed offered and refused. Ruminal pool sizes (kg) of NDF, iNDF, and pdNDF were determined by multiplying the concentration of each component in rumen samples by the ruminal digesta DM mass (kg). Duodenal fluxes (kg/d) of NDF and pdNDF 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.

Reduction rate of iNDF (rate of transfer of iNDF from the L pool to the S pool), passage rates of iNDF L and iNDF S, and relative size threshold for escape from the rumen were calculated as follows:

Reduction rate (k_r) from L pool to S pool:

$$\text{iNDF } k_r \text{ L} = [\text{iNDF}_{\text{Intake L}}\ (\text{kg/d}) - \text{iNDF}_{\text{Duodenal flux L}}\ (\text{kg/d})] / \text{iNDF}_{\text{Rumen pool L}}\ (\text{kg}),$$

Passage rate (k_p):

$$\text{iNDF } k_p \text{ L} = \text{iNDF}_{\text{Duodenal flux L}}\ (\text{kg/d}) / \text{iNDF}_{\text{Rumen pool L}}\ (\text{kg}), \text{ and}$$

$$\text{iNDF } k_p \text{ S} = \text{iNDF}_{\text{Duodenal flux S}}\ (\text{kg/d}) / \text{iNDF}_{\text{Rumen pool S}}\ (\text{kg}),$$

Relative size threshold:

$$\text{iNDF}_{\text{Duodenal flux L}}\ (\text{kg/d}) / \text{iNDF}_{\text{Duodenal flux total}}\ (\text{kg/d}).$$

Passage rates and relative size threshold were calculated similarly for pdNDF. Rate of particle size reduction was calculated for iNDF only because it can leave the pool only by breakdown or by passage, whereas pdNDF can leave the pool by digestion as well as by particle size reduction and passage.

Statistical Analysis

All data were analyzed by using the fit model procedure of JMP (Version 8, SAS Institute, 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: $Y_{ijk} = \mu + C_i + P_j + T_k + PT_{jk} + pDMI + T_kpDMI + pDMI^2 + T_kpDMI^2 + e_{ijk}$ where 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_kpDMI^2 is the interaction of treatment and pDMI (quadratic), and e_{ijk} is the residual error. Statistical significance for T_kpDMI and T_kpDMI^2 indicated 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. Tendencies for treatment effects and their interactions were declared at $P \leq 0.10$ and $P \leq 0.15$, respectively.

RESULTS AND DISCUSSION

Results of forage family and its interaction with pDMI did not affect DMI, milk yield, or milk composition (Kammes and Allen, submitted). The proportions of NDF L and NDF S intake as a percent of the total NDF intake were essentially the same as cows consumed approximately 75% of total NDF as L and 25% as S for both treatments (Figure 36) despite the significant differences detected between AL and OG ($P < 0.001$) and was related to pDMI. As pDMI increased, AL decreased the proportion of NDF L intake (interaction $P = 0.03$, Figure 37A) and increased the proportion of NDF S intake (interaction $P = 0.03$, Figure 37B), whereas both remained relatively constant for OG across the entire range of pDMI. This indicated that cows consuming AL were likely sorting the feed offered and selecting against long NDF particles in favor of short NDF particles as nutrient demand increased. This might have been allowed by the greater mean particle size and percent of particles > 19 mm (top sieve of Penn State Particle Separator) for alfalfa silage compared with orchardgrass silage (Table 63). Alfalfa increased the proportion of NDF consumed as iNDF L and iNDF S and decreased the proportion of NDF consumed as pdNDF L and pdNDF S compared with OG ($P < 0.001$, Table 65) because of the differences in chemical composition of forage families as alfalfa had a higher concentration of iNDF and lower concentration of pdNDF than orchardgrass (Table 63).

Alfalfa increased the rate of particle size reduction of iNDF from L to S compared with OG (7.16 vs. 4.67%/h, $P < 0.001$, Table 66). This is consistent with the greater resistance of grass cell walls to particle breakdown than for alfalfa cell walls because of chemical and structural differences (Wilson and Hatfield, 1997) and contributed to a greater proportion of total NDF in the rumen as S for AL compared with OG ($P < 0.001$, Figure 36). The faster reduction rate for AL occurred despite less rumination time spent per unit of forage NDF intake for AL

than OG (78.4 vs. 84.7 min/kg forage NDF, $P = 0.02$, Kammes and Allen, submitted). These responses indicated that alfalfa NDF was more fragile than orchardgrass NDF. However, 63.1 and 55.8% of NDF particles in the rumen for AL and OG, respectively, were below the threshold size for passage (Table 65), which suggested that particle size was not a limiting constraint to passage for either treatment.

Passage rates of L from the rumen were less than 1.2%/h for iNDF and pdNDF for both treatments (Table 66). Forage family and its interaction with pDMI did not affect ruminal passage rate of iNDF L, but response of ruminal passage rate of pdNDF L was related to pDMI (Table 66). As pDMI increased, AL increased the passage rate of pdNDF L and OG decreased it (interaction $P = 0.10$, Figure 38). An increase in passage rate with greater intakes was expected, but the reason for the reduction in passage rate of pdNDF L with increased level of intake for OG is not known. Responses of passage rates of S were not related to pDMI (Table 66), but AL increased the rates of ruminal passage of iNDF S (3.85 vs. 2.66 %/h, $P < 0.001$) and pdNDF S (3.80 vs. 2.50 %/h, $P = 0.002$) compared with OG. Despite the faster passage rate of iNDF S for AL, ~50% of the particles in the rumen for AL were iNDF S compared with ~30% for OG ($P = 0.001$, Table 65), which is because of the greater intake of iNDF for AL than OG. In contrast, ~13% of the particles in the rumen for AL were pdNDF S compared with ~26% OG ($P = 0.001$, Table 65) because of lower pdNDF intake and faster pdNDF digestion rate for AL than OG (Kammes and Allen, submitted). These responses contributed to the higher rumen pool size of iNDF and lower rumen pool sizes of pdNDF, NDF, DM, and digesta wet weight and volume for AL compared with OG, which ultimately resulted in lower rumen fill for AL than OG (Kammes and Allen, submitted).

Passage rates for pdNDF S and iNDF S were similar within forage family (Figure 39). If

buoyancy was a factor limiting passage, we would expect pdNDF to be selectively retained in the rumen and have a slower rate of passage than iNDF. Although both iNDF and pdNDF fractions are contained in the same particle, the probability of particles to pass should increase as the particle increases in iNDF because there is less gas from fermentation of pdNDF associated with the particle thereby decreasing buoyancy. Although overall passage rate (not fractionated into L and S) of iNDF was greater than pdNDF for AL and OG (Kammes and Allen, submitted), the similar passage rates for iNDF S and pdNDF S within forage species suggested buoyancy was likely not a constraint to the passage of S particles from the rumen in the current experiment.

Few studies in the literature report rates of passage of various size particles for iNDF and pdNDF fractions. In a study using the same method as the one used in this experiment, passage rate of iNDF was greater than pdNDF for particles <2.36 mm for cows fed low fiber or high fiber diets including alfalfa silage and corn silage as forage sources (Voelker Linton and Allen, 2007). Another study reported faster passage rates for iNDF than pdNDF for particles <2.50 mm for cows fed a mixed timothy and meadow fescue grass silage, but this study used rumen evacuations and fecal output, rather than duodenal flow, to calculate passage rates (Rinne et al., 2002). These results are in agreement with the above stated logic regarding buoyancy; however, we did not obtain similar findings in this experiment.

Alfalfa decreased rumen pool size of NDF L compared with OG (2.30 vs. 2.95 kg, P = 0.001, Table 65). The greater pools of L fibrous particles for OG likely function to entrap S particles and prevent their sedimentation, which reduces their probability of escape. Results on the distribution of particles within the rumen of cows (Evans et al., 1973) and sheep (Sutherland, 1982) have indicated that the ruminal mat functions very effectively as a retaining mechanism and escape from the mat has been identified as a rate-limiting component of passage of forage

particles (Poppi et al., 2001). In addition to the size of particles, shape of particles within the rumen mat is probably important. The cubodial-shaped fragments of legumes usually pass from the rumen faster than grass particles, which are elongated and needle-like (Buxton et al., 1996). The intertwining of long, thin grass particles within the rumen mat might be more efficient at retaining S particles than those of legumes. These factors likely contributed to the slower passage rate of pdNDF S and iNDF S observed for OG.

Rumen pools (kg) of NDF S were similar for AL and OG ($P = 0.17$, Table 65). Despite the faster passage and digestion rates of S particles for AL compared with OG as previously discussed, the pool of NDF S was not smaller for AL than OG because the rate of reduction was also faster such that S particles passed from the rumen or digested in the rumen were replaced by the reduction of L particles into S particles. The composition of the rumen mat and its effect on particle passage is likely a balance between passage, digestion, and reduction rates. If the rates of passage and digestion are slower than the rate of reduction, rumen pools of L particles will decrease and S particles will increase until more L particles are consumed. The accumulation of S particles at the expense of L particles will decrease the ability of the rumen mat to retain S particles.

Alfalfa tended to decrease or decreased the proportion of iNDF L (15 vs. 19% iNDF L duodenal flux/total iNDF duodenal flux, P = 0.09) and pdNDF L (17 vs. 23% pdNDF L duodenal flux/total pdNDF duodenal flux, P = 0.02) particles that escaped the rumen compared with OG (Table 66). This is consistent with the lower proportion of total NDF in duodenal flow as L particles for AL compared with OG ($P = 0.02$, Figure 36). Alfalfa increased the proportion of iNDF S particles that escaped the rumen compared with OG (67.1 vs. 44.3% of total NDF, $P < 0.001$), and this fraction comprised the greatest proportion of particles at the duodenum for both

treatments (Table 65). However, AL decreased the proportion of pdNDF S particles compared with OG (17.3 vs. 34.2% of total NDF, $P < 0.001$, Table 65). Lower intake and greater digestion rate of pdNDF for AL than OG resulted in the reduction of pdNDF S at the duodenum for AL despite the faster passage rate of pdNDF S (Table 66) and lower NDF digestibility (38.3% vs. 53.3%/h, 30 h in vitro fermentation, Table 63) for AL than OG.

CONCLUSIONS

Alfalfa increased rates of reduction of iNDF L to S and passage of pdNDF S and iNDF S compared with OG. The passage rate of particles was not likely limited for either treatment by the rate of reduction or particle size because the proportion of NDF in the rumen below the threshold for passage was greater than 55%. Additionally, it was not likely limited by buoyancy because of similar passage rates for pdNDF S and iNDF S within forage family. The slower passage rate of pdNDF S and iNDF S for OG was likely because of greater entrapment of NDF S within the rumen mat by the larger pool of NDF L. Particle size reduction was a prerequisite to ruminal passage but not a limiting constraint in this experiment. When alfalfa or orchardgrass silage was the only source of forage in diets formulated to contain similar concentrations of forage NDF, selective retention of S particles was less for legume than cool-season grass resulting in lower rumen fill and less effective fiber.

APPENDIX

Table 62. 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	Standard deviation	Minimum	Maximum
Parity	3	3.31	1.16	2	5
BW[1], kg	591	587	51	489	710
BCS	2.00	2.35	0.69	1.58	4.00
DIM	132	157	90	64	337
Milk, kg/d	41.4	41.5	10.8	22.6	57.1
3.5% FCM, kg/d	43.1	42.1	11.9	24.3	60.3
DMI, kg/d	26.7	25.9	3.0	19.6	29.5

[1]Empty BW (ruminal digesta removed).

Table 63. Chemical composition and particle size distribution of the alfalfa silage and orchardgrass silage included in the treatment diets

	Silage	
Item	Alfalfa	Orchardgrass
Chemical composition		
DM, %	43.5	33.7
OM, % DM	91.9	90.3
NDF, % DM	42.3	58.2
iNDF[1], % DM	23.0	16.1
iNDF, % of NDF	54.5	27.7
ADF, % of DM	35.0	36.4
ADL[2], % of DM	7.56	6.03
CP, % DM	22.5	11.4
Starch, % DM	1.87	1.37
NDF digestibility[3], %/h	38.3	53.3
Particles size distribution[4]		
Wet sieving, % DM retained		
19.0 mm	21.4	12.3
9.50 mm	18.0	18.4
4.75 mm	30.8	37.2
2.36 mm	17.0	21.2
1.18 mm	5.72	6.15
0.600 mm	3.09	2.08
0.300 mm	1.97	1.02
0.150 mm	1.16	0.94
0.075 mm	0.40	0.37
0.038 mm	0.50	0.37
Mean particle size[5], mm	11.6	9.66
Penn State Particle Separator, % DM retained		
> 19.0 mm	29.3	17.1
19.0 to 8.0 mm	48.5	50.2
< 8.0 mm	22.2	32.7

[1]iNDF = indigestible NDF.

[2]ADL = acid detergent lignin.

[3]30 h in vitro NDF digestibility.

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

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

Table 64. Ingredients and chemical composition of preliminary and treatment diets (as analyzed) containing either alfalfa (AL) or orchardgrass (OG) silage as the sole source of forage

	Preliminary	AL	OG
Ingredients, % DM			
Alfalfa silage	30.0	59.9	---
Orchardgrass silage	21.5	---	42.7
Dry ground corn	36.2	33.6	36.6
Soybean meal (48% CP)	5.81	---	11.8
SoyPlus®	1.82	2.50	3.39
Vitamin mineral mix[1]	3.99	3.99	3.99
Urea	0.15	---	0.30
Limestone	0.60	---	1.20
Chemical composition			
DM, %	51.6	54.5	52.3
OM, % DM	92.4	92.7	91.1
NDF, % DM	29.1	29.2	30.2
% forage NDF	24.7	25.3	24.9
% NDF from forage	84.8	86.8	82.3
iNDF[2], % DM	NA[3]	14.8	8.24
iNDF, % of NDF	NA	50.7	27.3
CP, % DM	17.5	18.4	17.0
Starch, % DM	33.5	27.3	29.6

[1]Vitamin mineral mix contained (DM basis) 16.5% sodium bicarbonate, 14.2% magnesium sulfate, 7.1% salt, 5.8% dicalcium phosphate, 2.4% trace mineral premix, 0.4% vitamin A, 0.4% vitamin D, 0.2% vitamin E, and 53.1% dry ground corn as a carrier.

[2]iNDF = indigestible NDF.

[3]NA = no analysis for preliminary diet.

Table 65. Particle size distribution of intake, rumen pool, and duodenal flux for cows fed treatment diets containing alfalfa (AL) or orchardgrass (OG) silage as the sole source of forage

Variable[3]	Treatment LSM[1]		SE	*P*[2]					
	AL	OG		Trt	Trt x Period	pDMI	Trt x pDMI	pDMI x pDMI	Trt x pDMI x pDMI
Intake									
NDF L, kg/d	5.49	5.81	0.16	0.05	0.08	0.02	NS	NS	NS
NDF S, kg/d	1.94	1.94	0.05	0.97	NS[4]	0.03	NS	NS	NS
iNDF L, kg/d	3.24	1.58	0.07	<0.001	NS	0.02	NS	NS	NS
iNDF S, kg/d	0.77	0.53	0.02	<0.001	0.10	0.01	0.11	NS	NS
pdNDF L, kg/d	2.25	4.23	0.09	<0.001	0.02	0.03	NS	NS	NS
pdNDF S, kg/d	1.16	1.40	0.03	<0.001	NS	0.03	NS	NS	NS
NDF L, % of NDF	73.7	74.7	0.2	<0.001	0.005	0.13	0.03	0.03	0.67
NDF S, % of NDF	26.3	25.3	0.2	<0.001	0.005	0.13	0.03	0.03	0.67
iNDF L, % of NDF	43.9	20.6	0.2	<0.001	<0.001	0.12	0.03	0.11	NS
iNDF S, % of NDF	10.4	6.91	0.07	<0.001	NS	0.17	0.06	NS	NS
pdNDF L, % of NDF	29.8	54.1	0.2	<0.001	<0.001	0.03	NS	0.009	NS
pdNDF S, % of NDF	15.9	18.4	0.1	<0.001	<0.001	0.002	0.08	0.001	NS
Rumen pool									
NDF L, kg/d	2.30	2.95	0.16	0.001	NS	0.14	NS	NS	NS
NDF S, kg/d	3.89	3.72	0.23	0.17	NS	NS	NS	NS	NS
iNDF L, kg/d	1.70	1.25	0.09	<0.001	NS	0.13	NS	NS	NS
iNDF S, kg/d	3.10	2.02	0.14	<0.001	NS	NS	NS	NS	NS
pdNDF L, kg/d	0.59	1.69	0.08	<0.001	NS	0.15	NS	NS	NS
pdNDF S, kg/d	0.79	1.71	0.12	<0.001	NS	NS	NS	NS	NS
NDF L, % of NDF	36.9	44.2	0.9	<0.001	NS	0.07	NS	NS	NS
NDF S, % of NDF	63.1	55.8	0.9	<0.001	NS	0.07	NS	NS	NS
iNDF L, % of NDF	26.6	19.4	0.8	<0.001	NS	0.16	0.35	0.69	0.10
iNDF S, % of NDF	50.6	30.3	0.7	<0.001	0.19	NS	NS	NS	NS
pdNDF L, % of NDF	9.37	25.3	0.50	<0.001	NS	0.03	NS	NS	NS

Table 65 (cont'd)

pdNDF S, % of NDF	12.6	25.5	0.8	<0.001	NS	0.19	0.08	NS	NS
Duodenal flux									
NDF L, kg/d	0.57	0.53	0.08	0.69	NS	0.32	0.16	NS	NS
NDF S, kg/d	3.30	2.12	0.20	<0.001	NS	0.31	0.002	0.40	0.07
iNDF L, kg/d	0.42	0.25	0.06	0.05	NS	NS	NS	NS	NS
iNDF S, kg/d	2.80	1.24	0.12	<0.001	NS	0.18	NS	NS	NS
pdNDF L, kg/d	0.15	0.28	0.02	0.001	NS	0.85	0.16	NS	NS
pdNDF S, kg/d	0.69	0.93	0.07	0.03	NS	0.31	0.15	NS	NS
NDF L, % of NDF	15.2	21.1	1.7	0.02	0.20	0.76	NS	0.10	NS
NDF S, % of NDF	84.8	78.9	1.7	0.02	0.20	0.76	NS	0.10	NS
iNDF L, % of NDF	11.5	10.6	1.4	0.59	NS	0.75	NS	0.10	NS
iNDF S, % of NDF	67.1	44.3	2.6	<0.001	NS	0.12	0.18	0.17	NS
pdNDF L, % of NDF	4.15	10.9	0.76	<0.001	NS	0.35	NS	0.17	NS
pdNDF S, % of NDF	17.3	34.2	1.9	<0.001	NS	0.05	NS	NS	NS

[1]Treatment least squares means.

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

[3]iNDF = indigestible NDF, pdNDF = potentially digestible NDF, L = large particles (≥2.36 mm), S = small particles (<2.36 mm).

[4]NS = not significant ($P > 0.20$); term removed from statistical model.

Table 66. Particle size kinetics for cows fed treatment diets containing alfalfa (AL) or orchardgrass (OG) silage as the sole source of forage

Variable[3]	Treatment LSM[1]		SE	*P*[2]					
	AL	OG		Trt	Trt x Period	pDMI	Trt x pDMI	pDMI x pDMI	Trt x pDMI x pDMI
Rate of reduction, %/h									
iNDF (L to S)	7.16	4.67	0.35	<0.001	0.18	NS[4]	NS	NS	NS
Passage rate, %/h									
iNDF L	1.18	1.01	0.16	0.38	NS	0.84	NS	0.13	NS
iNDF S	3.85	2.66	0.23	<0.001	NS	NS	NS	NS	NS
pdNDF L	1.05	0.75	0.11	0.07	NS	0.88	0.10	NS	NS
pdNDF S	3.80	2.50	0.28	0.002	0.13	NS	NS	NS	NS
Duodenal flux L/total duodenal flux									
iNDF	0.15	0.19	0.02	0.09	NS	0.91	NS	0.06	NS
pdNDF	0.17	0.23	0.01	0.02	0.03	0.06	NS	NS	NS

[1]Treatment least squares means.

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

[3]iNDF = indigestible NDF, pdNDF = potentially digestible NDF, L = large particles (≥2.36 mm), S = small particles (<2.36 mm).

[4]NS = not significant ($P > 0.20$); term removed from statistical model.

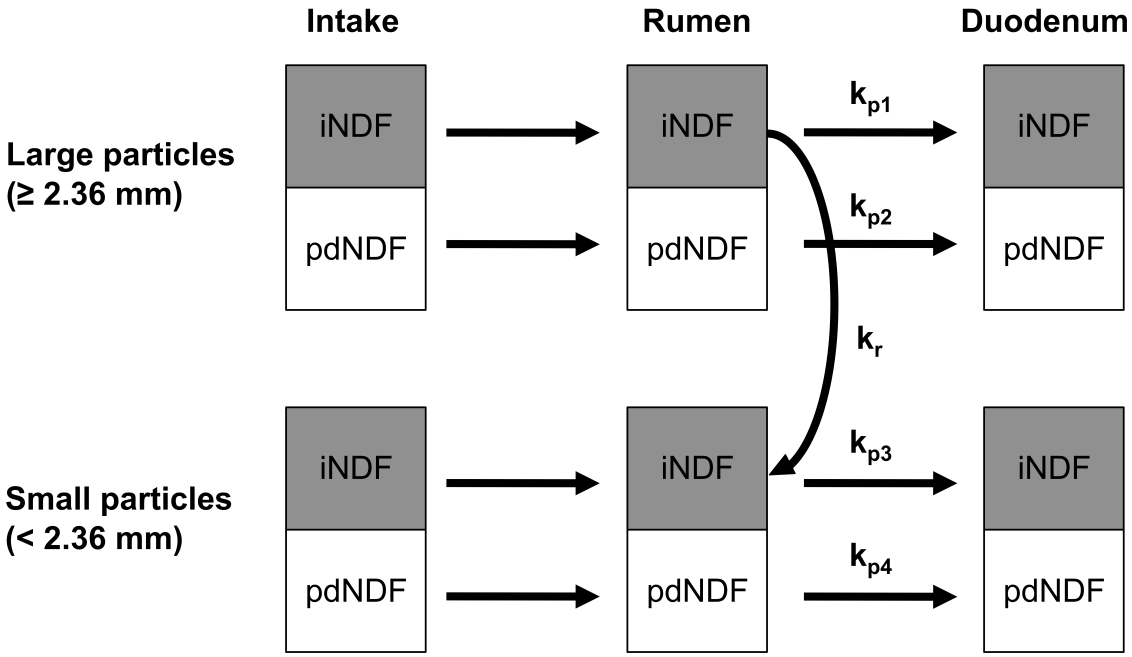

Figure 35. Model of ruminal particle size reduction and passage. Reduction of particle size during eating is included in the rate of particle size reduction (k_r). Passage rates (k_{pi}) are calculated for indigestible NDF (iNDF) and potentially digestible NDF (pdNDF); k_r is calculated for iNDF only. Figure reprinted from Voelker Linton and Allen (2007).

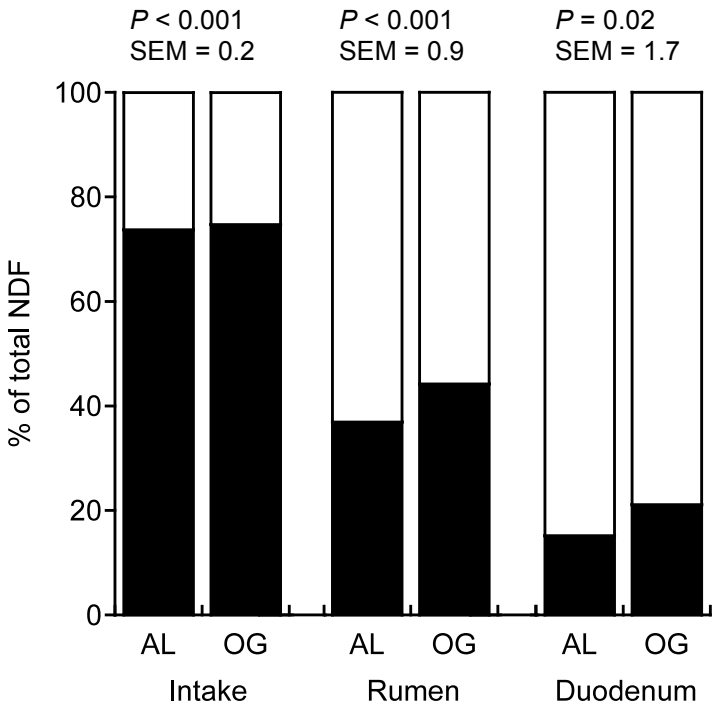

Figure 36. Particle size distribution of NDF in intake, rumen, and duodenum. Proportion of total NDF as large (≥2.36 mm; denoted by black) or small (<2.36 mm; denoted by white) particles for cows fed diets containing alfalfa (AL) or orchardgrass (OG) as the sole source of forage. Least squares mean and standard error of the mean (SEM) are shown. *P*-values above each set of columns indicate significance for comparison of AL and OG within NDF large and small particles.

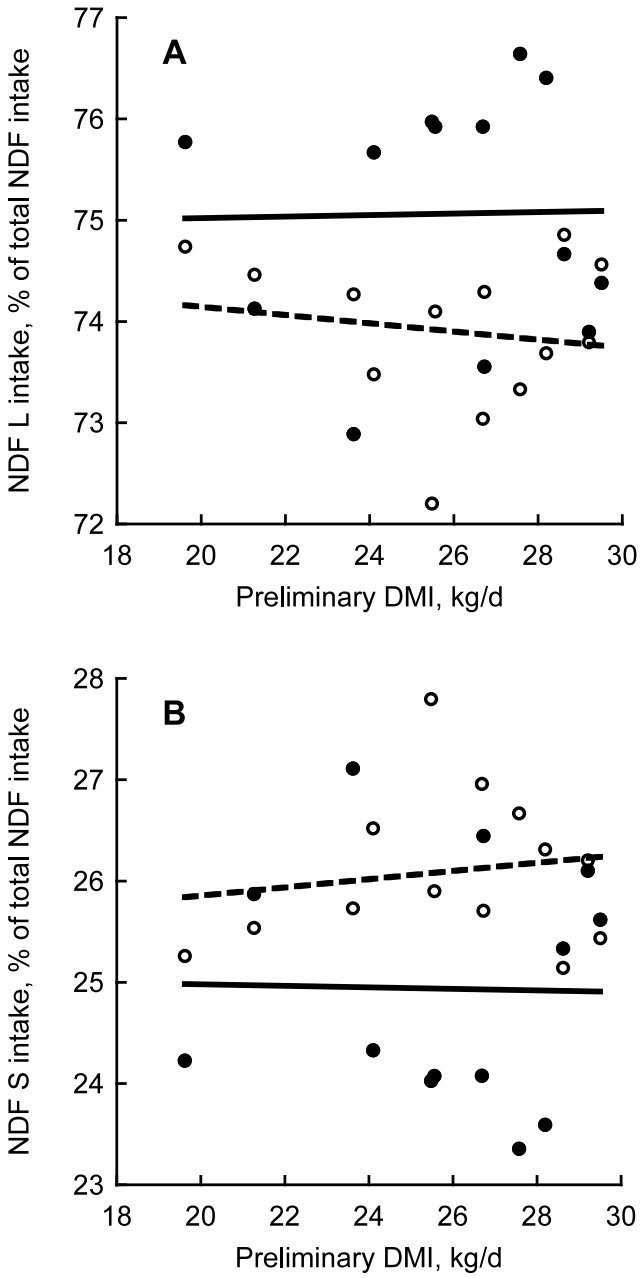

Figure 37. Interaction of alfalfa (open circles, dashed line) and orchardgrass (closed circles, solid line) with preliminary DMI for the proportion of NDF intake as A) large particles (NDF L; ≥2.36 mm; $P = 0.03$) or B) small particles (NDF S; $P = 0.03$). The preliminary DMI on the x-axis are the mean DMI of individual cows during the final 4 d of the preliminary period when all cows were fed a common diet.

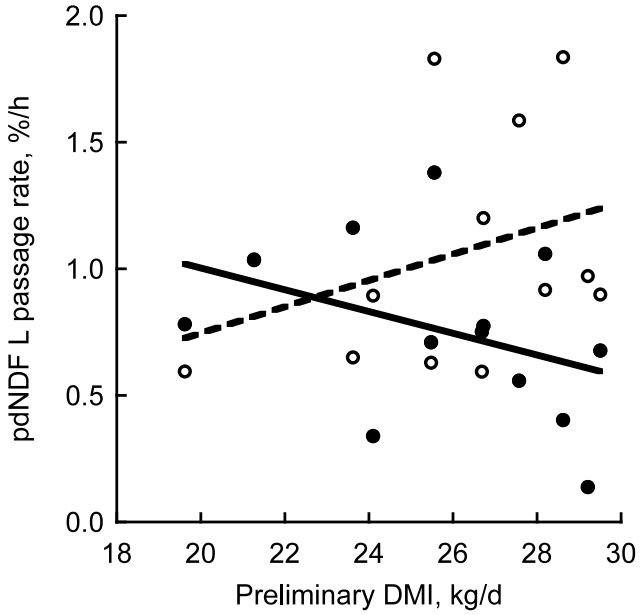

Figure 38. Interaction of alfalfa (open circles, dashed line) and orchardgrass (closed circles, solid line) with preliminary DMI for passage rate of large particles (≥2.36 mm) of potentially digestible NDF (pdNDF L; $P = 0.10$). The preliminary DMI on the x-axis are the mean DMI of individual cows during the final 4 d of the preliminary period when all cows were fed a common diet.

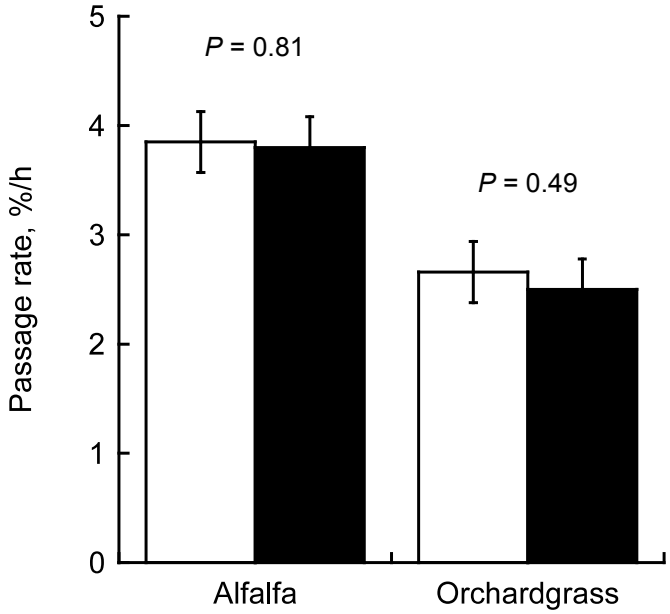

Figure 39. Passage rates of indigestible NDF small particles (iNDF S; <2.36 mm; white bars) and potentially digestible NDF small particles (pdNDF S; <2.36 mm; black bars) for cows fed diets containing alfalfa or orchardgrass as the sole source of forage. Least squares mean are shown and error bars represent standard error of the mean. *P*-values above each set of column indicate significance for comparison of iNDF S and pdNDF S within alfalfa and orchardgrass based on analysis using paired t-test.

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CHAPTER 8

Epilogue

RECAP

Understanding how the level of feed intake and forage characteristics interact to affect passage rate of individual feed fractions was the primary goal of the research presented in this dissertation. Level of intake ranged from approximately 20 to 30 kg DM per day among cows on the five experiments, and the forage characteristics evaluated included legume particle size (10 vs. 19 mm), grass particle size (10 vs. 19 mm), legume maturity (41 vs. 53% NDF), grass maturity (45 vs. 54% NDF), and forage family (legume vs. grass). Alfalfa and orchardgrass were selected as a representative legume and grass, respectively. We hypothesized that the normal variation in diet characteristics related to forage alters both the passage rates of feed fractions and the extent to which passage rates of these fractions are affected by voluntary DMI. This chapter provides key results including some findings that challenge conventional wisdom, application of the information gained from this research, and direction for future research.

SUMMARY OF RESULTS: EFFECTS OF FORAGE CHARACTERISTICS, LEVEL OF INTAKE, AND THEIR INTERACTIONS

Passage rates of individual feed fractions were highly variable at a given level of voluntary DMI across cow periods and depended upon the forage characteristic being evaluated. Forage particle size, level of intake, and their interaction were not related to passage rates of potentially digestible NDF (pdNDF), indigestible NDF (iNDF), or starch when cows consumed alfalfa or orchardgrass silage-based diets. Passage rate of iNDF was greater for early compared with late maturity alfalfa and was quadratically related to level of intake independent of

treatment, such that the passage rate of indigestible NDF (iNDF) increased as level of intake increased from low to mid range of preliminary DMI (pDMI) and remained relatively constant from mid to high range of pDMI (Figure 40A). Alfalfa maturity, level of intake, and their interaction were not related to passage rates of potentially digestible NDF (pdNDF) or starch. Similar to alfalfa maturity, passage rate of iNDF was greater for early compared with late maturity orchardgrass and was quadratically related to level of intake independent of treatment. However, the pattern for orchardgrass maturity was different than that for alfalfa maturity with the passage rate of iNDF decreasing from low to mid range of pDMI and increasing from mid to high range of pDMI (Figure 40B). Additionally, passage rate of pdNDF was lower for early compared with late maturity orchardgrass and was quadratically related to level of intake dependent upon treatment, such that passage rate of pdNDF decreased as pDMI increased for cows consuming early maturity orchardgrass but increased as level of intake increased from low to mid range of pDMI and decreased from mid to high range of pDMI for cows consuming late maturity orchardgrass (Figure 40C). Furthermore, orchardgrass maturity and its interaction with level of intake was related to passage rate of starch, which remained constant for early maturity orchardgrass but increased linearly for late maturity orchardgrass as pDMI increased (Figure 40D). Passage rate of iNDF was faster for alfalfa compared with orchardgrass and was linearly related to level of intake dependent upon treatment, such that the passage rate of iNDF increased slightly for alfalfa and decreased for orchardgrass as pDMI increased with the greatest difference for cows with high pDMI (Figure 40E). Passage rate of pdNDF was also faster for alfalfa compared with orchardgrass but was not related to level of intake. Additionally, forage family, level of intake, and their interactions were not related to the passage rate of starch. Although it has become conventional wisdom that ruminal passage rate increases with feed intake (NRC,

2001), these results demonstrate that the extent to which passage rates of individual feed fractions vary with level of intake depends upon forage characteristics.

It has also been widely accepted that microbial nitrogen flow to the duodenum increases with voluntary DMI (NRC, 2001); however, this was not evident in our research. Forage characteristics, level of intake, and their interactions were not related to microbial nitrogen flow or efficiency. Relationships were detected between microbial efficiency and passage rates of starch and pdNDF, digestion rate of starch, and amount of true ruminally digested OM (TRDOM, kg/d) for various experiments but no consistent patterns were observed. In the experiment with orchardgrass particle size, efficiency of microbial synthesis was positively related to passage rates of starch (Figure 41A) and pdNDF (Figure 41B) for long and short orchardgrass. When comparing alfalfa maturity, microbial efficiency was negatively related to digestion rate of starch for early and late alfalfa (Figure 41C), and microbial efficiency quadratically decreased as TRDOM increased for early maturity alfalfa but was not affected for late maturity alfalfa (Figure 41D). In contrast to alfalfa maturity, microbial efficiency linearly decreased as TRDOM increased for late maturity orchardgrass but was not affected for early maturity orchardgrass (Figure 41E). When comparing forage family, microbial efficiency was positively related to passage rate of pdNDF for orchardgrass but not alfalfa (Figure 41F). Based on our results, microbial efficiency was not related to level of intake but was related to other factors that varied among the experiments, and these inconsistencies suggest efficiency of microbial production is affected by multiple factors.

We expected the more filling diets (i.e. increased forage particle size, advanced forage maturity, and grass) to cause greater ruminal distention and limit feed intake of cows with high DMI to a greater extent than cows with low DMI. However, we were unable to detect this in any

of the five experiments presented here. Visual assessment illustrated that ruminal distention caused by the more filling diet may be more likely to limit feed intake for cows with high intake compared to cows with low intake for alfalfa particle size (Figure 42A), orchardgrass particle size (Figure 42B), and forage family (Figure 42C) but these interactions were not statistically significant. There was no evidence that the filling effect of diets affected feed intake differently for cows with high intake compared to cows with low intake due to alfalfa maturity (Figure 42D) or orchardgrass maturity (Figure 42E). Lack of significant interactions are likely because of the high variability among cows and are not surprising after evaluation of relationships between DMI and ruminal NDF pool, the primary feed constituent associated with ruminal fill, and total ruminal digesta wet weight, which were also inconsistent (Table 67). At best, NDF pool size and total digesta wet weight explained 74% and 53% of the variation in DMI (Table 67). These results indicate numerous factors affect physical fill in the rumen and control feed intake.

In conclusion, the research presented in this dissertation illustrates the complexity of ruminal passage. Results demonstrate that the extent to which passage rates of feed fractions vary with level of intake depends upon forage characteristics. The high variability among individual cow responses at a given level of intake emphasizes the difficulty involved in accurately predicting ruminal passage and digestibility: it is not a "one size fits all" concept that can be easily incorporated into nutrition models. Some of the relationships presented are weak, quadratic, and unexplainable at this time but important to report nonetheless. This body of work provides the foundation for additional research in this area.

APPLICATION OF DATA

Although effects of DMI on passage rates are not consistent, these experiments provide absolute measurements of passage rates of digesta fractions, which are necessary for the development of equations to predict digesta passage from the rumen. The data provided by this series of experiments can be summarized and used for modeling the effects of voluntary DMI and forage characteristics on passage rate of digesta fractions. While modeling ruminal passage can be improved, the degree of accuracy is limited because of the high variability and inconsistencies noted among the five experiments. In addition to the data from these experiments, data obtained in our laboratory from other experiments utilizing the pool and flux method for estimating passage rate of digesta fractions can be compiled and used in a meta-analysis, which has the potential of discovering relationships. Results may be useful in the development of new regression equations predicting passage rates of iNDF, pdNDF, and starch. The data collected from these experiments allow equations to be developed using not only data obtained in ruminal metabolism experiments (e.g. rumen pools) but also data that can be obtained by commercial dairy farms (e.g. DMI and diet composition). Data sets containing more easily measured parameters along with passage rates will increase the accuracy of the prediction of passage rates in models intended for use on commercial dairy farms. These results can be used to improve existing models to increase accuracy of prediction of ruminal digestibility and nutrient flow to the duodenum or used in the development of new nutrition models.

FUTURE RESEARCH

Future experiments utilizing the same experimental design and pool and flux method used in our research should be conducted to evaluate the effects of other dietary treatments including factors related to grain type, conservation method and physical form, non-forage fiber

sources (high fiber byproducts), and supplemental fat on passage rates of digesta fractions. More experiments evaluating forage characteristics are needed to validate or refute previous results. Furthermore, compiling databases from additional studies would allow sufficient numbers of observations to help overcome the lack of statistical power from single studies due to the large variation among animals and forages. Further research examining rates of particle size breakdown and particle passage from the rumen would be beneficial to determine the primary constraint limiting passage. These research efforts will further improve the accuracy of nutrition models to predict nutrient intake and utilization in dairy cows. Ultimately, improved accuracy of ruminal digestion models from the incorporation of this and future research will enhance our ability to formulate dairy cow diets to increase milk yield, decrease feed costs, and minimize excretion of nutrients as waste products.

APPENDIX

Table 67. Relationship between DMI (kg/d) and ruminal NDF pool (kg) or ruminal digesta wet weight (kg)

	DMI and NDF pool			DMI and digesta wet weight		
	P	R^2	Linear (L) or Quadratic (Q)	*P*	R^2	Linear (L) or Quadratic (Q)
Legume particle size						
Long alfalfa	0.13	0.19	L	0.41	0.06	L
Short alfalfa	<0.001	0.74	L	0.006	0.52	L
Grass particle size						
Long orchardgrass	0.09	0.21	L	0.05	0.27	L
Short orchardgrass	0.55	0.03	L	0.14	0.16	L
Legume maturity						
Early alfalfa	0.87	0.01	L	0.08	0.21	L
Late alfalfa	<0.001	0.63	L	0.002	0.53	L
Grass maturity						
Early orchardgrass	0.33	0.09	L	0.16	0.17	L
Late orchardgrass	0.93	0.001	L	0.56	0.03	L
Forage family						
Alfalfa	0.11	0.22	L	0.02	0.38	L
Orchardgrass	0.005	0.66	Q	0.03	0.51	Q

Figure 40

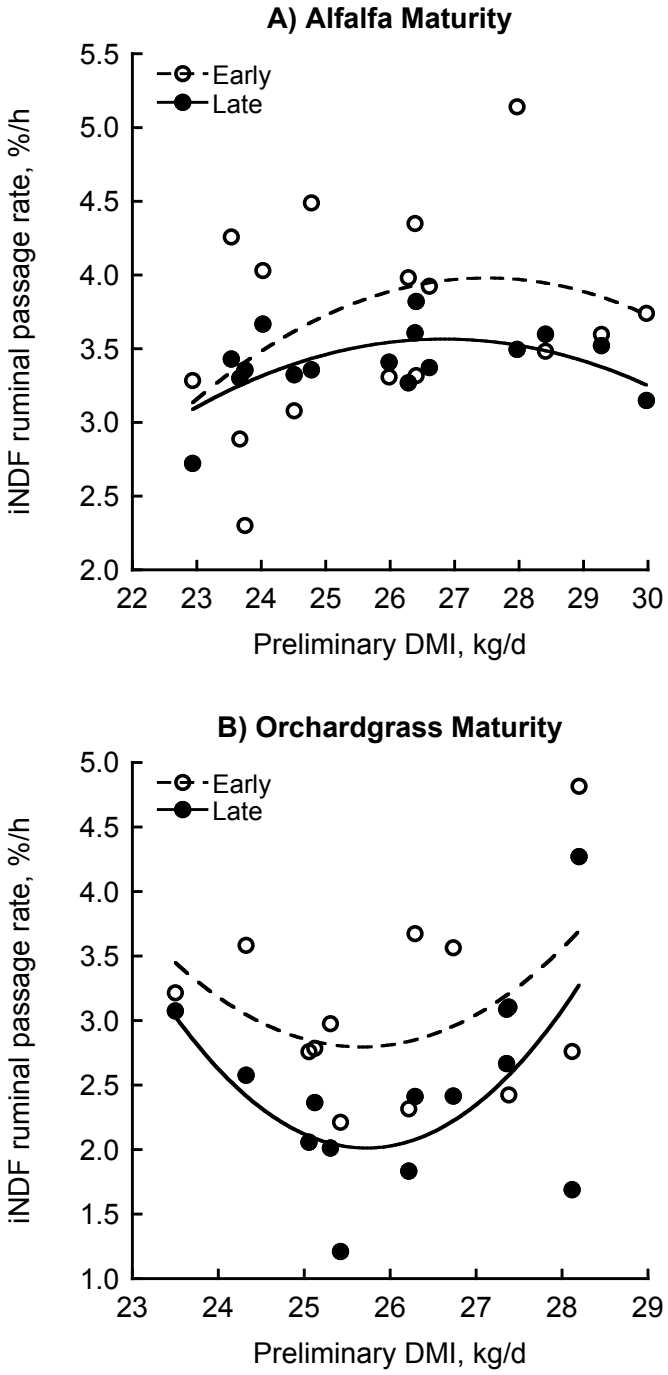

A) Alfalfa Maturity
Early
Late
iNDF ruminal passage rate, %/h
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
22
23
24
25
26
27
28
29
30
Preliminary DMI, kg/d
B) Orchardgrass Maturity
Early
Late
iNDF ruminal passage rate, %/h
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
23
24
25
26
27
28
29
Preliminary DMI, kg/d

Figure 40 (cont'd)

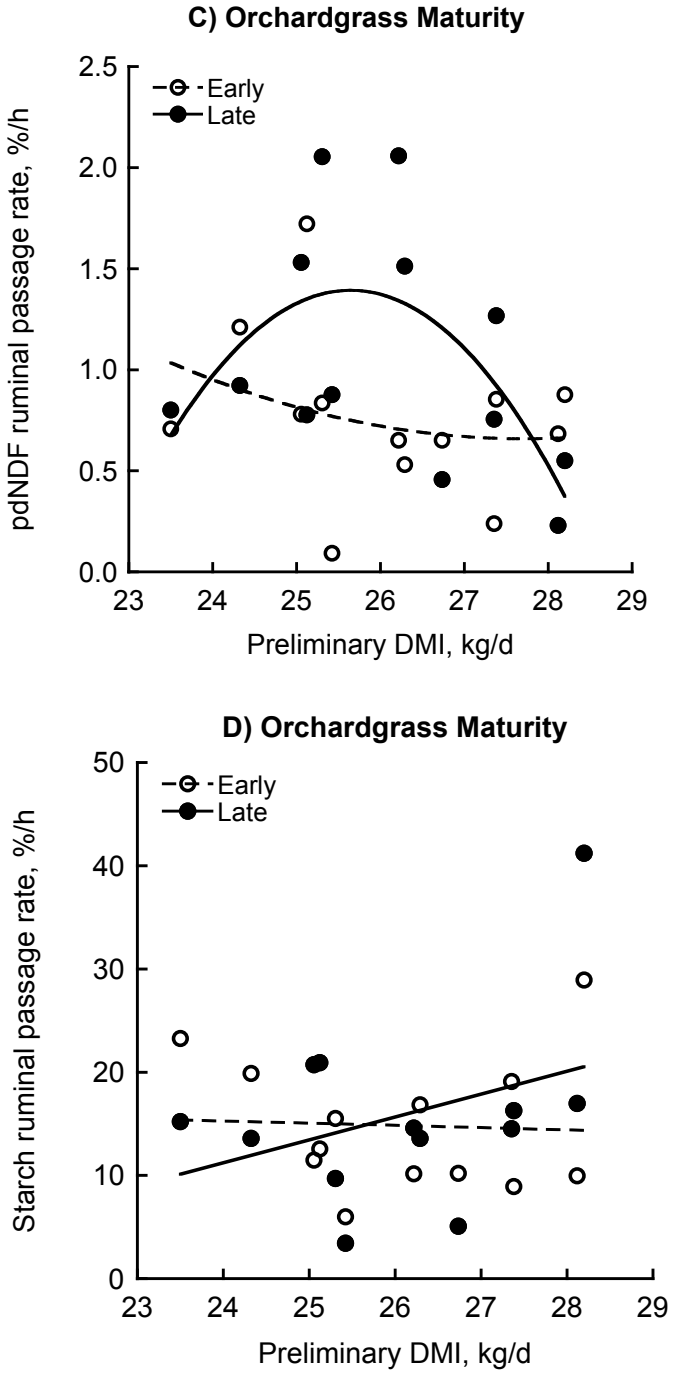


Figure 40 (cont'd)

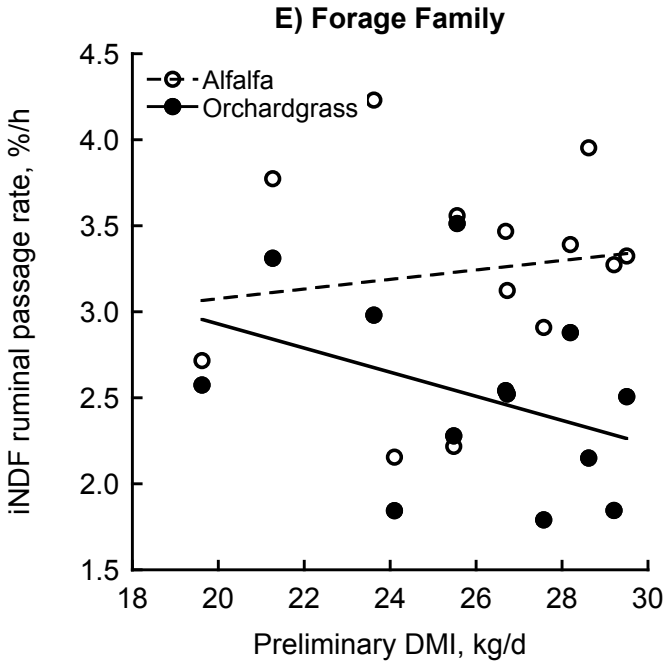


Figure 40. Relationship between preliminary DMI and A) indigestible NDF (iNDF) passage rate for alfalfa maturity (treatment: $P = 0.01$, preliminary DMI: $P = 0.07$ quadratic, interaction: nonsignificant (NS)), B) iNDF passage rate for orchardgrass maturity (treatment: $P < 0.001$, preliminary DMI: $P = 0.08$ quadratic, interaction: NS), C) potentially digestible NDF (pdNDF) passage rate for orchardgrass maturity (treatment: $P = 0.04$, preliminary DMI: $P = 0.09$ quadratic, interaction: $P = 0.11$ quadratic), D) starch passage rate for orchardgrass maturity (treatment: NS, preliminary DMI: $P = 0.01$ quadratic, interaction: $P = 0.03$ linear), and E) iNDF passage rate for forage family (treatment: $P < 0.001$, preliminary DMI: NS, interaction: $P = 0.09$ linear). 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.

Figure 41

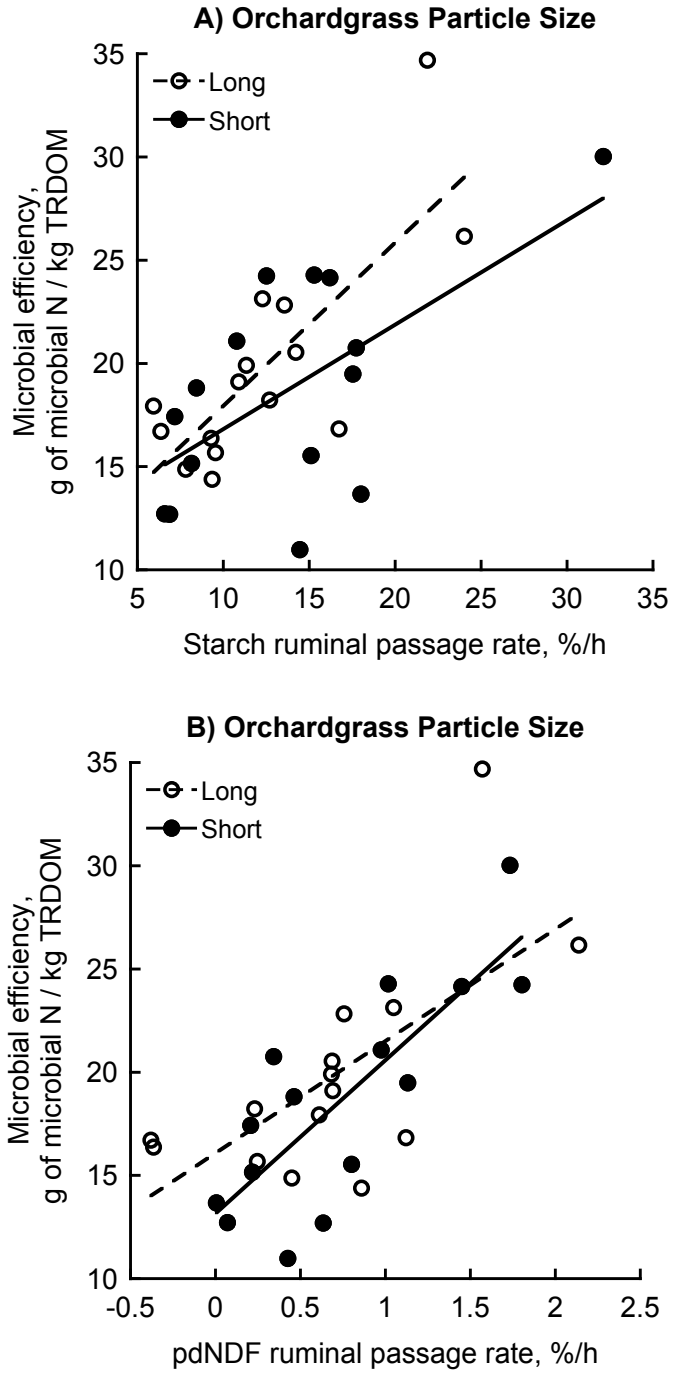
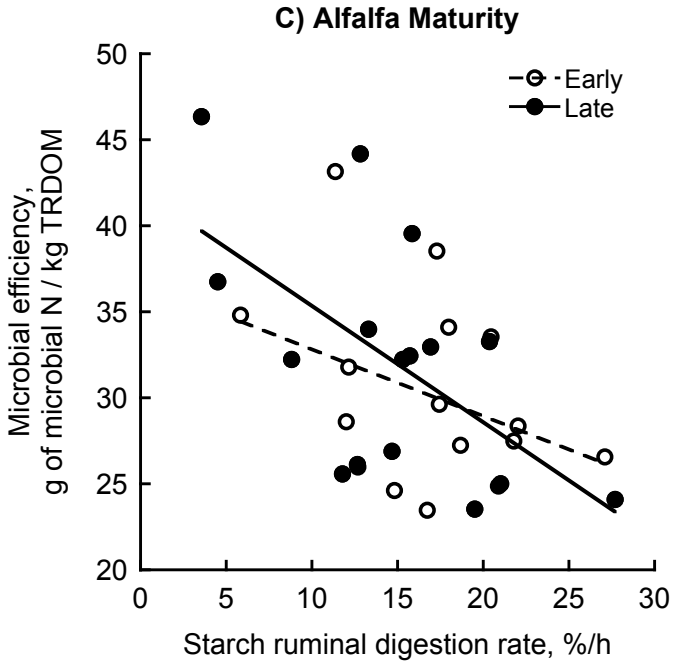

A) Orchardgrass Particle Size
Long
Short
Microbial efficiency, g of microbial N / kg TRDOM
Starch ruminal passage rate, %/h
B) Orchardgrass Particle Size
Long
Short
Microbial efficiency, g of microbial N / kg TRDOM
pdNDF ruminal passage rate, %/h

Figure 41 (cont'd)



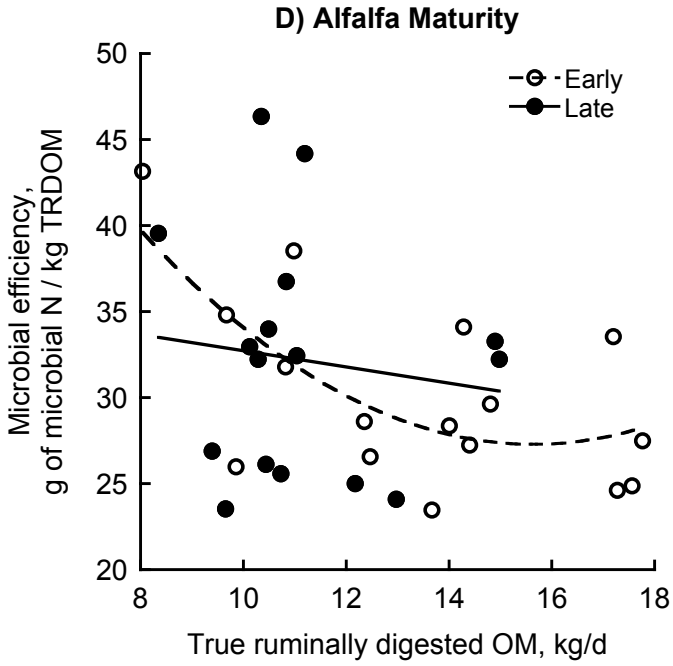
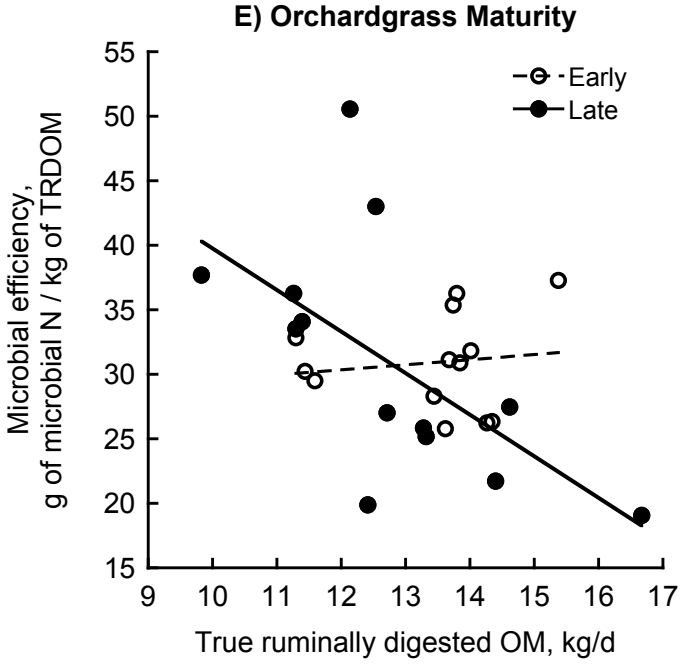


Figure 41 (cont'd)



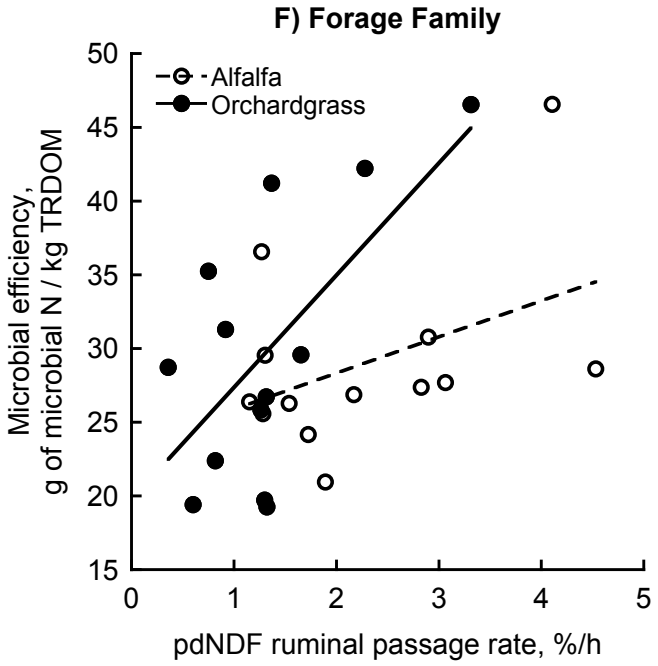


Figure 41. Relationship between microbial efficiency expressed as g of microbial N per kg true ruminally digested OM (TRDOM) and A) starch passage rate for orchardgrass particle size (Long: $P < 0.001$, $R^2 = 0.61$; SHORT: $P = 0.01$, $R^2 = 0.38$), B) potentially digestible NDF (pdNDF) passage rate for orchardgrass particle size (Long: $P = 0.006$, $R^2 = 0.45$; SHORT: $P < 0.001$, $R^2 = 0.64$), C) starch digestion rate for alfalfa maturity (Early: $P = 0.16$, $R^2 = 0.14$; Late:

Figure 41 (cont'd)

$P = 0.02$, $R^2 = 0.35$), D) TRDOM for alfalfa maturity (Early: $P = 0.03$, $R^2 = 0.42$; Late: $P = 0.65$, $R^2 = 0.02$), E) TRDOM for orchardgrass maturity (Early: $P = 0.68$, $R^2 = 0.02$; Late: $P = 0.03$, $R^2 = 0.37$), and F) pdNDF passage rate for forage family (Alfalfa: $P = 0.15$, $R^2 = 0.18$; Orchardgrass: $P = 0.02$, $R^2 = 0.42$).

Figure 42

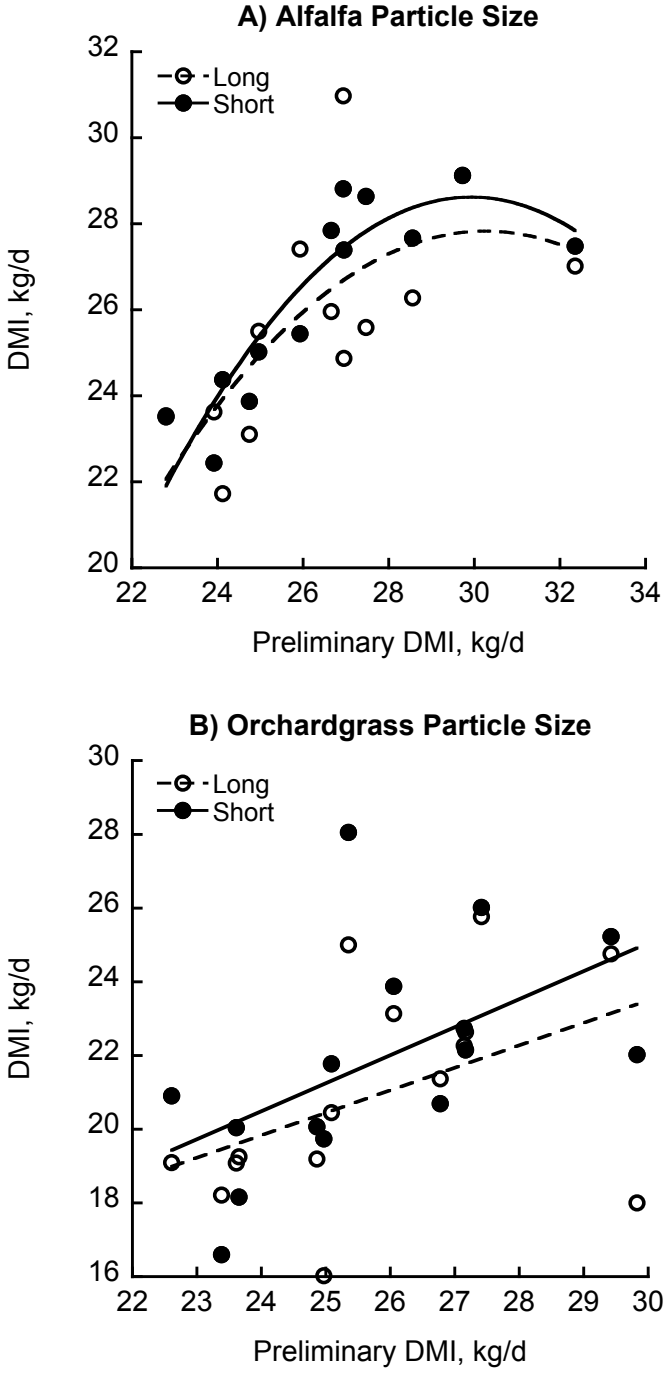
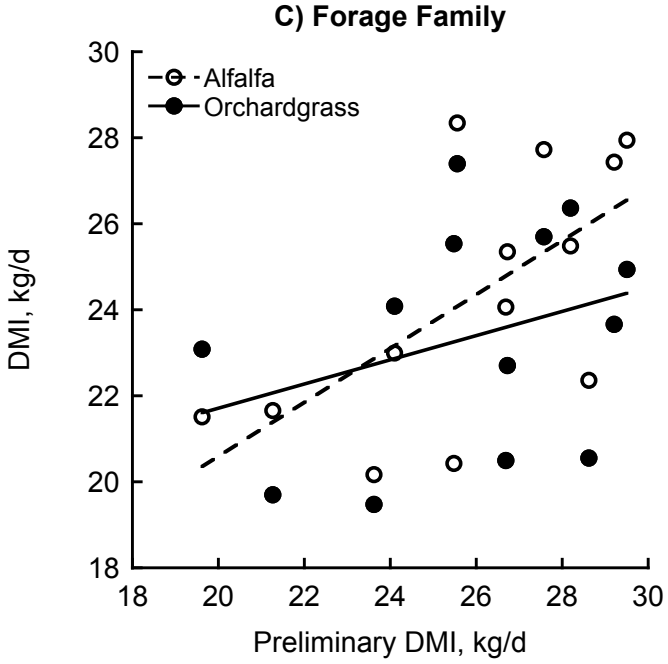

A) Alfalfa Particle Size
Long
Short
DMI, kg/d
Preliminary DMI, kg/d
B) Orchardgrass Particle Size
Long
Short
DMI, kg/d
Preliminary DMI, kg/d

Figure 42 (cont'd)



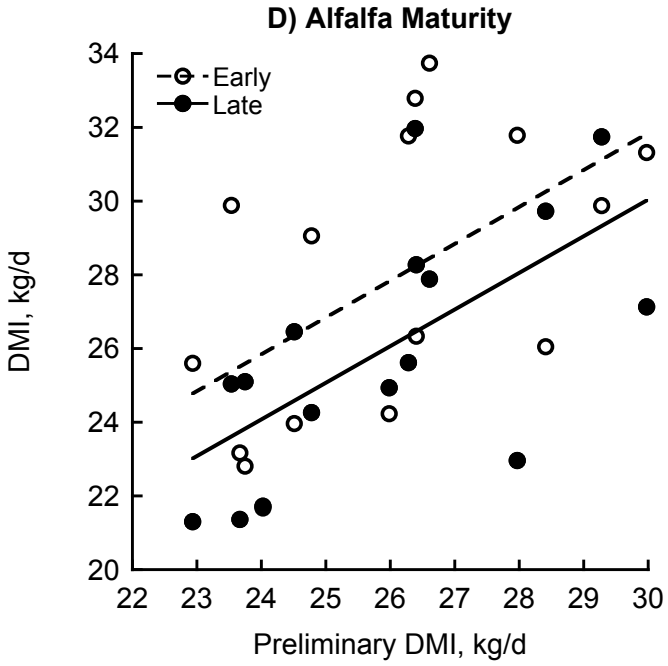


Figure 42 (cont'd)

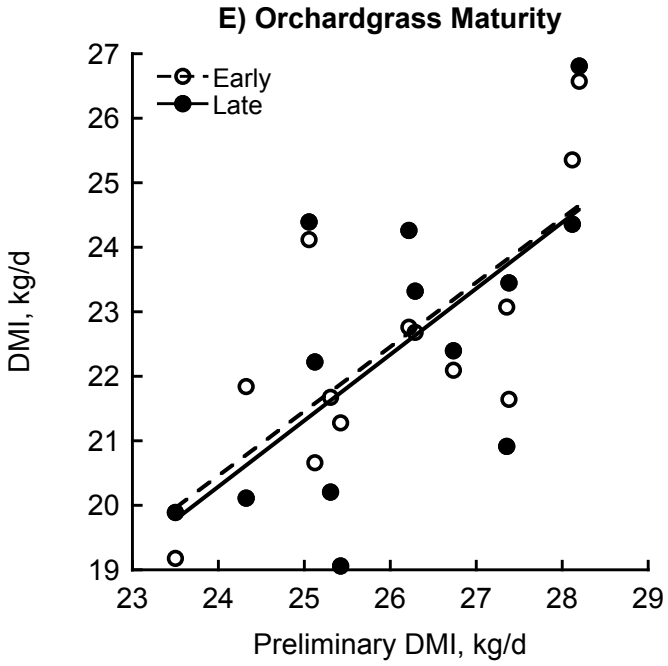


Figure 42. Relationship between preliminary DMI (pDMI) and DMI for: A) alfalfa particle size (treatment: $P = 0.10$, treatment by pDMI interaction: nonsignificant (NS)), B) orchardgrass particle size (treatment: $P = 0.06$, treatment by pDMI interaction: NS), C) forage family (treatment: $P = 0.13$, treatment by pDMI interaction: NS), D) alfalfa maturity (treatment: $P = 0.003$, treatment by pDMI interaction: NS), and E) orchardgrass maturity (treatment: $P = 0.70$, treatment by pDMI interaction: NS). 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.

REFERENCES

REFERENCES

National Research Council. 2001. Nutrient Requirements of Dairy Cattle, 7th rev. ed. Natl. Acad. Washington, DC.