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dissertation entitled

EFFECTS OF DIETARY FORAGE CHARACTERISTICS ON
DIGESTA PASSAGE RATE IN DAIRY COWS

presented by

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has been accepted towards fulfillment
of the requirements for the

Doctoral degree in Animal Science

Major Professor's Signature

11/10/06

Date

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EFFECTS OF DIETARY FORAGE CHARACTERISTICS ON DIGESTA PASSAGE RATE IN DAIRY COWS

by

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A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Animal Science

2006

ABSTRACT

EFFECTS OF DIETARY FORAGE CHARACTERISTICS ON DIGESTA PASSAGE RATE IN DAIRY COWS

By

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Important factors regulating nutrient availability for ruminants include feed intake, ruminal digestibility, and microbial protein flow to the duodenum. These factors are directly affected by ruminal passage rate, which is, in turn, affected by dietary characteristics. However, response to treatment also depends on nutrient demand; one index for nutrient demand is voluntary feed intake. Therefore, two experiments were conducted to quantify the effects of preliminary voluntary dry matter intake (pVDMI) on the response of passage rate in dairy cows to a change in forage source and a change in dietary forage fiber concentration. Both experiments utilized a crossover design in which pVDMI was measured during a preliminary period. Then, using data from these and similar experiments conducted in our laboratory, empirical models were developed for the prediction of passage rates of starch and of indigestible and potentially digestible fractions of neutral detergent fiber (iNDF and pdNDF, respectively).

In the first experiment, treatments were a diet containing alfalfa silage (AL) and a diet containing orchardgrass silage (OG). A more positive dry matter intake (DMI) response to AL over OG among cows with greater pVDMI was permitted by a more positive response in ruminal NDF turnover rate. Intake and duodenal flow of N also increased more for AL than for OG with increasing pVDMI.

However, among cows with greater pVDMI, a decreasing proportion of consumed N was used for milk production or body tissue gain on AL compared to OG.

In the second experiment, treatments were a high-forage diet (HF) and a low-forage diet (LF). Contrary to the hypothesis, differences in DMI responses to LF and HF did not depend on pVDMI. Neutral detergent fiber digestion and(or) passage might have been inhibited on LF among high-pVDMI cows, possibly as a result of lower ruminal pH.

Equations were developed to predict k_p of iNDF, pdNDF, and starch using data that can be obtained by commercial dairy farms. These equations explained 68% and 53% of variation in k_p of iNDF and pdNDF, respectively. Important predictors included dietary starch concentration, DMI, 30-h in vitro digestibility of NDF in forages in the diet (NDFD), dietary NDF concentration, and 3.5% fat-corrected milk yield. The prediction equation for starch passage rate explained 42% of variation in starch passage rate. Important predictors included dietary NDF concentration, NDFD, DMI, starch intake, milk yield, change in body condition score, and dietary starch concentration.

The results of this research demonstrate that the feed intake, digestion kinetics, and nutrient utilization of cows with higher and lower nutrient demands respond differently to changes in forage source and dietary forage NDF concentration. In addition, data resulting from these experiments and equations resulting from these empirical models can be used to improve models of feed digestion in dairy cows.

For Brian Linton, a fellow steward of good gifts.

ACKNOWLEDGEMENTS

This research could not have carried out without the financial, intellectual, and emotional investment of my advisor, Dr. Mike Allen. The wisdom and experience I heard in the advice he offered while I was investigating graduate school was a main reason I came to MSU; his skill in advising was a primary factor in my decision to stay for a Ph.D.; and his ability to adjust his tactics and advice as he encounters students of different temperaments and at different points in life facilitated my navigation of this doctoral program.

I would like to thank Dr. David Beede, Dr. Margaret Benson, Dr. Thomas Herdt, and Dr. Dale Romsos for their advice and suggestions as members of my graduate committee, not only with regard to my dissertation but also my career and professional development. Thanks also to Dr. Ames for performing the ruminal and duodenal cannulation surgeries.

To the Dairy Nutrition Research Lab (Dave Main, Dewey Longuski, Jackie Ying, Dr. C. Steve Mooney, and Dr. Barry Bradford): thank you for countless hours of labor, guidance in all aspects of these research projects, and perhaps most importantly, your varied senses of humor. I can only hope that the next team I work with is as effective and as kind-hearted as you all have been. Thanks also to the undergraduate students who took 2 a.m. samplings, helped make duodenal snow, and weighed innumerable crucibles and beakers for these projects. I'm also very grateful to the crew at the MSU dairy farm for making the

day-to-day aspects of this research happen. They played an irreplaceable role in this research, and I appreciate their patience, flexibility, and hard work.

My parents, Howard and Mary Voelker, have been my teachers inside and outside the classroom throughout my life. They model for me the value of welcoming change and risk, whether it's launching a new career, making new friends in a different state, or tackling a major remodeling project on their own. Thank you also, Mom and Dad, for your example of working together, with each other, all these years on all those different projects.

Many other family and friends provided emotional support while I was working on this degree. I depended greatly on the rest of the Voelker family, the Linton/Morgan clan (who taught me the meaning of the word "relax"), Joanna Spanos, and friends in Graduate InterVarsity and at Martin Luther Chapel. Their friendship and advice have encouraged me and helped maintain my priorities.

My husband, Brian, contributed to this research materially, by helping me with sampling, lab work, and statistical advice. But more importantly, we have shared the development of both of our understandings of how we can be, individually and together, "cultivators of the garden" (Genesis 2:15). Thank you, Brian, for journeying with me through this first volume of a lifelong adventure.

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KEY TO ABBREVIATIONS

AIC	Akaike's Information Criterion
AL	Alfalfa Silage-based Diet
BCS	Body Condition Score
bm3	Brown-midrib 3 mutation
BW	Body Weight
CP	Crude Protein
DIM	Days in Milk
DM	Dry Matter
DMI	Dry Matter Intake
F:C	Forage-to-concentrate Ratio
FA	Fatty Acids
FCMY	Fat-corrected Milk Yield
forNDF	Forage NDF
forNDFD	30-h In Vitro Digestibility of Forage NDF
HF	High-forage Diet
iNDF	Indigestible NDF
k_d	Digestion Rate
k_p	Passage Rate
k_r	Rate of Particle Size Reduction
LF	Low-forage Diet
LSM	Least-squares mean

MN	Microbial N
MNE	Microbial N Efficiency
MUN	Milk Urea N
MY	Milk Yield
NAN	Non-ammonia N
NANMN	Non-ammonia, Non-microbial N
NDF	Neutral Detergent Fiber
NE_L	Net Energy of Lactation
OG	Orchardgrass Silage-based Diet
OM	Organic Matter
pdNDF	Potentially Digestible Neutral Detergent Fiber
pVDMI	Preliminary Voluntary Dry Matter Intake
RDN	Ruminally Degraded N
RMSE	Root Mean Square Error
SD	Standard Deviation
SEM	Standard Error of the Mean
TMR	Total Mixed Ration
TOT	Ruminal Turnover Time
TRD	Truly Ruminally Degraded
TRDOM	Truly Ruminally Degraded Organic Matter
TRSD	True Ruminal Starch Digestibility
TTDOM	Total tract digested Organic Matter
VDMI	Voluntary Dry Matter Intake

CHAPTER 1

A Review of Literature

INTRODUCTION

In ruminants, the rates at which nutrients escape the rumen greatly affect feed intake, ruminal digestibility, and microbial protein production in the rumen and flow to the duodenum. Therefore, passage rate is a primary factor determining the amounts and types of fuel and protein absorbed by the animal. In order to accurately predict nutrient availability, passage rate must be accurately estimated. Estimates of passage rate are incorporated into many mathematical models of dairy cow digestion used in formulating diets for dairy cows (Baldwin et al., 1987; Russell et al., 1992; NRC, 2001). These models estimate the availability of nutrients for milk production and other needs, given a particular set of feed ingredients, cow characteristics, milk production data, and environmental factors. The accuracy of these models is reduced by their inability to account for the effects of dietary characteristics on voluntary feed intake and on the passage rate (k_p) of particles from the rumen (Illius and Allen, 1994; Firkins et al., 1998). Without an accurate prediction of passage rate, models cannot account for the effects of particle passage rate on feed intake, ruminal nutrient digestibility, and flow of true protein to the duodenum.

Most models overestimate both digestion rate and passage rate, and underestimate ruminal pool size, because they rely on *in vitro* digestion and rare-earth or chromium marker passage data (Allen, 1996). Also, nearly all passage

data available in the literature were measured by analysis of fecal excretion curves of external markers that are applied to intact forages and(or) concentrates and then pulse dosed. The results of these fecal excretion curves are difficult to interpret. Two or more significant pools and rates can be determined, but it is not clear which rate represents passage from the rumen or even that assignment of the resulting mathematically defined pools to specific biological pools is valid. Furthermore, most data for digestion and passage kinetics have been collected using sheep and cattle with low feed intake, so their ability to predict diet effects on intake and passage rate in high-producing animals is limited. Although both digestion rate and passage rate need to be predicted accurately, the inaccuracy of passage rate prediction has a greater effect because passage rate affects not only ruminal digestibility but also feed intake and microbial protein flow to the duodenum.

Finally, current predictions of ruminal digestibility of digesta fractions (e.g., starch, neutral detergent fiber (NDF), and protein fractions) are calculated using the digestion rates of those fractions and the passage rates of the individual feed ingredients that contain those fractions. Using the passage rates of feed ingredients produces inaccurate predictions for ruminal digestibility of digesta fractions, because the different fractions within a feed ingredient escape the rumen at different rates. Ruminal digestibility is determined for digesta fractions, not for feed ingredients, so both the digestion rate and the passage used to predict digestibility ideally should be for digesta fractions, not for ingredients. Passage rate data for the various fractions have been either completely

unavailable or limited until recently, when the development and increasing use of the pool and flux method resulted in the production of much more data for passage rate of digesta fractions.

Therefore, this is a suitable time to explore the use of this new method to improve predictions of ruminal passage rate. This review of literature will describe the importance of predicting ruminal passage rate accurately, evaluate the methods available to measure passage rate, and describe some effects of nutrient demand and dietary factors on passage rate that could be measured using the pool and flux method.

PHYSICAL FILLING EFFECTS ON FEED INTAKE

Dietary characteristics affect feeding and digestion in dairy cows through both physical and chemical mechanisms. Physical controls include gut distension (Lehman, 1941), and limitations to time spent eating and ruminating (Allen, 2000). Ruminal fiber digestibility, rate of particle size breakdown, rate of increase in particle specific gravity, and the resulting fiber passage rate primarily determine the "filling" effect of a diet. Rates of digestion and passage for fiber are determined predominately by forage characteristics such as forage family (e.g., grass or legume), particle size, and quality (e.g., maturity, genetics, or environmental effects), so the physical control of feed intake depends largely on forage characteristics and the concentration of forage fiber in the diet. However, physical filling effects are not the only factors contributing to feed intake regulation. Altered fermentation acid production in the rumen, from a higher

proportion of grain in the diet or from inclusion of more rapidly-fermented grain, may also affect intake through chemical mechanisms and alter the digestion of a diet (Forbes, 1995). Excess production of fermentation acids, resulting in lower ruminal pH, can decrease fiber digestibility (Hoover, 1986), and excess ruminal propionate production can result in lower feed intake (Anil et al., 1993), possibly because of metabolism in the liver (Allen, 2000). The effects of most major dietary characteristics on feed intake and digestibility have been investigated extensively. Although the mechanisms have not been unraveled completely, the general effects of major dietary characteristics on nutrient availability are included in most models of ruminant metabolism used for diet evaluation. However, models do not attempt to predict the effects of major dietary characteristics on feed intake. Predicting the effects of diet on passage rate, and the subsequent effect on feed intake through physical filling effects, is beyond the capability of models currently in use.

RUMINAL NUTRIENT DIGESTIBILITY

Ruminal digestibility of a nutrient is a function of the fraction that is potentially digestible, the rate at which that fraction is digested, and the rate at which that fraction passes from the rumen. Digestibility increases as digestion rate increases or as passage rate decreases. Passage rate of digesta from the rumen increases with DMI resulting in lower digestibility of diets at greater DMI (NRC, 2001). Rates of digestion and passage also vary greatly across feed fractions such as starch and fiber. Ruminal digestion influences the chemical

form and temporal pattern in which fuels, amino acids, and other nutrients are absorbed (Allen, 2000). Slow fiber digestion can lead to increased ruminal distention and decreased VDMI (Jung and Allen, 1995). However, products of ruminal digestion such as propionate from starch fermentation can depress VDMI and thus reduce total nutrient supply. Supply of nutrients to the duodenum is affected directly by ruminal digestion. For example, individual FA flux to the duodenum is determined by the FA content of the diet, as well as rates of passage and biohydrogenation (Allen, 2000). In particular, as discussed below, protein absorption is highly dependent on ruminal fermentation because of microbial protein production in the rumen and extensive protein degradation by ruminal microbes. However, duodenal flux of microbial and non-microbial protein are highly variable across diets and are predicted poorly by current models (Firkins et al., 1998).

Because ruminal processes and products greatly affect nutrient intake and availability, digestion in and passage from the rumen must be predicted as accurately as possible (Firkins et al., 1998). Partitioning of feeds in a model into more homogeneous, chemically or biologically defined fractions would allow the use of table values, combined with empirical relationships for rates of digestion and passage. This is of key importance for models that can be adopted to formulate and evaluate diets. Models that predict digestibility using basic feed characteristics (e.g., feed type, particle size, or maturity) and empirically determined relationships for passage rate and digestion rate eventually should

be able to substitute for direct measurements of ruminal nutrient digestibility for individual feed ingredients (Firkins et al, 1998).

NITROGEN UTILIZATION AND EFFICIENCY

The effect of increasing the capability to predict (and manipulate) passage rate with the greatest likely potential environmental benefit is the connection between passage rate and microbial protein flow and efficiency. Ruminal microbial protein is the most significant source of amino acids for the lactating dairy cow, not only because of the quantity produced, but also because microbial protein is highly digestible and because its amino acid profile resembles ruminants' amino acid requirements (O'Connor et al., 1993). Within the rumen, proteins and amino acids are not only synthesized but are also constantly degraded as a result of bacterial cell death, enzymatic action of some bacterial species, and predation by ruminal protozoa. Much of the N in microbial amino acids is derived from non-protein N, adding to the economic and environmental value of microbial protein. Ammonia produced by amino acid degradation is absorbed into the blood through the rumen wall and is converted to urea in the liver, excreted in urine, or absorbed into the large intestine and excreted in feces, particularly as microbial protein. Urea may be secreted into saliva for reentry into the rumen milieu or excreted in urine (Owens and Zinn, 1988). Nitrogen recycling through urea and saliva is metabolically expensive and reduces the efficiency of feed energy and N utilization (Wells and Russel, 1996). Faster escape of bacteria from the rumen (shorter residence time) increases efficiency

of N and energy utilization by decreasing bacterial death and breakdown in the rumen. Until lately, this theory was supported primarily by *in vitro* and liquid dilution studies (Isaacson et al., 1975; Stouthamer and Bettenhaussen, 1973; Kennedy and Milligan, 1978), but recent experiments in our laboratory (Oba and Allen, 2003c; Voelker and Allen, 2003b) have confirmed the positive relationship between microbial efficiency and the passage rates of starch and of potentially digestible NDF *in vivo*. Microbial protein flow to the duodenum is limited by the availability of readily fermented feed for growth and by the ability of bacteria to avoid lysis and escape the rumen. Therefore, increasing passage rate of particles and bacteria from the rumen should cause increased microbial protein flow to the duodenum as a result of greater feed intake and microbial protein efficiency. Because the passage rate of fiber from the rumen also depends on nutrient demand (Voelker and et al., 2002), developing equations that reflect the relationships between a parameter measurable on commercial farms (DMI), passage rate, and microbial flow and efficiency should be possible and beneficial. Reducing N excretion and(or) N recycling both within the rumen and through the cardiovascular system can reduce the amount of N excreted per pound of milk produced, and therefore can reduce the amount of N fed. More efficient N utilization also increases the utilization of energy consumed, thus reducing not only nitrogenous waste but also the amount of total manure per pound milk produced.

In the future, improving the capability to predict passage rate of digesta fractions will lead to more accurate estimates of microbial protein flow and can

lead to more efficient N utilization for microbial protein production. Currently, estimated passage rates of feedstuffs are used in order to predict ruminal degradation and passage of N fractions in a diet evaluation model (NRC, 2001). Following the example of the Cornell Net Carbohydrate and Protein System (Sniffen et al., 1992) the NRC protein and Amino Acid model uses passage rate, along with degradation rate, to predict the extent of ruminal degradation of the fraction of feed N that is degradable, but not immediately degraded, in the rumen (labeled "fraction B"). Passage rate is predicted for individual feedstuffs using equations created for wet forages, dry forages, and concentrates from data obtained using rare earth markers. Increasing estimated passage rate results in lower estimated ruminally degraded N (RDN; NRC, 2001). Estimated RDN is used to determine the efficiency of production of microbial N (MN) from organic matter digested in the total tract (TTDOM). Because MN efficiency is adjusted for RDN, an increase in estimated passage rate leads to a decrease in estimated RDN, which, when used in the equation for predicting MNE, leads to an increase in estimated microbial efficiency. No direct adjustment is made to MN efficiency for the reduced ruminal proteolysis that would be caused by greater passage rate. The adjustment for RDN for passage rate does change microbial efficiency in the same direction (positive vs. negative) in which a direct adjustment for passage rate would change microbial efficiency. Therefore, the adjustment for RDN could reduce the need to adjust estimated MNE for an increase or decrease in estimated passage rate (e.g., increase estimated MNE when estimated passage rate increases). However, passage rates are estimated for individual

feedstuffs in order to estimate passage of undegraded feed N, not microbe-bearing digesta particles or liquid. Therefore, adjustment of RDN for feedstuff passage rate may not represent the effects of passage rates of all feed fractions –or of all feed ingredients- on microbial escape from the rumen. The use of digesta passage rates to predict MN efficiency would require the accumulation of a larger database, and likely requires the measurement of passage rates of digesta fractions such as solid and liquid, or feed fractions, rather than of individual feed ingredients.

The ability to predict and manipulate ruminal passage rate can greatly improve our ability to predict and exploit the effects of various feed ingredients on feed intake, site of digestion, and microbial protein flow and efficiency. While theories regarding the relationships between these parameters currently are integrated into models of dairy cow metabolism, little data exists to provide accurate prediction equations. Most importantly, these models do not account for the interactions between voluntary feed intake level and major dietary characteristics (such as forage and grain type, quality, and preservation or processing method, dietary forage fiber concentration, and particle size) in their effects on passage rate from the rumen.

TECHNIQUES FOR MEASURING PASSAGE RATE

The relationships between nutrient demand, voluntary feed intake and parameters such as particulate passage rate, ruminal digestibility, and ruminal microbial protein production and flow, have not been quantified because of the

practical difficulties of carrying out such experiments. Currently, models rely on *in vitro* digestion data using dry, ground feeds for rate of digestion and therefore overestimate digestion rate. Ruminal passage rate of an individual feed (wet forage, dry forage, or concentrate) is predicted using external markers that overestimate passage rate, underestimate ruminal pool size, and do not account for the existence of components within a single feed ingredient that have different ruminal digestion and passage kinetics.

External Passage-rate Markers

Nearly all passage data available in the literature were measured by analysis of fecal excretion curves of external markers applied to intact forages and(or) concentrates and pulse dosed. Furthermore, the majority of these data were obtained using external markers, and problems with these markers have been well-documented (Firkins et al., 1998). External passage-rate markers include rare earth labeled feeds, ruthenium, and chromium mordanted fiber. Usually, they are pulse-dosed into the rumen and their rate of disappearance from the rumen or their concentration in feces is used to estimate the passage rate of the feed with which they were dosed or assumed to travel. With this method, three assumptions must be made regarding the marker: (1) the marker flows with the intended feed ingredient, (2) the marker does not affect the rate of digestion or passage of the labeled feed, and (3) the marker reflects the passage rate of different chemical components (soluble and insoluble, digestible and indigestible) of the feed (Firkins et al., 1998). Metals increase the density of the particles to which they are attached, so if they remain attached to the feed, they

increase the passage rate of the feed particles. Markers also usually migrate extensively from the labeled feeds (Teeter et al. 1984; Combs et al., 1992), especially to microbial cell bodies, mucin, or other feeds, or into the liquid phase (Erdman and Smith, 1985; Allen, 1982; Van Soest et al., 1988), where their small size, weight and density cause them to escape the rumen quickly. Therefore, rare-earth-labeled feeds not only overestimate passage rate but also fail to represent the actual passage rate of the feed ingredient with which they are originally dosed. However, fecal excretion curves require no surgery or other invasive techniques, and ruminal marker disappearance techniques require only ruminal cannulation. Therefore these techniques carry relatively low cost and low risk, which allows data to be collected for many different feeds under many different conditions.

Pool and Flux Determination of Kinetics

An alternate method for estimating both ruminal passage and digestion rates uses the pool and flux of chemical components –indigestible NDF (iNDF), potentially digestible NDF (pdNDF) and starch- in the rumen and duodenum (Oba and Allen, 2000). In this method, a marker is dosed continuously or approximately continuously and used to estimate the flow of digesta dry matter at the duodenum, which is then used to calculate flow of pdNDF, iNDF, and starch. The fractional passage rates of a individual, uniform digesta fraction is calculated by dividing duodenal flux of the fraction by its ruminal pool size. The total rate of disappearance (turnover rate) of a digesta fraction from the rumen at steady state is calculated by dividing its rate of intake by its ruminal pool size. Then

digestion rate of the fraction is calculated by subtracting its passage rate from its turnover rate in the rumen. For example, if duodenal flux of pdNDF is 58 g/h and ruminal pool of pdNDF is 2300 g, then passage rate of pdNDF is 58/2300 h^{-1}, or 0.025 h^{-1}. If pdNDF intake is 188 g/h, then turnover rate of pdNDF is 188/2300 h^{-1}, or 0.082 h^{-1}. Digestion rate of pdNDF is the difference between turnover rate and passage rate, digestion rate is 0.082 h^{-1} – 0.025 h^{-1}, or 0.057 h^{-1}. Because the digestion rate of each fraction is calculated simultaneously with passage rate, digestion and passage rates are consistent with both pool size and ruminal digestibility (Oba and Allen, 2000).

The pool and flux technique is the only method that can be used to measure passage rate of individual feed fractions (e.g., starch, iNDF, and pdNDF) (Firkins et al., 1998). Although a marker (either external or intrinsic) is still required to determine duodenal flux, digesta flux markers have a great advantage compared to passage markers because they are not required to associate with any particular digesta or feed fraction. Passage markers must remain associated with the labeled feed and must not affect its passage rate; in contrast, digesta flux markers simply must not be absorbed and must represent a constant fraction of the duodenal digesta, requirements that can be attained with proper procedures (Firkins et al., 1998). This method cannot be used to determine the passage rate of an individual feed ingredient, and it assumes that (1) marker concentration in duodenal digesta is constant and (2) markers do not interfere with digestion or passage of digesta. In at least seven experiments in our laboratory, this method has given reasonable estimates of passage and

digestion rates that do not conflict with ruminal and whole-tract digestibility (calculated using internal and external markers) or with directly measured ruminal pool sizes (Oba and Allen, 2000; Oba and Allen, 2003a; Voelker and Allen, 2003a; Taylor and Allen, 2005; Harvatine and Allen, 2006; Ying and Allen, 1998, 2005; Ying et al., 1998; Mooney and Allen, 2004).

Alternatively, iNDF (determined by 240-h in vitro fermentation) can be used as an internal marker to determine pool size, and duodenal flux of iNDF is assumed to equal iNDF intake. This strategy removes the possibility of marker effects on passage or dosing problems, but it requires the assumptions that measured iNDF is truly indigestible and not created in the digestive tract, and that iNDF flows with other digesta fractions. The primary drawback to the pool and flux method is the expense and complexity of ruminally and duodenally cannulating high-producing dairy cattle.

Duodenal and Omasal Sampling

One attempt to reduce the cost and risk required to directly measure ruminal digestibility is to sample digesta in the omasal canal (Huhtanen et al., 1997). Omasal sample digesta samples can be obtained through the ruminal cannula, so cows do not need to have a second cannula added at the duodenum (or abomasum, or omasum). Also, digesta obtained at the omasal canal has not been exposed to absorption in the omasum or the secretions and digestion of the abomasum. This is particularly important for studying the absorption of water, minerals, or ruminal fermentation acids (i.e., volatile fatty acids and lactate). Furthermore, less-invasive and lower-cost animal preparation may permit greater

numbers of animals to be used to measure ruminal digestibility (Firkins et al., 1998). However, a major shortcoming of omasal sampling is the collection of samples that are not representative of digesta that enters the omasal canal. Because the samples are obtained under vacuum, differential collection of digesta particles of various sizes, and of fluid, occurs (Ahvenjärvi et al., 2001). Double and triple markers have been used to "reconstitute" omasal digesta from two or three phases (i.e., fluid, small particles, and large particles; Huhtanen et al., 1997; Ahvenjärvi et al., 2000). However, because some markers appear to demonstrate affinity not only for particle sizes but also for particular chemical fractions of digesta (Ahvenjärvi et al., 2003), the marker selected might measure the flow of only one of a group of fractions of interest.

A second complication of omasal canal sampling is evidence of significant backflow of particles from the omasum to the reticulorumen (Mathison et al., 1995). If backflow occurs, then even if samples are "correctly" collected during the second reticular contraction, they still likely contain particles that will be returned to the reticulorumen again before finally passing through the omasum and arriving in the duodenum (Firkins et al., 1998). In a comparison of omasal and duodenal sampling (Ahvenjärvi et al., 2000), OM flow was lower, and NDF flow was greater, at the omasal canal than at the duodenum. This could have been caused by either backflow or marker affinity. Finally, placement of sampling equipment in the omasal canal might interfere with flow through the omasum (Firkins et al., 1998) and the residing tube through which samples are

obtained at the ruminal cannula might interfere with movement of digesta within the rumen.

Nonetheless, the omasal sampling technique introduced in 1997 (Huhtanen et al.) and modified in 2000 (Ahvenjärvi et al.) has been utilized to study ruminal protein degradation and utilization (Choi et al., 2002; Reynal and Broderick, 2003; Reynal et al., 2003; Noftsger et al., 2005), the effects on ruminal digestion of forage type (Onetti et al., 2004; Ahvenjärvi et al., 2006) or supplementation of forage diets with grain (Ahvenjärvi et al., 2002), and ruminal digestion of ruminal biohydrogenation of fatty acids (Lundy et al., 2004). Because of the differential sampling of particulate digesta, the potential for backflow, and the likelihood of marker affinity for chemical constituents within particulate digesta, the omasal sampling technique is not appropriate for measuring the effects of forages on passage rate, microbial efficiency, and particle size reduction.

Most models of dairy cow digestion and metabolism rely on data obtained using *in vitro* data for rate of digestion and labeled feeds in ruminally cannulated animals for passage rate. Furthermore, most data in the literature from duodenal experiments involve non-lactating or low-producing cattle, or sheep, so they cannot accurately predict responses for high-producing dairy cattle with much higher feed intake and passage rate, and are therefore not sufficient for use in models. If the relationships between nutrient demand, digestion and passage can be described in high-producing cows using equations containing easily

measured parameters, a large gap in the mathematical models of the metabolism of dairy cows will be filled.

PASSAGE RATE RESPONSE DEPENDS ON NUTRIENT DEMAND

Responses of digestion parameters to dietary characteristics and the extent to which physical or metabolic factors limit intake are dependent on individual energy balance (Mertens, 1994; Allen, 1996). Therefore, testing only treatment means may not detect important responses in intake, digestibility, and production (Allen, 2000). Because cows are now frequently grouped and fed according to milk yield, models that predict the effects of nutrient demand on response to diet are even more necessary. Oba and Allen (1999a) fed diets containing a normal corn silage and one with a greater in vitro fiber digestibility, and used pretrial milk yield as an indicator of nutrient demand independent of experimental treatments. They found that individual milk yield and feed intake responses to the diet containing corn silage with greater fiber digestibility increased linearly with pretrial milk yield (Figure 1). That is, cows for whom intake was more likely to be limited by fill (high pretrial milk yield) responded more positively to greater fiber digestibility than did cows whose intake was less likely to be limited by fill (low pretrial milk yield).

This varied response to a diet characteristic was confirmed by another experiment in our laboratory (Voelker et al., 2002) that was designed specifically to test the relationship between milk yield measured during a preliminary period and response to a pair of treatments. Individual responses of intake and milk

production to a change in forage-to-concentrate ratio were dependent on preliminary milk yield (Figure 2). Furthermore, feed intake and milk production were not the only responses affected by preliminary milk yield. The same experiment also demonstrated that individual responses of NDF turnover in the rumen to concentration of forage fiber in the diet depend on preliminary milk yield (Voelker et al., 2002). This suggests that the ruminal digestion and passage kinetics of at least fiber, and probably other nutrients, respond to changes in dietary characteristics in a manner dependent upon nutrient demand.

It has been demonstrated that passage rate from the rumen increases with increased DMI (Riewe and Lippke, 1970). This is likely through effects of increased distension on rate of reticular contractions (Dado and Allen, 1995) and on amplitude and duration of reticular contractions (Okine and Mathison, 1991). An important implication of this relationship is the reduction of diet digestibility at DMI above 4X maintenance (NRC, 2001). However, data regarding this relationship are lacking for high producing cows, and the linear relationship should not be extrapolated beyond the limits of the VDMI in the existing data (NRC, 2001). In fact, Van Soest et al. (1992) suggested that the decline in digestibility with increasing VDMI is not linear, but rather that digestibility decreases at a decreasing rate as VDMI increases. Therefore, any measurement of the effects of diet characteristics on ruminal passage rate and the resulting effects on digestibility must also account for effects of diet on the relationship between VDMI and passage rate.

However, a large number of duodenally cannulated, lactating dairy cows with a wide range of DMI (including high producing animals) is required to detect statistically the effect of voluntary feed intake on passage rate and other ruminal and post-ruminal digestion parameters in lactating dairy cows. This type of experiment previously was nearly impossible physically (because of the usually inevitable negative effects of cannulation on milk yield) or fiscally (because of the large number of cannulated animals required). During previous experiments conducted in our laboratory, ruminally and duodenally cannulated cows have maintained unusually high VDMI (up to 32 kg/d) and milk yields (up to 57 kg/d), and duodenal cannulae are left in place at the end of experiments. This presents a unique opportunity to generate data that are not only essential for further improvement of models of dairy cow metabolism and diet evaluation but are also very difficult to obtain given the typical low milk production of duodenally cannulated animals and the difficulty of maintaining the number of cannulated cows necessary to conduct such experiments. At the same time, we have the capability of testing hypotheses that have existed for decades as assumptions in applied ruminant intake and digestion as well as new hypotheses raised by recent intake-related experiments in our own laboratory.

This experimental design has already been used in additional experiments in our laboratory to measure the effects of individual nutrient demand on response to treatments within a group of cows (Harvatine and Allen, 2002; Bradford and Allen, 2004). Most of these previous experiments have used preliminary milk yield to represent nutrient demand; however, preliminary VDMI

also can be used for the same purpose. Milk yield and feed intake are inextricably linked, both statistically (Fuentes-Pila et al., 2003) and biologically, and the two parameters are both interdependent and influenced by other factors. That is, feed intake does, to some extent, determine milk yield, and the demand for nutrients to produce milk does drive feed intake. Although preliminary milk yield was selected as the predictor of responses to diet forage concentration for publication (Voelker et al., 2002), responses were also dependent on preliminary DMI (data not shown). For future experiments addressing passage rate and nutrient digestion responses, DMI is the more logical predictor of passage rate because, with respect to physiology, DMI is more directly linked to passage rate. Although DMI typically is not measured for individual cows on dairy farms, DMI is often measured at the same level of aggregation for which diets are formulated: by pen or by group. Therefore, the use of DMI to predict passage rate, ruminal digestion, and nutrient flux to the duodenum under practical conditions bears no disadvantage compared to the use of milk yield.

DIETARY FACTORS AND PASSAGE RATE

The NRC subcommittee recognized that "intrinsic properties of feeds such as particle size and density" affect passage rate but concluded that "data are too sparse to make adjustments for those factors" (NRC, 2001). Feed properties of forages include forage family (grass versus legume), diet forage-fiber concentration, maturity, NDF lignification, and particle size.

Grass Versus Legume Forage

Because perennial grass fiber generally is more thoroughly digested than legume fiber, we might expect greater feed intake, passage rate, and milk yield for cows fed grass. However, despite greater digestibility for grass fiber, a meta-analysis of data from experiments using dairy cows demonstrated lower feed intake and milk yield for grass-based diets than for legume-based diets, across maturities, despite greater fiber digestibility for grass (Oba and Allen, 1999b). This indicates that cows usually will produce less milk when fed grass due to reduced feed intake. We believe that feed intake is more limited for grass forage because of its filling effect, caused by slow particle breakdown and slow passage rate. Grass fiber and alfalfa fiber have different chemical compositions, anatomical characteristics, and digestion characteristics that affect both the rate and extent of their digestion (Allen, 1996; Wilson and Kennedy, 1996). Because grass fiber generally contains less lignin than alfalfa at the same maturity, grass fiber ultimately is more digestible. However, grass fiber is also digested more slowly, and its cell walls are more resistant to particle breakdown than are alfalfa cell walls (Wilson and Hatfield, 1997). Because grass fiber is more digestible but also more slowly digested, it is also hypothesized that more gas is associated with grass particles than with alfalfa particles, thus reducing the specific gravity of grass over time relative to alfalfa (Allen, 1996). Passage rate increases as density increases and as particle size decreases, so alfalfa particles probably escape the rumen more quickly than grass particles.

Dietary Forage Fiber Concentration

The concentration of forage fiber in a diet affects feeding and digestion in dairy cows through both physical and chemical mechanisms, including gut distension (Lehman, 1941), limitations to time spent eating and ruminating (Allen, 2000), altered fermentation acid production in the rumen, and additional propionate production (Anil et al., 1993; Allen, 2000). Others investigating the effects of varying forage to concentrate ratios have reported either increased DM digestibility for diets with lower forage contents (Dado and Allen, 1995) or no significant effect (Alhadhrami and Huber, 1992; Wheeler et al., 1975). Diets with high forage fiber contents produce a larger rumen mat than do low forage diets, so feed particles of any size may be retained in the rumen longer and, therefore, be more completely digested (Grant, 1997). High-forage diets also generally result in higher rumen pH, which permits more extensive rumen microbial fermentation (Hoover, 1986). As discussed previously, responses of feed intake, milk yield, and fiber turnover kinetics to dietary forage content were dependent on preliminary milk yield (Voelker et al., 2002). Differences between low-forage and high-forage diets for whole-tract digestibility of starch and NDF were not related to preliminary milk yield, possibly due to post-ruminal compensatory digestion. However, the cows in that experiment were ruminally, but not duodenally, cannulated, so ruminal digestibility, ruminal passage rates, and microbial efficiency were not measured. It is anticipated that the responses of starch and NDF passage rates, ruminal digestibility, and microbial efficiency will also depend on preliminary milk yield.

Forage Maturity

With greater maturity, the concentration of fiber in a plant typically increases, and fiber digestibility decreases due to greater lignification. This not only decreases the potentially digestible NDF (pdNDF) concentration but also decreases digestion rate of the remaining pdNDF (Smith et al., 1972). This suggests that more mature forages will result in slower passage rates from the rumen and greater ruminal distension. 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, contrary to the expected effects of distention. Therefore, it is likely that a combination of factors including ruminal distension, rate of particle breakdown, and the individual animal's drive to eat will determine the responses of passage rate and intake to forage maturity. Increased maturity is expected to have a greater negative effect on intake and passage rate as milk production and nutrient demand increases, when fill is more likely to limit feed intake.

Fiber Digestibility

The brown midrib 3 mutation (*bm3*) in corn reduces lignin by ~40% with little effect on NDF or other components (Allen et al., 2003). When *bm3* corn silage and an isogenic normal corn silage were fed to 32 lactating cows, the response of feed intake and milk yield to the more-digestible *bm3* was positive, but a much greater response was elicited among cows with greater preliminary milk yield so that a positive, linear relationship existed between the individual milk

yield averaged over 14 d prior to the start of the experiment and the individual responses of intake or milk yield to *bm3* (*bm3* – control; Oba and Allen, 1999a). These cows were not cannulated, so ruminal digestion kinetics and microbial protein flow and efficiency were not measured. A second experiment (Oba and Allen, 2000) among relatively high-producing cows found that the more digestible fiber of *bm3* resulted in faster passage, possibly due to increased particle fragility, but did not change rate of NDF digestion or NDF digestibility. When fed to cows with a wide range in milk yields and feed intake, *bm3* should have a greater positive effect on feed intake and particulate passage rate among higher-producing cows but might actually slow passage rate for low-producing cows. The more rapidly fermented diet could reduce pH or cause other metabolic responses that would reduce ruminal motility, decrease reticular contractions, or shorten the duration of reticular contractions. Among cows with lower milk yield, more rapid ruminal fermentation of *bm3* might cause a negative response to *bm3* versus normal silage because for those cows intake likely will be limited more by metabolic factors than by physical fill. Therefore, for low-producing cows, greater fermentation acid production from *bm3* might reduce passage rate and feed intake.

Particle Size

Forage particle size influences the rate of particulate passage (Rodrigue and Allen, 1960) because ruminal retention time increases greatly with increasing digesta particle size (Dixon and Milligen, 1985) and because mat formation by long forage particles increases ruminal retention and digestibility of smaller

particles from other feeds (Grant, 1997). Longer particles generally are retained longer in the rumen and therefore reduce passage rate and feed intake. Greater ruminal distention caused by longer forage particles is more likely to affect passage rate and feed intake among cows with the highest nutrient demand, when feed intake becomes increasingly limited by ruminal fill.

Other Dietary Factors

Forages are not the only dietary ingredients that might affect passage rate. Passage rate also may be influenced by physical and chemical variations in grain (Oba and Allen, 2003a), non-forage fiber sources (Voelker and Allen 2003a), dietary fat and its composition (Nicholson and Omer, 1983; Allen, 2000; Harvatine and Allen, 2006), and ionic supplements such as sodium bicarbonate (Okeke et al., 1983; Woodford and Murphy, 1988; Martin and Michalet-Doroeau, 1996). Compared to forages, fewer data exist to describe the effects of these factors on passage rate.

CONCLUSION

Nutrient availability in the rumen is affected by ruminal rates of digestion and passage of feeds. Although it is accepted that ruminal passage rate of nutrients increases with voluntary feed intake and also is affected by forage characteristics, existing models do not adequately account for these effects because the necessary data do not exist. The recently developed pool and flux method allows direct, simultaneous measurement of the rates of digestion and passage for nutrient fractions, so data obtained using this method can greatly

improve the accuracy of models in predicting nutrient availability. Improving these models will aid in reducing excretion of nutrients as waste products.

RESEARCH OBJECTIVES

Therefore, the research reported in this dissertation was designed to further examine the interactions of feed intake and forage-related diet characteristics in affecting passage rate, and to summarize the passage rate data currently available in our laboratory in a form that can be used on dairy farms or in further research. Toward that end, the objective of the first experiment (Chapters 2 and 3) was to determine the effects of voluntary feed intake on responses to a grass forage (orchardgrass silage) compared with a legume forage (alfalfa silage). The objective of the second experiment (Chapter 4) was to investigate the effects of voluntary feed intake on responses to diets containing either high or low concentrations of forage NDF. Finally, empirical models were developed to predict the passage rates of the two NDF fractions, iNDF and pdNDF (Chapter 5), and the passage rate of starch (Chapter 6), using data from 11 experiments conducted in our laboratory using the pool and flux method.

Figure 1. Relationship between mean 3.5% fat-corrected milk yield (FCMY) over 4 d prior to the beginning of the experiment and the response to the brown-midrib (*bm3*) over normal corn silage (*bm3* - control) in (A) DMI ($DMI_{bm3} - DMI_{control}$ = -4.4 + 0.15 x pretrial milk yield; $P < 0.06$) or (B) FCMY ($FCMY_{bm3} - FCMY_{control}$ = -13.2 + 0.37 x pretrial milk yield; $P < 0.03$). (Oba and Allen, 1999a)

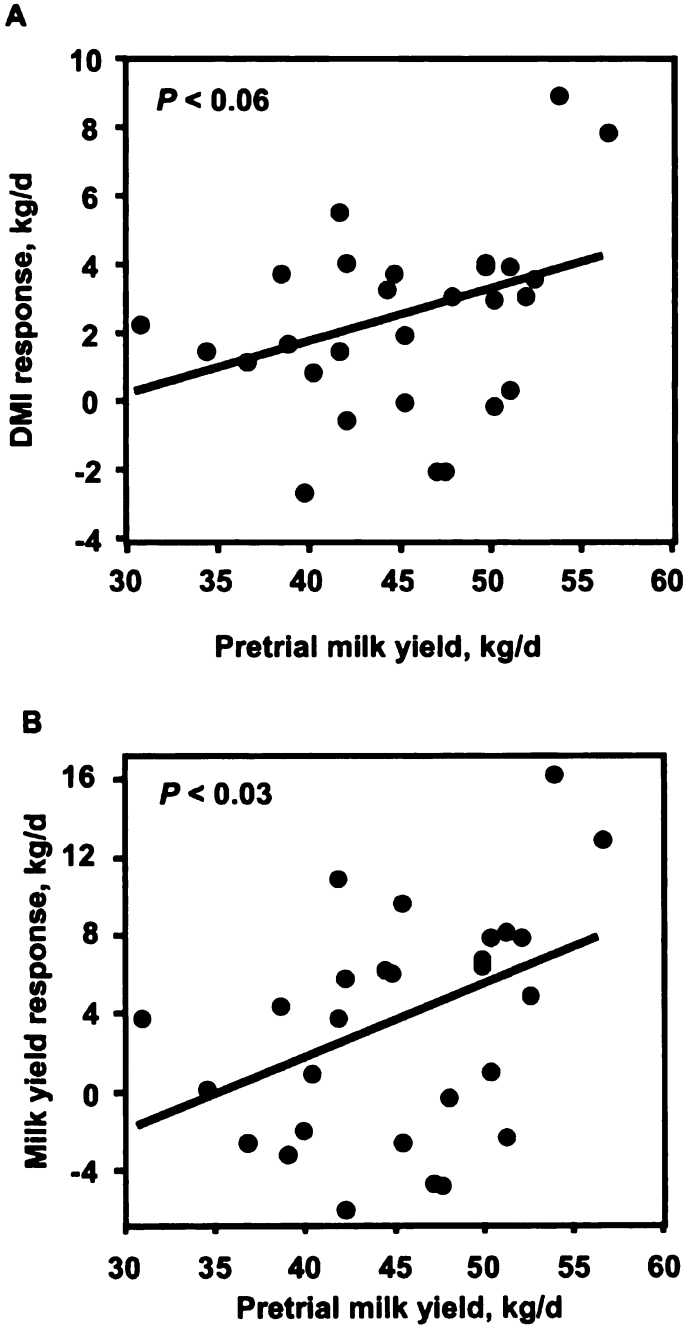
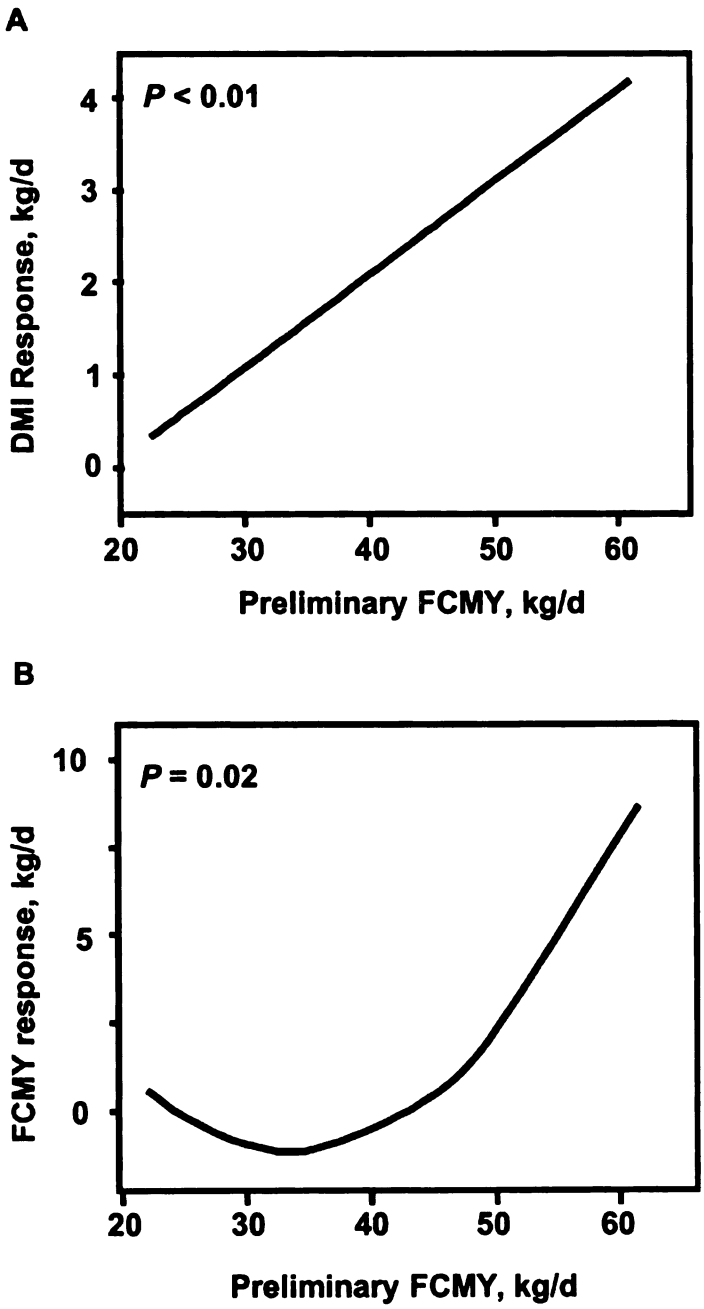


Figure 2. Relationship between mean 3.5% fat-corrected milk yield (FCMY) over 4 d prior to the beginning of the experiment and the response to the low-forage over the high-forage diet (LF - HF) in (A) DMI ($DMI_{LF} - DMI_{HF} = -2.1 + 0.10$ x preliminary milk yield; $P < 0.01$) or (B) FCMY ($FCMY_{LF} - FCMY_{HF} = 18.2 - 1.13$ x preliminary milk yield + 0.02 x (preliminary milk yield)^2; $P = 0.03$). (Voelker et al., 2002)



CHAPTER 2

Nutrient Demand of Lactating Dairy Cows Affects Feed Intake and Nutrient Utilization Responses to Diets Containing Alfalfa or Orchardgrass

ABSTRACT

The effect of preliminary feed intake on responses to diets containing alfalfa silage or orchardgrass silage was evaluated using eight ruminally and duodenally cannulated Holstein cows in a crossover design with two 15-d periods. Responses measured were DMI, rates of fiber digestion and passage, and milk production. Cows were 139 ± 83 (mean ± SD) DIM at the beginning of the preliminary period. During the 14 d preliminary period, milk yield ranged from 24.5 to 46.0 kg/d (mean = 37.0 kg/d) and preliminary voluntary DMI (pVDMI) ranged from 11.4 to 21.0 kg/d (mean = 17.5 kg/d). The two treatments were a diet containing alfalfa silage as the sole forage (AL) and a diet containing orchardgrass silage as the sole forage (OG). Alfalfa silage contained 43% neutral detergent fiber (NDF; DM basis) and orchardgrass silage contained 48% NDF; diets contained ~23% forage NDF and 27% total NDF, so forage-to-concentrate ratio was 53:47 for AL and 48:52 for OG. Digestibility of NDF was lower for AL in the rumen and whole tract, and milk fat concentration was greater for OG than for AL. Mean 3.5% fat-corrected milk yield (FCMY) and DMI were not different between AL and OG, but individual FCMY and DMI responses to AL over OG were correlated positively with individual pVDMI values. A more positive DMI response to AL over OG among high-pVDMI cows was permitted by

a more positive response in ruminal NDF turnover rate for AL over OG as pVDMI increased. This response in NDF turnover rate was because of a differential response in rate of passage rather than digestion; indigestible NDF passage rate response tended to increase with increasing pVDMI, but potentially digestible NDF digestion rate response did not change as pVDMI increased. Therefore, the effects of alfalfa and grass forages on intake, fiber digestion, and milk production depended on the extent to which fill limited intake of an individual cow.

INTRODUCTION

A meta-analysis of data from experiments using dairy cows demonstrated lower voluntary DMI (VDMI) and milk yield for grass-based diets than for legume-based diets, across maturities, despite greater NDF digestibility for grass (Oba and Allen, 1999b). Although grass NDF usually is more digestible than alfalfa NDF, grass NDF is digested more slowly than alfalfa NDF, and grass cell walls are more resistant to particle breakdown than are alfalfa cell walls (Wilson and Hatfield, 1997). Therefore, we hypothesize that the reduction in VDMI seen for grass-based diets is because of the filling effect caused by slower particle breakdown and slower passage rate in grass forages.

However, individual energy balance influences both feed intake responses to forage characteristics and the extent to which physical or metabolic factors limit VDMI (Mertens, 1994; Allen, 1996). The effects on feed intake of diet characteristics (such as forage family) that influence ruminal passage rate of digesta will depend on the extent to which physical filling effects limit feed intake

in an individual animal. As a result, 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 the effects of nutrient demand on response to diet are even more necessary. We developed and have successfully used an experimental model to evaluate effects of pVDMI, an index of nutrient demand, on animal responses to dietary treatments (Oba and Allen, 1999a; Burato et al., 2001; Voelker et al., 2002; Harvatine and Allen, 2002; Bradford and Allen, 2004). This model was utilized to test our hypothesis that pVDMI affects individual responses of VDMI and digesta passage rate to diets containing grass silage or alfalfa silage as the sole forage.

MATERIALS AND METHODS

Cows and Treatments

Experimental procedures were approved by the All University Committee on Animal Use and Care at Michigan State University. Eight 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 a 14 d preliminary period and two 15 d experimental periods. These eight cows were 138 ± 83 (mean ± SD) DIM at the beginning of the preliminary period (Table 1) and were selected deliberately to provide a wide, uniform distribution of preliminary milk yield and DMI (Figure 1). During the 14 d preliminary period, milk yield ranged from 24.5 to 46.0 kg/d (mean = 37.0 kg/d)

and pVDMI ranged from 11.4 to 21.0 kg/d (mean = 18.6 kg/d). Cows were cannulated ruminally and duodenally prior to calving. Surgery was performed at the Department of Large Animal Clinical Science, College of Veterinary Medicine, Michigan State University. Cows were housed in tie-stalls and fed once daily (1100 h) at 110% of expected intake.

The two treatments were a diet containing alfalfa silage as the sole forage (AL) and a diet containing orchardgrass silage as the sole forage (OG). Alfalfa and orchardgrass forages were raised at the campus farm at Michigan State University (East Lansing) and ensiled in Ag-Bags (Ag-Bag Systems, Inc., St. Nazianz, WI). Alfalfa was harvested at early bud stage, chopped at 3/8" (0.95 cm) theoretical length of cut, and ensiled at 36% DM. Orchardgrass was harvested at early boot stage, chopped at 1/4" (0.64 cm) theoretical length of cut, and ensiled at 37% DM. Cut lengths were selected to yield similar particle size distributions using the Pennsylvania State Particle Size Separator (NASCO, Fort Atkinson, WI). Proportions of fresh-chopped forage retained on the top pan varied greatly by sample batch and averaged 20.1% for alfalfa and 28.1% for orchardgrass. Mean total mass retained on the top and middle pans were similar for alfalfa (62%) and orchardgrass (58%).

Nutrient composition for alfalfa silage and orchardgrass silage are shown in Table 2. During the sample collection periods, alfalfa silage contained 43% NDF (DM basis) and orchardgrass silage contained 48% NDF. Diets AL and OG were formulated to contain 23% forage NDF and 27% total NDF, so forage-to-concentrate ratios (DM basis) were 53:47 for AL and 48:52 for OG (Table 3).

The diet fed during the preliminary period was formulated so that alfalfa silage and orchardgrass silage each contributed 50% of forage NDF. Diets also contained dry ground corn, soybean meal (48% CP), expeller-processed soybean meal, a vitamin-mineral premix, and blood meal; limestone, urea, and bloodmeal were used to compensate for lower measured CP and anticipated Ca concentrations in orchardgrass silage than in alfalfa silage. All diets were formulated for 18% dietary CP and fed once daily as totally mixed rations. During the experimental periods, orchardgrass silage CP concentration was similar to alfalfa silage CP concentration, so dietary CP was 0.5% higher on a diet DM basis in OG than in AL.

Data and Sample Collection

Amounts of feed offered and orts were weighed for each cow daily. Samples of all dietary ingredients (0.5 kg) and orts from each cow (12.5% of orts) were collected daily on d 11 to 13 and combined into one sample per period. Cows were milked twice daily in a milking parlor (0300 and 1500 h); milk yield was measured, and milk was sampled, at each milking on d 11 to 13. Rumen-empty BW was measured after evacuation of ruminal digesta on d 14 of the preliminary period, and on d 15 of each experimental period. Body condition score was determined on the same days by three trained investigators blinded to treatments (Wildman et al., 1982; five-point scale where 1 = thin and 5 = fat).

Duodenal samples (1,000 g), fecal samples (500 g), and rumen fluid samples for pH (100 mL) were collected every 9 h from d 11 to d 13 so that eight samples were taken for each cow in each period, representing every 3 h of a 24-

hour period to account for diurnal variation. Rumen fluid was obtained by combining digesta from 5 different sites in the rumen and straining it through a layer of nylon mesh (~1 mm pore size). Fluid pH was recorded immediately. All samples were stored at -20°C.

Ruminal contents were evacuated manually through the ruminal cannula at 1600 h (5 h after feeding) on d 14 and at 0700 h (4 h before feeding) on d 15 of each period. Total ruminal content mass and volume were determined. During evacuation, 10% aliquots of digesta were separated to allow accurate sampling. Aliquots were squeezed through a nylon screen (1 mm pore size) to separate 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 and Statistical Analyses

Diet ingredients, orts, and feces were dried in a 55°C forced-air oven for 72 h. All samples were ground with a Wiley mill (1mm screen; Authur 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 into "snow" using a commercial food processor (84142 Food cutter, Hobart Manufacturing Co., Troy, OH) and sub-sampled in the frozen state to obtain representative samples. These duodenal subsamples and the 350 mL ruminal solid and liquid samples were lyophilized (Tri-Philizer™ MP, FTS Systems, Stone Ridge, NY) 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), CP, and starch. Ash concentration was determined after 5 h oxidation at 500°C in a muffle furnace. Concentrations of NDF were determined according to Van Soest et al. (1991, method A). Indigestible NDF was estimated as NDF residue after 120-h in vitro fermentation (Goering and Van Soest, 1970). Ruminal fluid for the in vitro incubations was collected from a non-pregnant dry cow fed only alfalfa hay. 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 a forced-air oven for more than 8 h. Milk samples were analyzed for fat, true protein, and lactose with infrared spectroscopy by Michigan DHIA (East Lansing).

Indigestible NDF was used as an internal marker to estimate nutrient digestibility in the rumen and in the total tract (Cochran et al., 1986), and to estimate rates of passage for iNDF, pdNDF, and starch, and rates of digestion for pdNDF and starch. Nutrient intake was calculated using the composition of feed offered and refused. Ruminal pool sizes (kg) of OM, NDF, iNDF, pdNDF, and starch were determined by multiplying the concentration of each component by

the ruminal digesta DM mass (kg). Turnover rate in the rumen, passage rate from the rumen, and ruminal digestion rate of each component (%/h) were calculated by the following equations:

Turnover rate in the rumen (%/h) =

100 x (Intake of component / Ruminal pool of component) / 24

Passage rate from the rumen (%/h) =

100 x (Duodenal flow of component / Ruminal pool of component) / 24;

and

Digestion rate in the rumen (%/h) =

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

To determine differences between treatments, all data were analyzed using the fit model procedure of JMP® (Version 5.1.2, SAS Institute, Cary, NC) according to the following model:

$$Y_{ijk} = \mu + C_i + P_j + T_k + PT_{jk} + e_{ijk}$$

where

μ = overall mean,

C_i = random effect of cow (i = 1 to 8),

P_j = fixed effect of period (j = 1 to 2),

T_k = fixed effect of treatment (k = 1 to 2),

PT_{jk} = interaction of period and treatment, and

e_{ijk} = residual, assumed to be normally distributed.

To correlate response to treatment with pVDMI, the response (Y) was calculated as follows:

$Y = y_{AL} - y_{OG}$.

where

y_{AL} = response for AL diet

y_{OG} = response for the OG diet

Preliminary VDMI was calculated as the mean of DMI values on d 11 to 14 of the 14-d preliminary period. Relationships between response to treatment and pVDMI were analyzed according to the following model:

$$Y_i = \mu + S_i + V + V^2 + e_i$$

Where

$Y_i = y_{AL} - y_{OG}$

μ = overall mean,

S_i = effect of sequence (i = 1 to 2),

V = pVDMI

V^2 = $pVDMI^2$

e_i = residual, assumed to be normally distributed.

Significance was declared at or below $P = 0.05$, and tendencies were declared at or below $P = 0.10$. In the pVDMI model, sequence effect (Seq) was removed when $P < 0.25$ and $pVDMI^2$ effect was removed when $P < 0.15$. Prediction equations reported are adjusted for Seq (Seq[a] = AL, OG; Seq[b] = OG, AL and was set at 0).

The original sample size was 13 cows; data from five cows were excluded from statistical analysis. One cow developed hypocalcemia during the experiment, two were removed from the trial due to duodenal cannula

malfunction, one was excluded because feed intake decreased by 50% on d 11 of Period 2 for undetermined reasons (intake slowly returned to normal on the same diet), and one was excluded because several key digestion parameters were outside the 95% confidence interval. None of the causes for removal or exclusion were believed to be associated with either of the two treatments. Among the remaining eight cows, each of the two treatment sequences was represented by four cows. Data in Table 1 and Figure 1 are for the eight cows used in the statistical analysis.

RESULTS AND DISCUSSION

In Vivo and In Vitro NDF Digestibility

Enhanced NDF digestibility of forages usually improves DMI in high-producing dairy cows (Oba and Allen, 1999b). The alfalfa silage used in this experiment was of moderate quality, containing 42.6% NDF and 25.2% iNDF; and the orchardgrass was high-quality, containing 48.0% NDF and 13.1% iNDF. In vitro NDF digestibility (30-h) was much greater for orchardgrass silage (61.1%) than for alfalfa silage (29.4%). Digestibility of NDF was lower for AL than for OG in both the rumen (37.4 vs. 57.1%; $P < 0.0001$) and the whole gastrointestinal tract (32.8 vs. 55.9%; $P < 0.0001$; Table 4). This is consistent with previous comparisons of grasses and legumes in lactating cows (Hoffman et al., 1998; Holden et al., 1994; Weiss and Shockey 1991).

Intake and Ruminal NDF Kinetics

Despite greater NDF digestibility of grass in many studies, VDMI and MY of lactating dairy cows are usually lower for grass-based diets than for legume-based diets (Oba and Allen, 1999b). However, in this experiment, mean DMI (20.4 kg/d) and NDF intake (5.4 kg/d) were not different between AL and OG ($P > 0.46$; Table 4). Feed intake is regulated by a combination of mechanisms, including physical filling effects and metabolic satiety, and the dominant limiting factor varies depending on nutrient demand. These cows represented a wide range in pVDMI, which was used as an estimate of nutrient demand, and their responses to forage family depended on pVDMI. Individual DMI responses to AL over OG (DMI_{AL} - DMI_{OG}) were related positively to individual pVDMI values (Figure 2a; DMI_{AL} - DMI_{OG} = -16.8 + 0.95 x pVDMI; $P < 0.01$). As pVDMI increased, DMI increased when cows were fed AL ($P = 0.05$) but not when they were fed OG ($P = 0.73$). This suggests that the mechanism by which cows are able to increase feed intake was more impaired among cows with higher pVDMI when they were fed OG. In fact, NDF turnover time (TOT) in the rumen decreased more for AL than for OG as pVDMI increased (Figure 2b; TOT_{AL} - TOT_{OG} = 157 + 2.30 x Seq - 1.60 x pVDMI; $P < 0.05$). The faster disappearance of NDF from AL reduced the physical filling effects for AL more than was possible for NDF from OG.

Decreased NDF turnover time may result from an increase in digestion rate and(or) passage rate. In this case, the decreased turnover time was solely because of a differential response in passage rate. Indigestible NDF passage

rate response ($k_{pAL} - k_{pOG}$) tended to increase with increasing pVDMI (Figure 2c; $k_{pAL} - k_{pOG}$ = -1.44 -0.10 x Seq + 0.10 pVDMI; $P = 0.06$), but pdNDF digestion rate response ($k_{dAL} - k_{dOG}$) did not change as pVDMI increased ($P = 0.47$). Therefore, among cows with greater drive to eat (greater pVDMI), mechanisms permitting greater passage rate of NDF for AL allowed actual DMI to more closely match demand.

Passage rate from the rumen can be increased by increased reticular contractions (Okine and Mathison, 1991), and this does occur with greater ruminal distention (Dado and Allen, 1995). Reticular contractions were not measured, because such measurements may interfere with flow of digesta within and from the reticulorumen (Kaske and Midasch, 1994). Among measures of rumen volume and mass, including NDF pool (kg), rumen pools were similar both between treatments (Table 4) and across the range of pVDMI (data not shown). This suggests that cows were unable to increase rumen pool size in order to allow greater feed intake; that is, physical fill likely was a primary factor limiting feed intake among cows with greater pVDMI. Without an increase in ruminal pool, the only means to increase feed intake was to increase rates of passage and(or) digestion. As demonstrated, cows with greater pVDMI were able to increase passage rate on AL, but not on OG, in order to allow greater intake.

Primary limitations to escape of particles from the rumen are particle size and particle density. Both rate of particle size reduction and rate of increase in particle specific gravity are likely faster in legume forages than in grasses. *Partic*les of legume, and specifically alfalfa, have been demonstrated to be more

fragile than particles of grass (Waghorn et al., 1989; Chai et al., 1984). Chewing during eating reduced 61% of alfalfa particulate DM, but only 46% of ryegrass particulate DM, to a size able to pass a 2 mm sieve, and ryegrass particles were cleared more slowly from the rumen (Waghorn et al., 1989). This is likely because of anatomical differences in cell wall structure between temperate grasses and legumes leading to differences in bacterial access to digestible components and the resulting particle shape (Wilson and Kennedy, 1996). Although grasses usually contain lower concentrations of lignin and therefore contain more potentially digestible NDF, the lignin generally is dispersed throughout most of the cell wall in both stem and leaf. Furthermore, in grass leaves, veins run the length of the leaf and the tube-shaped cells can be several centimeters long (Wilson and Kennedy, 1996; Wilson and Hatfield, 1997). Therefore, bacterial access to potentially digestible NDF is limited, reducing the rate of NDF digestion and reducing the rate at which particle fragility increases (Wilson and Kennedy, 1996).

Not only does the geometry of lignification limit bacterial access to the digestible inner cell wall surface, but it also provides fewer natural fracture points and causes grass particles to break into long, narrow particles that are easily trapped within the rumen mat. Although legumes generally contain more lignin than do grasses, that lignin is localized within the xylem and interbundular cells, leaving the mesophyll essentially unlignified. Legume leaves also have shorter, more reticulate veins, so they fracture rapidly into short particles that are less likely to be trapped in the rumen mat (Wilson and Kennedy, 1996). As a result,

the mesophyll of both stem and leaf can be degraded rapidly and completely, increasing particle fragility and quickly producing small particles of indigestible NDF that can escape the rumen more readily (Akin, 1989). Therefore, both rate of NDF digestion and rate of particle size reduction usually are greater, and retention time usually is shorter, in legumes than in grasses (Hoffman et al., 1993; Holden et al., 1994; Waghorn et al., 1989).

Differences in structure and digestion rate affect not only rate of particle size reduction but also specific gravity of particles. Particle density is decreased by fermentation gasses, so particles with more associated gasses will have longer rumen retention times (Sutherland, 1988). In legumes, once the rapidly-digestible mesophyll is digested, the production rate of fermentation gasses decreases drastically; then the remaining highly lignified tissue, in the form of short, dense particles, will rapidly increase in density and escape the rumen quickly (Allen, 1996; Wilson and Kennedy, 1996). Grass particles, by contrast, have associated fermentation gasses over a longer period of time due to slower digestion and a greater potentially digestible fraction (Allen, 1996). Also, their long, tubular structure might prevent gas from escaping the particle, further reducing the specific gravity and increasing the ruminal retention time of grass particles (Wilson et al, 1989).

Anatomical characteristics that lead to more gas retention, longer particles, and slower NDF digestion combine to explain the greater ruminal filling effects usually observed for grasses compared to legumes. Therefore, grass-based diets have little negative effect on cows that have lower pVDMI and for

whom intake is less likely to be limited by fill. Animals with greater pVDMI, however, need to compensate for greater ruminal NDF retention time. These animals could increase chewing when fed grass and thus increase the rate of particle size reduction. Chewing behavior was not measured in this study, and previous comparisons of chewing time for grasses and legumes are rare and have not utilized high-producing dairy cows for whom total chewing time might be a primary limiting factor (Beauchemin and Iwaasa, 1993; McLeod et al., 1990). Given the lower fragility of grasses, total chewing time would have to increase greatly in order to bring retention time of grass forage equal to the retention time of legume forage. In the present study, this apparently only occurred among cows with lower pVDMI and not among cows with greater pVDMI. As a result, feed intake of cows with greater pVDMI, for whom ruminal filling effects more often limit feed intake, is much lower for grass-based diets than for alfalfa-based diets.

Milk Production

Milk yield averaged 29.3 kg/d and was similar across treatments ($P = 0.77$; Table 4). Mean 3.5% fat-corrected milk yield (FCMY) was numerically, but not statistically ($P = 0.19$) greater when cows were fed OG (33.8 kg/d) than when they were fed AL (31.4 kg/d; Table 4). This is in contrast with the increase commonly seen in MY or FCMY when legume forage is substituted for grass forage (Oba and Allen, 1999b), and it occurred because milk fat concentration was greater for OG (4.40%) than for AL (3.99%; $P = 0.03$; Table 4). Milk fat concentration response has varied in previous comparisons of grass- and

legume-based diets (Zimmerman et al., 1991; Hoffman et al., 1998; Broderick et al., 2002; Dewhurst et al., 2003a; Al-Mabruk et al., 2004), probably because of differences in forage NDF concentrations, total dietary NDF and diet concentration of forage NDF. Most diets comparing forages are formulated to contain equal forage-to-concentrate ratios, equal total dietary NDF, or equal estimated NE_L, or are fed as separate components, all of which eliminate the possibility of directly comparing the specific effects of forage fiber on intake and production parameters.

Milk fat concentration is determined by many factors, including the profile of fatty acids (FA) removed from blood by the mammary gland (Bauman and Griinari, 2003). Although the FA concentrations of grass and alfalfa forages are very low, and their FA profiles quite similar (Dewhurst et al., 2003a), faster passage rate for alfalfa-based diets relative to grass-based diets (as discussed above) likely result in greater escape of rumen biohydrogenation intermediates for alfalfa-based diets (Harvatine and Allen, 2006). Milk FA profiles were not measured in this experiment, but Dewhurst et al. (2003a,b) reported greater concentrations of the intermediates of ruminal FA biohydrogenation, such as $C_{18:2}$, in milk from cows fed legumes, including alfalfa, compared to milk from cows fed grass. Some biohydrogenation intermediates have inhibited milk fat synthesis (Bauman and Griinari, 2003) and may have caused the reduction in milk fat concentration observed for cows fed alfalfa-based diets. That is, the effect of forage type on milk fat concentration may have been mediated by diet effect on passage rate and ruminal retention time.

Just as the effect of diet on passage rate depended on pVDMI, so also the effect of diet on milk fat concentration tended to differ with increasing pVDMI (% Fat_{AL} - % Fat_{OG} = 21.7 - 0.16 x seq – 2.56 x pVDMI + 0.07 x $pVDMI^2$; $P = 0.07$). Previous investigations of the effects of diet on milk fat yield and composition have focused on FA composition of the diet and products of fermentation, and on endocrine responses to diet (Bauman and Griinari, 2003). It is possible that the effects of diet on physical aspects of ruminal digestion and passage may also affect digesta FA profile at the small intestine and therefore influence milk fat synthesis. Because of different responses in passage rate with increasing pVDMI, nutrient demand may also alter the extent to which diet affects the production of milk and its components.

The effect of pVDMI on diet utilization was further illustrated by the response of the partitioning of nutrients toward milk production and body tissue accretion. Individual FCMY responses to AL over OG were related to individual pVDMI values ($FCMY_{AL}$ - $FCMY_{OG}$ = 263 – 31.4 pVDMI + 0.90 $pVDMI^2$; $P = 0.02$; Figure 3a). Similar quadratic relationships with pVDMI were demonstrated for milk yield ($P = 0.05$) and milk fat percentage ($P = 0.07$). The quadratic response of FCMY suggests that different factors controlled responses to diet of nutrient partitioning among cows with different pVDMI. Milk yield generally is correlated with DMI (NRC, 2001). Among cows with greater pVDMI, the increase in DMI response to AL with increasing pVDMI resulted in increased 3.5% FCMY on AL compared to OG, but among cows with low to moderate pVDMI, the smaller increase in DMI for AL resulted in similar or lower 3.5% FCMY on AL compared

to OG. Cows may have used additional nutrients obtained from slightly greater DMI on AL to replenish body tissue rather than to increase milk production. This is supported by the response of BCS change, which was the opposite of the FCMY response ($\Delta BCS_{AL} - \Delta BCS_{OG} = -15.9 + 1.9\ pVDMI - 0.05\ pVDMI^2$, $P < 0.01$; Figure 3b). Blood metabolites and hormones were not measured, so the variation in endocrine response to diet across pVDMI could not be determined. However, it is apparent that the changes in ruminal filling effects and in passage rate from the rumen caused by differences in forage fiber digestion had different effects on nutrient utilization depending on the pVDMI of the individual cow.

SUMMARY

As hypothesized, DMI on AL became increasingly greater than DMI on OG with greater pVDMI. This occurred because NDF turnover time in the rumen decreased more for AL than for OG as pVDMI increased. The faster disappearance of NDF on diet AL, caused primarily by a greater increase in passage rate of iNDF on AL with increasing pVDMI, reduced the physical filling effects for AL more than was possible for NDF from diet OG. This likely was caused by differences in both rate of particle size reduction and rate of increase in particle specific gravity, which have been demonstrated to be faster in legume forages than in grass forages. Through its effect on passage rate responses, pVDMI also altered the extent to which diet affected the production of milk and its components. Individual milk fat concentration, FCMY and BCS responses to AL over OG were related to individual pVDMI values.

CONCLUSIONS AND IMPLICATIONS

Cows with the greatest drive to eat, as estimated by pVDMI, responded the most positively in feed intake and milk production to alfalfa versus orchardgrass as the primary dietary fiber source. These results corroborate previous research suggesting that intake is more limited by physical fill effects with increasing nutrient demand and on grass forages compared to legume forages.

Many models of feed intake, digestion, and metabolism in dairy cows may be improved by incorporating the quantified effects of nutrient demand and feed sources on feed intake and passage rate, which can be provided by this experiment and future experiments testing other important variations in diet characteristics. Finally, the results of this experiment reinforce the need to provide separate diets for cows with higher and lower nutrient demand, in order to maximize the efficiency of nutrient utilization among the whole herd.

Table 1. Status of eight cows during the final 4 d of the preliminary period, when cows were fed a common diet.

Parameter	Mean	Standard Deviation
Parity	4.0	2.6
BW, kg	538	17
BCS	2.5	0.4
DIM	139	83
Milk yield, kg/d	40.1	5.5
DMI, kg/d	18.6	2.8

Figure 1. Distribution of DMI and 3.5% fat-corrected milk yield of eight cows during the final 4 d of the preliminary period, when cows were fed a common diet.

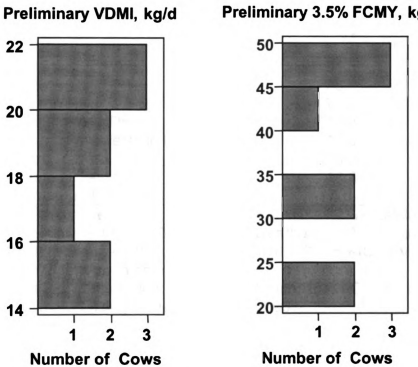


Table 2. Chemical characterization of alfalfa silage and orchardgrass silage.

	Alfalfa Silage	Orchardgrass Silage
DM (% as fed)	30.6	35.3
Nutrient, % DM		
OM	88.7	89.2
NDF	42.6	48.0
Indigestible NDF	25.2	13.1
Potentially digestible NDF	17.4	34.9
Starch	4.0	2.3
Crude protein	20.5	20.4
30-h in vitro NDF digestibility, %	29.4	61.1

Table 3. Ingredient and nutrient composition of treatment diets, one diet (AL) containing alfalfa silage and another diet (OG) containing orchardgrass silage.

	AL	OG
Ingredient	% of DM	
Alfalfa silage	53.0	------
Orchardgrass silage	------	47.9
Dry ground corn	36.3	40.3
Soybean meal (48% CP)	6.5	7.0
Vitamin mineral mix[1]	4.2	4.2
Expeller-processed soybean meal[2]	1.3	1.3
Bloodmeal	0.3	0.9
Limestone	------	0.4
Urea	------	0.2
Nutrient		
DM (% as fed)	43.6	50.6
	% of DM	
OM	91.5	91.5
NDF	26.7	27.5
Forage NDF	22.5	23.0
Indigestible NDF	14.8	7.9
Potentially digestible NDF	11.9	19.7
Starch	30.2	32.1
Crude protein	18.3	18.8
Rumen-undegraded CP[3]	5.6	6.3

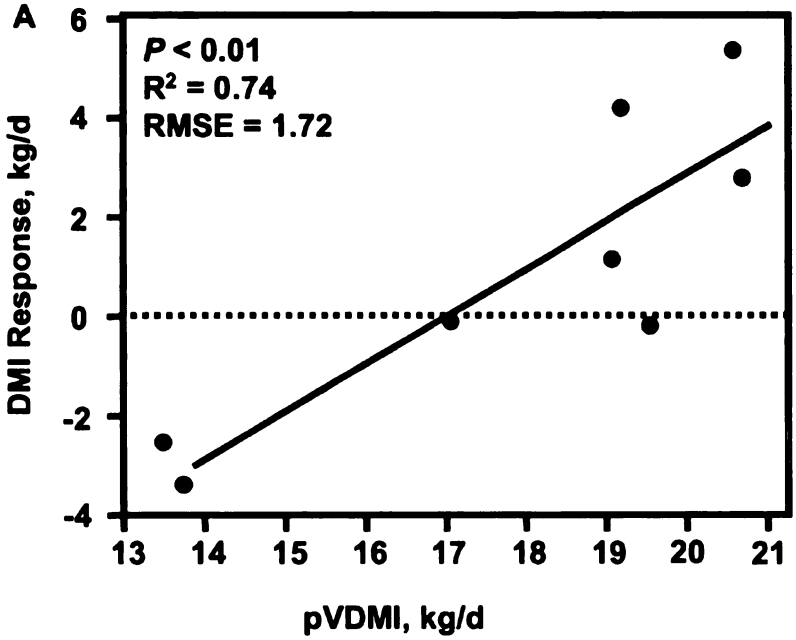
[1] Vitamin mineral mix contained (DM basis) 11.7% dicalcium phosphate, 11.1% trace-mineral premix, 8.8% sodium bicarbonate, 2.3% magnesium oxide, 134.3 KIU/kg vitamin A, 35.53 KIU/kg vitamin D, 895.5 KIU/kg vitamin E, and 65.2% ground corn grain as a carrier.
[2] Nutrient composition: 86% DM, 7% ash, 16% NDF, 5% starch, 51% CP.
[3] Estimated using values from NRC (2001).

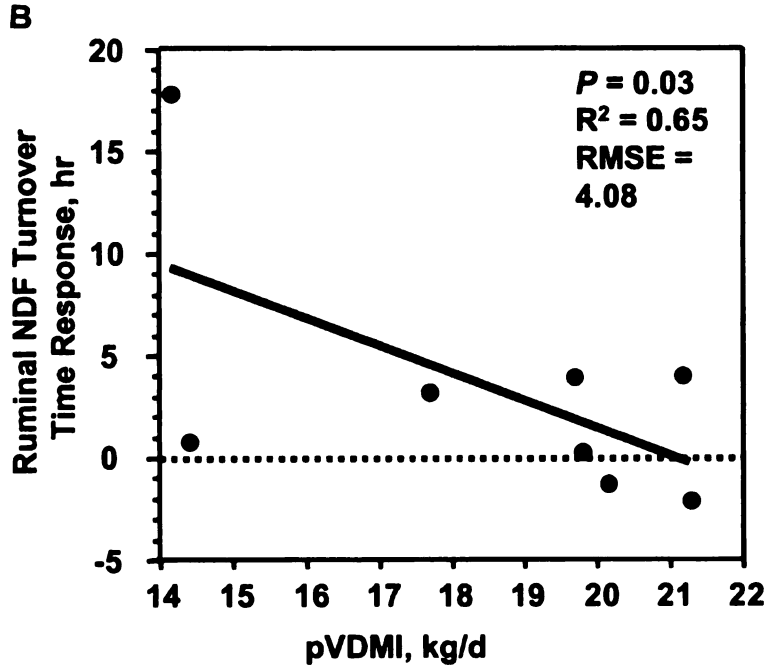
Table 4. Least-squares means of feed intake, digestion, and production responses in response to diets containing alfalfa (AL) or orchardgrass (OG).

	Treatment LSM[1]			
	AL	OG	SEM[2]	*P*
Yield, kg/d				
Milk	29.1	29.4	2.6	0.77
3.5% fat-corrected milk	31.4	33.8	3.3	0.19
Fat	1.17	1.31	0.14	0.13
Milk composition, %				
Fat	3.99	4.40	0.16	0.03
BW change, kg/15 d	-1.2	-17.5	5.6	0.04
BCS change, /15 d	-0.04	-0.16	0.08	0.23
Intake, kg				
DM	21.3	20.4	1.3	0.46
NDF	5.4	5.3	0.3	0.69
iNDF[3]	3.0	1.6	0.1	< 0.0001
Forage NDF	4.7	4.6	0.3	0.67
Rumen Pool, kg				
DM	10.3	11.3	0.8	0.31
NDF	5.6	5.1	0.4	0.24
iNDF	4.4	2.7	0.2	< 0.0001
Passage rate from rumen, hr^{-1}				
iNDF[3]	2.9	2.4	0.2	0.01
pdNDF[4]	1.2	1.3	0.4	0.81
Starch	11.4	12.3	1.8	0.70
Ruminal digestion rate, hr^{-1}				
pdNDF	6.9	5.2	0.6	0.06
Starch	21.1	18.3	3.2	0.42
NDF digested in the rumen				
kg	2.0	3.0	0.2	< 0.01
%	37.4	57.1	2.6	< 0.001
NDF digested in the whole tract				
kg	1.8	3.0	0.2	< 0.001
%	32.8	55.9	1.5	< 0.0001

[1] Treatment least-squares means.
[2] Standard error of the mean.
[3] Indigestible NDF.
[4] Potentially digestible NDF = NDF – iNDF.

Figure 2. Relationship between mean DMI during the final 4 d of the preliminary period (pVDMI) and the response to the alfalfa diet (AL) over the orchardgrass diet (OG) of (A) DMI ($DMI_{AL} - DMI_{OG}$ = -16.8 + 0.95 pVDMI), (B) ruminal NDF turnover time (TOT_{AL} - TOT_{OG} = 157 + 2.30 seq - 1.60 pVDMI), and (C) iNDF ruminal passage rate ($k_{pAL} - k_{pOG}$ = -1.44 -0.10 seq + 0.10 pVDMI). Equations B and C include the effect of treatment sequence (seq).





C

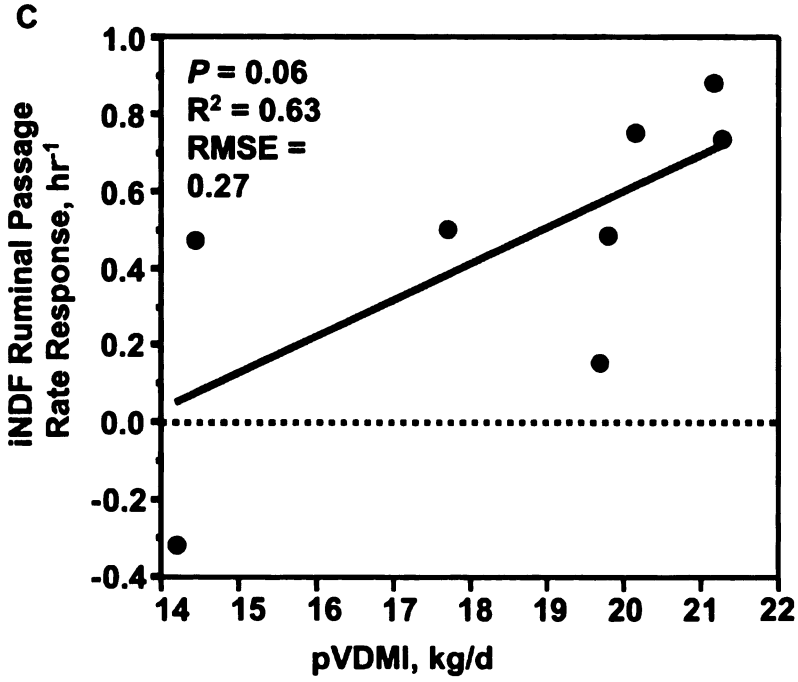
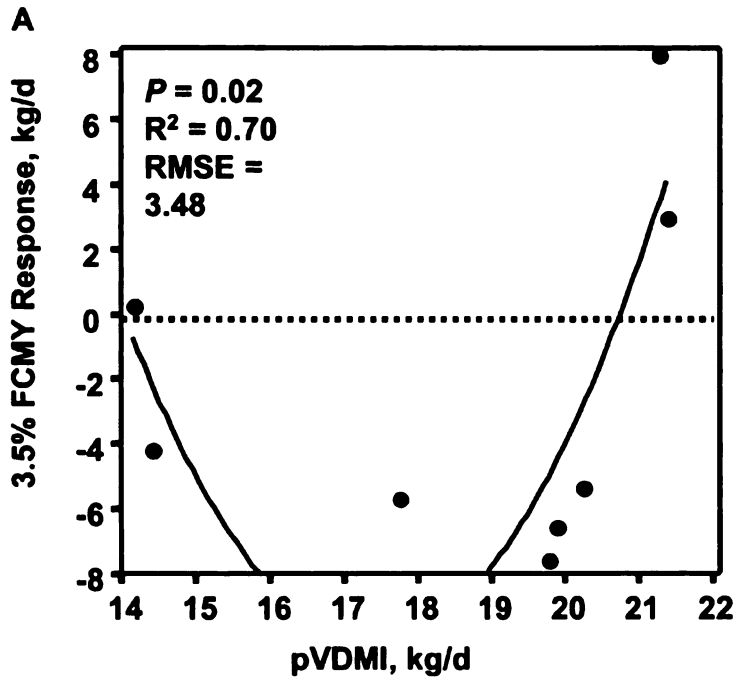
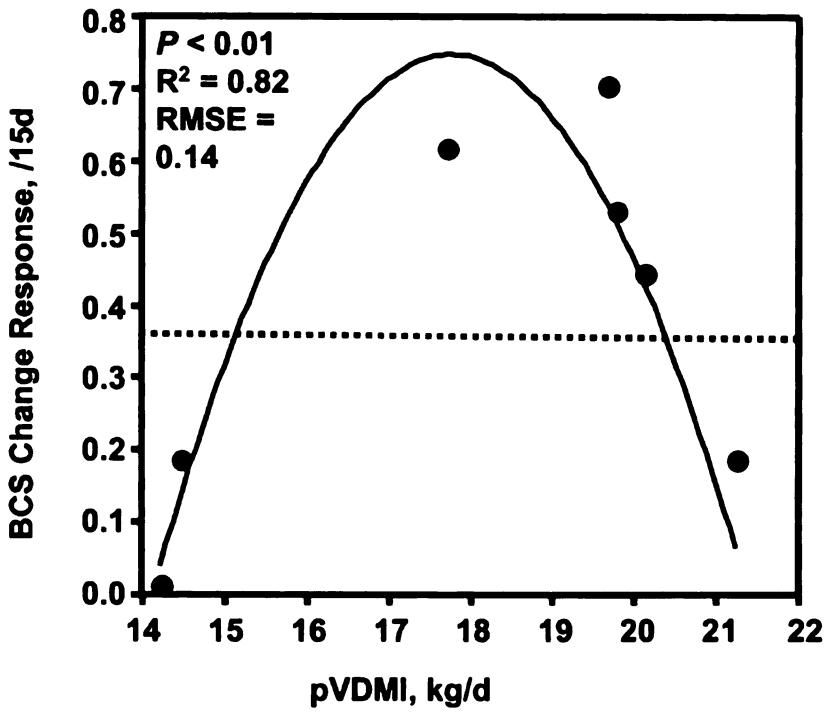

iNDF Ruminal Passage Rate Response, hr-1
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
P = 0.06
R2 = 0.63
RMSE = 0.27
14
15
16
17
18
19
20
21
22
pVDMI, kg/d

Figure 3. Relationship between mean DMI during the final 4 d of the preliminary period (pVDMI) and the response to the alfalfa diet (AL) over the orchardgrass diet (OG) in (A) 3.5% FCM yield ($FCMY_{AL} - FCMY_{OG} = 263 - 31.4$ pVDMI + 0.90 $pVDMI^2$) and (B) change in BCS ($\Delta BCS_{AL} - \Delta BCS_{OG} = -15.9 + 1.9$ pVDMI – 0.05 $pVDMI^2$).



B


P < 0.01
R2 = 0.82
RMSE = 0.14
BCS Change Response, /15d
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
14
15
16
17
18
19
20
21
22
pVDMI, kg/d

CHAPTER 3

Nutrient Demand of Lactating Dairy Cows Affects Nitrogen Intake and Utilization Responses to Diets Containing Alfalfa or Orchardgrass

ABSTRACT

The effect of preliminary feed intake on responses to diets containing alfalfa silage or orchardgrass silage was evaluated using eight ruminally and duodenally cannulated Holstein cows in a crossover design with two 15-d periods. Responses measured were N intake, digestion, and utilization. Cows were 139 ± 83 (mean ± SD) DIM at the beginning of the preliminary period. During the 14 d preliminary period, milk yield ranged from 24.5 to 46.0 kg/d (mean = 37.0 kg/d) and preliminary voluntary DMI (pVDMI) ranged from 11.4 to 21.0 kg/d (mean = 17.5 kg/d). Treatments were a diet with alfalfa silage as the sole forage (AL) and a diet with orchardgrass silage as the sole forage (OG). Alfalfa silage contained 20.5% CP (DM basis) and orchardgrass silage contained 20.4% CP; AL contained 18.3% CP and 5.6 estimated rumen-undegraded CP, and OG contained 18.8% CP and 6.3% estimated rumen-undegraded CP. Mean N intake was similar between treatments ($P = 0.95$), ruminal N digestibility was greater ($P = 0.03$) for AL (30.4%) than for OG (17.7%), and whole-tract N digestibility did not differ between treatments ($P = 0.50$). With increasing pVDMI, intake and duodenal flow of N increased more for AL than for OG because of increasingly greater DMI for AL compared to OG. However, among cows with greater pVDMI, a decreasing proportion of the additional N consumed from AL

was digested and used for increased milk production or body tissue gain. Although feeding diets containing alfalfa instead of orchardgrass can increase yields of milk and milk protein among cows with greater pVDMI, increasing N intake at the same rate as DMI likely will lead to less efficient utilization of dietary N for production of microbial protein, muscle, or milk protein. When feeding less-filling diets, such as those containing large proportions of legume forage, to high-producing cows, reducing dietary N concentration could increase the efficiency of N utilization and reduce the extent to which greater DMI leads to greater N excretion.

INTRODUCTION

Although alfalfa generally is considered to have a higher nutritional value than grass because of its higher crude protein and lower fiber concentrations, the addition of grass to the forage component of a diet for dairy cows can increase the efficiency of alfalfa N use. Using alfalfa alone to meet requirements for forage fiber often results in excess dietary N in a form that is degraded rapidly to ammonia in the rumen. Grasses reduce the ratio of N to fiber in forage (Spandl and Hesterman, 1997) and therefore can reduce fecal and urinary N waste excreted by cows.

Grass fiber and alfalfa fiber also have different chemical compositions, physical characteristics, and digestion characteristics that affect both the rate and extent of their digestion (Allen, 1996). Because grass fiber generally contains less lignin than alfalfa at the same maturity, grass fiber is ultimately more

digestible. However, grass fiber also is digested more slowly, and its cell walls break down more slowly than alfalfa cell walls. Passage rate increases as density increases and as particle size decreases, so fibrous alfalfa particles might escape the rumen more quickly than fibrous grass particles (Allen, 1996). Faster escape of bacteria from the rumen (shorter residence time) increases efficiency of N and energy utilization (Oba and Allen, 2003c; Voelker and Allen, 2003b) by decreasing bacterial death and breakdown in the rumen (Isaacson et al., 1975; Stouthamer and Bettenhaussen, 1973; Kennedy and Milligan, 1978). Microbial protein flow to the duodenum is limited by the availability of readily fermented feed for growth and by the ability of bacteria to avoid lysis and escape the rumen. Therefore, increasing passage rate of particles and bacteria from the rumen should cause increased microbial protein flow to the duodenum and increased efficiency of microbial protein synthesis. Thus, adding grass to a legume forage would likely reduce passage rate and lower microbial protein efficiency.

Finally, the passage rate of fiber from the rumen also depends on nutrient demand (Voelker et al., 2002). We developed and have successfully used an experimental model to evaluate effects of indices of nutrient demand, such as preliminary milk yield, on responses to dietary treatments (Oba and Allen, 1999a; Burato et al., 2001; Voelker et al., 2002; Harvatine and Allen, 2005; Bradford and Allen, 2004). This model was utilized to test our hypothesis that preliminary VDMI (pVDMI) affects individual responses of N intake, digestion, and utilization to diets containing orchardgrass silage or alfalfa silage as the sole forage.

MATERIALS AND METHODS

Cows and Treatments

Experimental procedures were approved by the All University Committee on Animal Use and Care at Michigan State University. Eight multiparous Holstein cows (139 ± 83 DIM; mean ± SD; Table 1) from the Michigan State University Dairy Cattle Teaching and Research Center were assigned randomly to treatment sequence in a crossover design experiment with a 14 d preliminary period and two 15 d experimental periods. These eight cows were selected deliberately to provide a wide, uniform distribution of milk yield and DMI (Figure 1). During the 14 d preliminary period, milk yield ranged from 24.5 to 46.0 kg/d (mean = 37.0 kg/d) and preliminary voluntary DMI (pVDMI) ranged from 11.4 to 21.0 kg/d (mean = 18.6 kg/d). Cows were cannulated ruminally and duodenally prior to calving. Surgery was performed at the Department of Large Animal Clinical Science, College of Veterinary Medicine, Michigan State University. Cows were housed in tie-stalls and fed once daily (1100 h) at 110% of expected intake.

Treatments were a diet with alfalfa silage as the sole forage (AL) and a diet with orchardgrass silage as the sole forage (OG). Alfalfa and orchardgrass forages were grown at the campus farm at Michigan State University (East Lansing) and ensiled in Ag-Bags (Ag-Bag Systems, Inc., St. Nazianz, WI). Alfalfa was harvested at bud stage, chopped at 3/8" (0.95 cm) theoretical cut length, and ensiled at 36% DM. Orchardgrass was harvested at early boot stage, chopped at 1/4" (0.64 cm) theoretical cut length, and ensiled at 37% DM.

Theoretical cut lengths were selected to yield similar particle size distributions using the Pennsylvania State Particle Size Separator (NASCO, Fort Atkinson, WI). Proportions of fresh-chopped forage retained on the top pan varied greatly by sample batch and averaged 20.1% for alfalfa and 28.1% for orchardgrass. Mean total mass retained on the top and middle pans were similar for alfalfa (62%) and orchardgrass (58%).

Nutrient composition for alfalfa silage and orchardgrass silage are presented in Table 2. During the sample collection periods, alfalfa silage contained 43% NDF (DM basis) and orchardgrass silage contained 48% NDF. Diets AL and OG were formulated to contain 23% forage NDF and 27% total NDF, so forage-to-concentrate ratios (DM basis) were 53:47 for AL and 48:52 for OG (Table 3). The diet fed during the preliminary period was formulated so that alfalfa silage and orchardgrass silage each contributed 50% of total forage NDF. Diets also contained dry ground corn, soybean meal, a vitamin-mineral premix, and blood meal; limestone, urea, and bloodmeal were used to compensate for greater measured CP and anticipated Ca concentrations in alfalfa silage than in orchardgrass silage. All diets were formulated for 18% dietary CP and fed once daily as totally mixed rations. During the experimental periods, orchardgrass silage CP concentration was similar to alfalfa silage CP concentration, so total dietary CP was 0.5% higher in OG than in AL.

Data and Sample Collection

Amounts of feed offered and orts were weighed for each cow daily. Samples of all dietary ingredients (0.5 kg) and orts from each cow (12.5% of orts)

were collected daily on d 11 to 13 and combined into one sample per period. Cows were milked twice daily (0300 and 1500 h) in a milking parlor; milk yield was measured, and milk was sampled, at each milking on d 11 to 13. Rumen-empty BW was measured after evacuation of ruminal digesta on d 14 of the preliminary period, and on d 15 of each experimental period. Body condition score was determined on the same days as BW, by three trained investigators blinded to treatments (Wildman et al., 1982; five-point scale where 1 = thin and 5 = fat).

Duodenal samples (1,000 g), fecal samples (500 g), and rumen fluid samples for microbial isolation (350 mL) were collected every 9 h from d 11 to d 13 so that eight samples were taken for each cow in each period, representing every 3 h of a 24-hour period in order to account for diurnal variation. Rumen fluid for microbial isolation was collected from the reticulum, near the reticular-omasal orifice, and strained. All samples were stored immediately at -20°C.

Ruminal contents were evacuated manually through the ruminal cannula at 1600 h (5 h after feeding) on d 14 and at 0700 h (4 h before feeding) on d 15 of each period. Total ruminal content mass and volume were determined. During evacuation, 10% aliquots of digesta were separated to allow accurate sampling. Aliquots were squeezed through a nylon screen (1 mm pore size) to separate into primarily solid and liquid phases. Samples (350 mL) were taken from both phases for determination of nutrient pool size. Samples were stored immediately at –20°C.

Sample and Statistical Analyses

Diet ingredients, orts, and feces were dried in a 55°C forced-air oven for 72 h and analyzed for DM concentration. All samples were ground with a Wiley mill (1mm screen; Authur 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 into "snow" using a commercial food processor (84142 Food cutter, Hobart Manufacturing Co., Troy, OH) and sub-sampled in the frozen state to obtain representative samples. These duodenal subsamples and the 350 mL ruminal solid and liquid samples were lyophilized (Tri-Philizer™ MP, FTS Systems, Stone Ridge, NY) and ground with a Wiley mill as above. Dried and ground 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), and starch, as described elsewhere (Chapter 2). Crude protein concentrations were determined according to Hach et al. (1987). Concentrations of all nutrients except DM were expressed as percentages of DM determined by drying at 105° C in a forced-air oven for more than 8 h.

Duodenal digesta were analyzed for purines and ammonia to estimate microbial N (MN) flow and non-ammonia non-microbial N (NANMN) flow to the duodenum. Purine concentration was used as a microbial marker, and purine to microbial N ratio was estimated by analysis of microbial pellets obtained by differential centrifugation of the rumen fluid collected in the reticulum. Total purines were measured by spectrophotometer (Beckman Instruments, Inc.,

Fullerton, CA) at 260 nm (Zinn and Owens, 1986). Ammonia concentration was determined for centrifuged duodenal and rumen fluid samples according to Broderick and Kang (1980). Milk samples were analyzed for true protein with infrared spectroscopy by Michigan DHIA (East Lansing). Milk true protein N yield was calculated as milk true protein yield / 6.38 (Jenness, 1985), and intake N was calculated as DMI x dietary N concentration. Milk samples from the first experimental period only were analyzed for milk urea N (MUN) with infrared spectroscopy by Michigan DHIA (East Lansing); therefore a t-test was used to determine the difference between treatments.

Indigestible NDF was used as an internal marker to estimate duodenal flow of nutrients in order to calculate nutrient digestibility in the rumen and in the total tract (Cochran et al., 1986), and to estimate passage rates of passage for iNDF, pdNDF, and starch, and rates of digestion for pdNDF and starch. Nutrient intake was calculated using the composition of feed offered and refused. Duodenal flow of microbial OM was determined as described by Oba and Allen (2003b), and true ruminally degraded OM (TRDOM) was calculated by subtracting duodenal flow of non-microbial OM from OM intake. Ruminal pool sizes (kg), turnover time in the rumen (h), passage rate from the rumen (h^{-1}), and ruminal digestion rate of each component (h^{-1}) were calculated as described elsewhere (Chapter 2).

To determine differences between treatments, all data were analyzed using the fit model procedure of JMP® (Version 5.1.2, SAS Institute, Cary, NC) according to the following model:

$$Y_{ijk} = \mu + C_i + P_j + T_k + PT_{jk} + e_{ijk}$$

where

μ = overall mean,

C_i = random effect of cow (i = 1 to 8),

P_j = fixed effect of period (j = 1 to 2),

T_k = fixed effect of treatment (k = 1 to 2),

PT_{jk} = interaction of period and treatment, and

e_{ijk} = residual, assumed to be normally distributed.

To determine the dependence of response to treatment on pVDMI, the response (ΔY) was calculated as follows:

$$\Delta Y = y_{AL} - y_{OG}$$

where

y_{AL} = response for AL diet, and

y_{OG} = response for the OG diet.

Preliminary VDMI was calculated as the mean of DMI values on d 11 to 14 of the 14-d preliminary period. Relationships between response to treatment and pVDMI were analyzed according to the following model:

$$Y_i = \mu + S_j + V + V^2 + e_i$$

Where

$Y_i = y_{AL} - y_{OG}$

μ = overall mean,

S_j = effect of sequence (j = 1 to 2),

V = pVDMI,

V^2 = $pVDMI^2$, and

e_i = residual, assumed to be normally distributed.

Significance was declared at or below $P = 0.05$, and trends were declared at or below $P = 0.10$. In the pVDMI model, sequence effect was removed when $P > 0.25$ and $pVDMI^2$ effect was removed when $P > 0.15$. Prediction equations reported are adjusted for Sequence (Seq[a] = AL, OG; Seq[b] = OG, AL and was set at 0) for N intake, rumen N turnover time, N digested in the rumen, ruminal N digestibility, N digested in the whole tract, whole-tract N digestibility, and g milk N/ g N intake.

The original sample size was 13 cows; data from five cows were excluded from statistical analysis. One cow developed hypocalcemia during the experiment, two were removed from the trial due to duodenal cannula malfunction, one was excluded because feed intake decreased by 50% on d 11 of Period 2 for undetermined reasons (intake slowly returned to normal on the same diet), and one was excluded because several key digestion parameters were outside the 95% confidence interval. None of the causes for removal or exclusion were believed to be associated with either of the two treatments. Among the remaining eight cows, each of the two treatment sequences was represented by four cows. Data in Table 1 and Figure 1 are for the eight cows used in the statistical analysis.

RESULTS AND DISCUSSION

Nitrogen Digestion: AL versus OG

Diet CP and estimated rumen-undegraded CP concentrations were similar for OG and AL (Table 3). Because mean DMI was similar for the two treatments ($P > 0.45$) and dietary N concentrations were similar, N intake was not affected by treatment ($P = 0.95$, Table 4). Ruminal N pool was greater for OG than for AL ($P = 0.01$), and ruminal turnover time of N was longer on OG than on AL ($P < 0.01$; Table 4). This was probably the result of a slower passage rate of digesta from the rumen on OG as indicated by the slower passage rate of iNDF (Chapter 2) and possibly because of greater proteolysis on AL. The two silages contained similar concentrations of N, but protein from legumes usually is degraded more rapidly and extensively in the rumen than is protein from grasses (Kwakkel et al., 1986; Kohn and Allen, 1995).

Consistent with greater proteolysis on AL, ruminal ammonia concentration was much greater for AL (29.3 mg/dl) than for OG (18.0 mg/dL; $P < 0.001$). This helps to explain the greater turnover time of N on OG. The effect of forage type on ammonia production rate likely was even greater than the difference in ruminal ammonia concentration, because rate of absorption likely was greater for AL. Mean pH was higher for AL than for OG ($P < 0.001$; Table 4), suggesting that rate of ruminal ammonia absorption was greater for AL than for OG for two reasons. First, decreasing pH causes a slower rate of absorption because more ammonia is converted to ammonium, which is not absorbed. Furthermore, lower

pH may inhibit rumen motility (Allen et al., 2006), which also reduces rate of ammonia absorption.

Rates of ammonia production, utilization by microbes, absorption from the rumen, and recycling were not measured. However, the higher concentration of ammonia on AL apparently promoted the absorption of more N from the rumen as ammonia, as indicated by a greater digestibility of N in the rumen ($P = 0.03$) and a greater amount of N disappearing from the rumen ($P < 0.01$) for AL than for OG (Table 4). Although more N was absorbed in the rumen for AL, compensatory postruminal digestion of N occurred for OG, so that in the whole tract, digestibility of N and the amount of N absorbed were similar on both treatments ($P > 0.50$; Table 4). Because the cows were not ileally cannulated, N disappearance from the small intestine and in the large intestine cannot be differentiated.

Nitrogen Digestion: Effects of pVDMI

Forage type was not the only factor that affected the site of N digestion. The nutrient demand of individual cows, as estimated by their pVDMI, also interacted with forage source to affect N digestion and utilization. As pVDMI increased, N intake was increasingly greater for AL over OG ($P = 0.01$, $R^2 = 0.83$, RMSE = 2.19; Figure 2a). As reported in Chapter 2, when cows were fed AL, DMI increased as pVDMI increased ($P = 0.05$); but when cows were fed OG, DMI was similar for all cows regardless of pVDMI ($P = 0.73$). Therefore, because of greater ruminal fill effects of OG, N intake likely was limited on OG relative to AL among cows with greater pVDMI. This was caused primarily by restriction of

digesta passage; as pVDMI increased, passage rate of iNDF increased for AL but not for OG (Chapter 2). Increased passage rate with increasing pVDMI combined with the likely greater ruminal degradation of nitrogenous compounds on AL, so that N turnover time in the rumen was not only shorter on AL than on OG but also decreased on AL relative to OG among cows with greater pVDMI (Figure 2b). That is, the increase in passage rate seen for AL with increasing pVDMI permitted shorter turnover time and greater intake of N, along with other nutrients, for cows with greater pVDMI. For OG, the inability to increase passage rate nearly eliminated the ability of cows with greater pVDMI to reduce turnover time and increase intake of N and other nutrients in order to meet nutrient demands.

Furthermore, forage source and pVDMI also interacted in their effects on ruminal absorption of N and microbial protein production. The difference in individual responses to diet ($y_{AL} - y_{OG}$) of N apparently digested and absorbed in the rumen depended on pVDMI in a quadratic relationship ($P = 0.05$; $R^2 = 0.91$; RMSE = 86.1; Figure 3a). The difference between ruminal N digestibility on AL and OG also demonstrated a tendency for a similar quadratic dependence on pVDMI ($P = 0.08$; $R^2 = 0.83$; RMSE = 5.69; Figure 3b). Therefore, the linear increase of N intake (Figure 2a) and the quadratic response of ruminal N digestibility (Figure 3b) for AL compared to OG with increasing pVDMI led to the quadratic response in the quantity of N absorbed in the rumen (Figure 3a). Among cows with lower pVDMI, a greater N absorption rate on AL relative to OG compensated for a lower N intake on AL relative to OG. As pVDMI increased

from moderate to high values, the increasing N intake on AL relative to OG and the slightly increasing ruminal digestibility on AL relative to OG resulted in a sharp increase in the quantity of N absorbed in the rumen on AL relative to OG. Mechanisms could include changes in the rate or extent of protein degradation, in the rate of absorption of ammonia from the rumen, in the rate or extent of incorporation of N into microbial protein, or in the rate of passage of N-containing digesta from the rumen. None of these potential mechanisms were measured directly. The difference in ruminal ammonia concentrations for cows fed the two diets did not depend on pVDMI ($P > 0.40$), but this does not eliminate the possibility of changes in rate of ammonia production or absorption. As mentioned earlier, lower ruminal pH can reduce absorption rate of ammonia. The difference in response of daily mean ruminal pH to treatment ($pH_{AL} - pH_{OG}$) tended to depend on pVDMI ($P = 0.07$; $R^2 = 0.53$; RMSE = 0.31; data not shown). When cows were fed AL, mean pH was between 6.1 to 6.6 regardless of pVDMI ($P = 0.55$). When cows were fed OG, mean pH tended to demonstrate a quadratic relationship to pVDMI, with a maximum at 6.2 ($P = 0.09$); pH was particularly lower, around 5.8, among cows with high pVDMI when they were fed OG. This lower ruminal pH may have reduced the rate of ammonia absorption from the rumen among cows with high pVDMI when they were fed OG. Therefore, diet effects and pVDMI effects on ruminal ammonia production and absorption may have contributed to the observed responses of ruminal N digestion.

Reflecting N intake, flow to the duodenum of total N for AL relative to OG increased linearly as pVDMI increased ($P = 0.01$, $R^2 = 0.69$; RMSE = 82.2; Figure 4a); flow of NAN responded similarly ($P = 0.01$; $R^2 = 0.69$; RMSE = 79.6; Figure 4b). Flow of MN to the duodenum also increased for AL relative to OG as pVDMI increased ($P = 0.05$; $R^2 = 0.51$; RMSE = 82.9; Figure 5a). This resulted from a numerical increase in MN flow with greater pVDMI ($P = 0.15$) when cows were fed AL and a numerical decrease in MN flow with greater pVDMI ($P = 0.14$) when cows were fed OG. Greater duodenal flow of MN for AL with increasing pVDMI reflects the greater DM and N intake and passage rate observed for AL compared to OG with greater pVDMI.

Response of efficiency of microbial protein production from truly ruminally degraded OM (MNE) demonstrated a quadratic relationship to pVDMI ($P = 0.03$; $R^2 = 0.76$; RMSE = 0.56; Figure 5b). When cows were fed OG, the production of microbial N from the ruminal digestion of OM decreased linearly with increasing pVDMI ($P = 0.05$), suggesting that fermentation and microbial growth were increasingly uncoupled on OG as pVDMI increased. Ruminal starch digestion likely played a significant role in determining the extent to which N was incorporated into MN. The relationship between pVDMI and the difference in ruminal starch digestion on the two diets (Figure 6a,b) was the opposite of the relationship between pVDMI and the response in MNE (Figure 5b). The amount of starch truly ruminally digested (TRD starch, kg/d) and true ruminal starch digestibility (TRSD, % of intake) demonstrated quadratic relationships between pVDMI and relative response to AL versus OG ($P = 0.05$ and $P = 0.04$, for TRD

starch (Figure 6a) and TRSD (Figure 6b), respectively). Opposite relationships to pVDMI for MNE and ruminal starch digestion indicate that among cows with low and high pVDMI, for whom ruminal starch digestion was greater on AL than on OG, MNE was lower on AL than on OG; the reverse was true for cows with moderate pVDMI. Across all 16 cow-period values, MNE was correlated negatively with true ruminal starch digestibility ($P = 0.02$, $R^2 = 0.32$), and MN flow to the duodenum (g/d) tended to be correlated negatively with true ruminal starch digestibility ($P = 0.07$, $R^2 = 0.22$).

In general, greater ruminal starch digestion did not result in greater or more efficient production of MN. Rather, it may have reduced efficiency of utilization of N and OM for MN production, probably by uncoupling fermentation and microbial growth (Strobel and Russell, 1986) and(or) by increasing the population of amylolytic bacteria, some of which are also very actively proteolytic (Russell et al., 1981). The negative effect of greater starch digestion on MNE was apparently the greatest among cows with high pVDMI when they consumed diet AL, where the greatest starch intake and ruminal starch digestion were observed. When cows were fed OG, ruminal starch digestion was not affected by pVDMI ($P > 0.25$) so differences in ruminal starch digestion probably did not cause the decrease in MN production and efficiency observed for OG with increasing pVDMI.

The less efficient production of microbial protein on OG as pVDMI increased was caused, in part, by the increasingly negative effect of OG on passage rate as pVDMI increased. As demonstrated earlier (Chapter 2),

passage rate of iNDF tended to be affected negatively by OG compared to AL as pVDMI increased. Decreasing passage rate can decrease the efficiency of N utilization for microbial protein production. With greater passage rate, microbes associated with particulate digesta can escape the rumen more rapidly, reducing microbial protein turnover by reducing the extent of autolysis (Wells and Russell, 1996) and protozoal predation (Wallace and McPherson, 1987). The efficiency with which N was incorporated into MN, and the turnover of MN in the rumen, likely contributed to the observed responses of ruminal N digestion for OG compared to AL.

Just as N intake and duodenal N flow increased on AL relative to OG with increasing pVDMI, the amount of N digested postruminally also increased on AL compared to OG with greater pVDMI ($P = 0.01$; $R^2 = 0.67$; RMSE = 67.7; Figure 7a), as did the amount of NAN digested postruminally ($P = 0.01$; $R^2 = 0.67$; RMSE = 66.2; Figure 7b). The amount of N digested in the whole tract also increased on AL relative to OG with increasing pVDMI ($P = 0.02$; $R^2 = 0.89$; RMSE = 42.2; Figure 8a). However, whole-tract N digestibility tended to become increasingly lower on AL relative to OG with increasing pVDMI ($P = 0.07$; $R^2 = 0.98$; RMSE = 1.35; Figure 8b). With increasing pVDMI, AL permitted increased DMI and N intake, and increased MN production. However, the decreases for AL compared to OG in microbial efficiency (MN, %TRDOM) and whole-tract N digestibility among cows with the highest pVDMI suggest that the efficiency of N utilization did not benefit from the increasingly greater intake and passage rate observed

for AL relative to OG with increasing pVDMI, because N supply probably was in excess of requirements, as discussed below.

Nitrogen in Milk Production

The form in which dietary N was absorbed might have affected its proportion and form in milk. Mean yield and concentration of true protein in milk did not differ across treatments (Table 4). Although yields of milk and true protein, and milk true protein concentration, were similar between treatments, more N was secreted in milk in the form of urea for AL than for OG (Table 4). Milk urea N was measured only during one period (n = 8), but MUN was much greater ($P < 0.01$) cows fed AL (23.5 mg/dL) than for cows fed OG (15.3 mg/dL). This is consistent with the greater disappearance of N from the rumen for AL compared to OG, probably as ammonia which is used to synthesize urea in the liver. Ruminal ammonia concentration and MUN were highly correlated ($P < 0.01$; $R^2 = 0.84$).

The effect of diet on the yield of true protein in milk varied and tended to depend on pVDMI ($P < 0.10$; $R^2 = 0.66$; RMSE = 0.09; Figure 9a). Among the cows with lower and moderate pVDMI, true protein yield was similar or lower on AL compared to OG, but among cows with the highest pVDMI, true protein yield increased on AL relative to OG as pVDMI increased (Figure 9a). Mean efficiency of utilization of N consumed in the diet for synthesis of true protein in milk (g milk true protein N / g N intake) did not differ between treatments (Table 4). However, with increasing pVDMI, N tended to be used less efficiently for milk protein production on AL compared to OG ($P = 0.10$; $R^2 = 0.49$; RMSE = 0.02; Figure 9b)

and might have been secreted increasingly as MUN instead of as true protein on AL, with increasing pVDMI. This was likely caused, at least in part, by the increase in N intake and decrease in whole-tract N digestibility observed on AL compared to GR with increasing pVDMI. In addition, this apparent decrease in efficiency of N utilization on AL with increasing pVDMI occurred despite the expected dilution of maintenance N with increasingly greater MY on AL as pVDMI increased.

Apparent efficiency of milk protein production from dietary N also can be increased through mobilization of body tissue protein to meet the demand for milk production. The relationship between pVDMI and response in estimated NE_L balance ($P = 0.03$; $R^2 = 0.52$; RMSE = 3.71; Figure 9d) supports tissue mobilization as a mechanism for increased apparent efficiency of milk true protein synthesis, but the relationship between pVDMI and response in BCS change ($P < 0.01$; $R^2 = 0.82$; RMSE = 0.142; Figure 9c) does not. Change in BCS is a direct measurement and NE_L balance is an indirect estimate, so it is unlikely that cows actually experienced increasingly positive body tissue gains on AL compared to OG with increasing pVDMI as is suggested by Figure 9d. Therefore, increased N intake with increasing pVDMI (Figure 2a) and decreasing N digestibility (Figure 8b) likely were primary factors in decreasing the efficiency of N utilization for milk protein production on AL compared to OG as pVDMI increased (Figure 9b). Urinary N output was not measured; it is possible that an increasingly greater amount of digested N was excreted in urine on AL than on OG as pVDMI increased. Although the increasingly greater DMI for AL

compared to OG allowed additional N intake among cows with greater pVDMI, that extra feed N apparently was digested and utilized less efficiently.

This assertion that utilization of N may be decreasingly efficient with increasing DMI bears important economic and environmental implications for dairy farms. For all the animals in this experiment, fuel availability was likely more limiting than protein for milk production. Diets were formulated to ensure that N and amino acid availability were not limiting to ruminal fermentation or milk production, and the actual dietary concentration of CP was high (approximately 20% of DM) for both diets. Even with excess dietary total and rumen-degradable CP in both diets, the effect of diet on whole-tract N digestibility and efficiency of N utilization for milk true protein depended on pVDMI. This implies that when practices are implemented to permit greater DMI, dietary N concentration might need to be reduced in order to avoid less-efficient digestion and utilization of N.

Furthermore, the effects of pVDMI on N digestion and utilization reinforce the need to group and feed animals according to some index of nutrient demand. Reducing the variation in energy and protein demand within the group for which a diet is formulated would reduce the extent to which fuels or N limit ruminal fermentation or milk production in all animals. This would allow diets to be formulated to more accurately meet each individual animal's demands and thus lead to more efficient utilization of N among all groups of animals on the farm. Increased N digestion and utilization and more accurate diet formulation will reduce the proportion and amount of N excreted in feces and urine. It should also reduce the likelihood of overfeeding N. Thus, adjusting feeding practices for

the effects of increasing DMI on efficiency of N utilization can contribute to the reduction of N waste.

SUMMARY AND CONCLUSIONS

As expected, ruminal N absorption was greater when the dietary forage was alfalfa than when it was orchardgrass. The effect of forage type on N intake, digestion and utilization depended on the pVDMI of individual animals. Because DMI responded increasingly more positively to AL than to OG as pVDMI increased, intake and duodenal flow of N also increased more for AL than for OG with increasing pVDMI. Site of digestion and efficiency of utilization of dietary N for microbial protein and for milk true protein depended not only on intake of N but also on responses of ruminal passage rate and ruminal starch digestion. The reduction of passage rate by OG, particularly among cows with high pVDMI, reduced the total amount of N consumed and utilized for microbial protein and milk true protein production. However, a decreasing proportion of the additional N consumption that was allowed by the increased DMI on AL among cows with greater pVDMI was digested and used for increased milk production or body tissue gain. Increasing passage rate and DMI by feeding a perennial legume forage instead of a perennial grass forage can increase yields of milk and milk protein among cows with greater nutrient demand. However, increasing N intake at the same rate as DMI is increased likely will lead to less efficient utilization of dietary N for production of microbial protein, muscle, or milk true protein. When feeding less-filling diets, such as those containing a legume forage, to high-

producing cows, reducing dietary N concentration could increase the efficiency of N utilization and reduce the extent to which greater DMI leads to greater N excretion. A better understanding of the different effects of perennial grass and legume forages on N utilization by cows with different nutrient demands will aid in field management decisions to minimize the turnover and loss of N on the whole farm.

Table 1. Status of eight cows during the final 4 d of the preliminary period, when cows were fed a common diet.

Parameter	Mean	SD
Parity	4.0	2.6
BW, kg	538	17
BCS	2.5	0.4
DIM	139	83
Milk yield, kg/d	40.1	5.5
DMI, kg/d	18.6	2.8

Figure 1. Distribution of DMI and 3.5% fat-corrected milk yield of eight cows during the final 4 d of the preliminary period, when cows were fed a common diet.

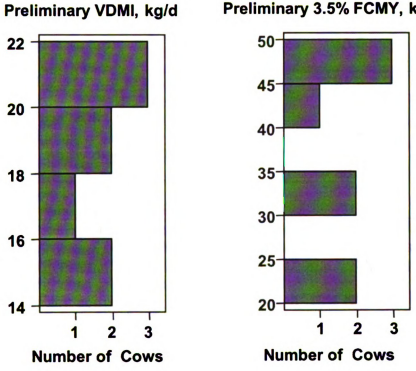


Table 2. Chemical characterization of alfalfa silage and orchardgrass silage.

	Alfalfa Silage	Orchardgrass Silage
DM (% as fed)	30.6	35.3
Nutrient, % DM		
OM	88.7	89.2
NDF	42.6	48.0
Indigestible NDF	25.2	13.1
Potentially digestible NDF	17.4	34.9
Starch	4.0	2.3
Crude protein	20.5	20.4
30-h in vitro NDF digestibility, %	29.4	61.1

Table 3. Ingredient and nutrient composition of treatment diets, one diet (AL) containing alfalfa silage and another diet (OG) containing orchardgrass silage.

	AL	OG
Ingredient	% of DM	
Alfalfa silage	53.0	------
Orchardgrass silage	------	47.9
Dry ground corn	36.3	40.3
Soybean meal (48% CP)	6.5	7.0
Vitamin mineral mix[1]	4.2	4.2
Expeller-processed soybean meal[2]	1.3	1.3
Bloodmeal	0.3	0.9
Limestone	------	0.4
Urea	------	0.2
Nutrient		
DM (% as fed)	43.6	50.6
	% of DM	
OM	91.5	91.5
NDF	26.7	27.5
Forage NDF	22.5	23.0
Indigestible NDF	14.8	7.9
Potentially digestible NDF	11.9	19.7
Starch	30.2	32.1
Crude protein	18.3	18.8
Rumen-undegraded CP[3]	5.6	6.3

[1] Vitamin mineral mix contained (DM basis) 11.7% dicalcium phosphate, 11.1% trace-mineral premix, 8.8% sodium bicarbonate, 2.3% magnesium oxide, 134.3 KIU/kg vitamin A, 35.53 KIU/kg vitamin D, 895.5 KIU/kg vitamin E, and 65.2% ground corn grain as a carrier.
[2] Nutrient composition: 86% DM, 7% ash, 16% NDF, 5% starch, 51% CP.
[3] Estimated using values from NRC (2001).

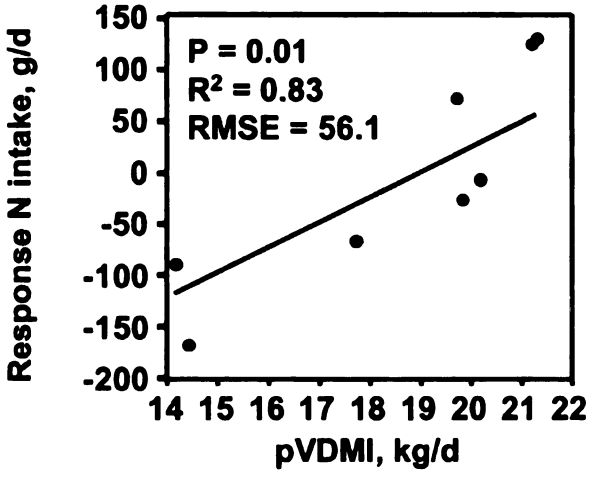
Table 4. Least-squares means, standard errors, and *P*-values of effects of forage source on N intake, digestion, and utilization.

Variable	Treatment LSM[1] AL	OG	SEM[2]	*P*
Intake, g/d	620	623	37	0.95
Rumen pool, g	269	371	20	0.01
Turnover time in rumen, h	10.7	14.4	0.8	<0.01
Rumen ammonia concentration, mg/dl	29.3	18.0	1.1	< 0.001
Mean pH	6.44	6.17	0.04	< 0.001
Ruminally digested				
g	196	110	42	< 0.01
%	30.4	17.7	4.3	0.03
N flow to duodenum				
Total, g/d	426	515	35	0.14
Ammonia N, g/d	19.9	14.5	1.1	< 0.01
Non-ammonia N (NAN)				
g/d	406	500	42	0.10
Microbial N				
g/d	271	293	26	0.56
% duodenal NAN	66.3	58.3	2.6	0.07
% TRDOM[3]				
Per 1	1.9	2.8	0.3	0.09
Per 2	2.7	2.5	0.3	0.69
NA, non-microbial N (NANMN)				
g/d	135	207	16	0.03
% duodenal NAN	33.7	41.7	2.7	0.09
N digested postruminally				
g	230	316	30	0.08
%	54.4	60.2	2.8	< 0.01
NAN digested postruminally				
g/d	210	301	29	0.06
% duodenal NAN	52.1	59.0	2.9	< 0.01
N digested in the whole tract				
g	425	424	23	0.99
%	68.6	68.1	1.1	0.50
Yield, kg/d				
Milk	29.1	29.4	2.6	0.77
3.5% fat-corrected milk	31.4	33.8	3.3	0.19
True protein	0.88	0.89	0.06	0.77
Milk true protein concentration, %	3.06	3.07	0.12	0.65
g milk true protein N / g intake N	0.21	0.22	0.01	0.89

[1] Treatment least-squares means.
[2] Standard error of the mean.
[3] Per x Trt: $P = 0.13$.

Figure 2. Relationship between mean DMI during the final 4 d of the preliminary period (pVDMI) and the response to the alfalfa diet (AL) over the orchardgrass diet (OG) of (A) N intake (N $intake_{AL}$ – N $intake_{OG}$ = -570 + 30.5 pVDMI), and (B) N turnover time (N TOT_{AL} – N TOT_{OG} = -106 + 12.8 pVDMI – 0.38 $pVDMI^2$). Equation A includes adjustment for Seq.

A



B

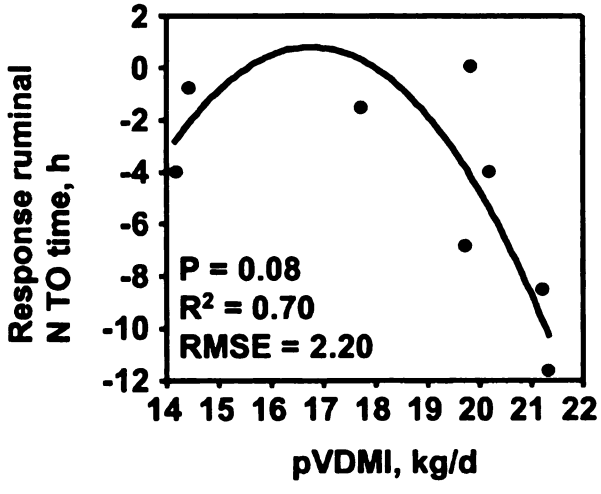
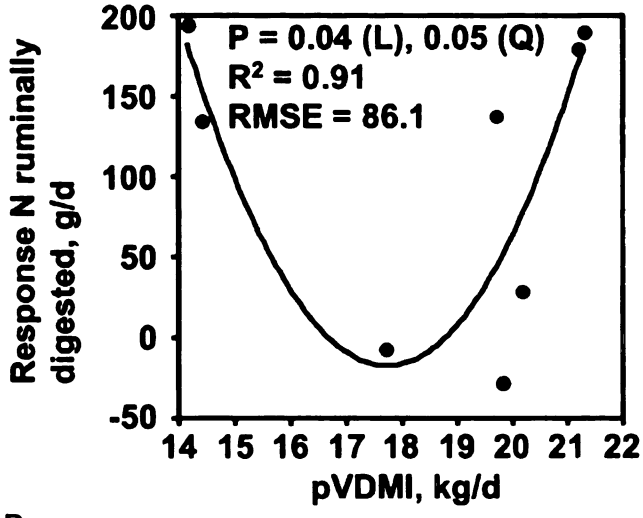


Figure 3. Relationship between mean DMI during the final 4 d of the preliminary period (pVDMI) and the response to the alfalfa diet (AL) over the orchardgrass diet (OG) in (A) N absorbed from the rumen (g N rumen digested$_{AL}$ – g N rumen digested$_{OG}$ = 2887 = 317 pVDMI + 8.75 pVDMI^2) and (B) ruminal digestibility of N (% of N intake) (ruminal N digestibility$_{AL}$ – ruminal N digestibility$_{OG}$ = 453 – 47.8 pVDMI + 1.27 pVDMI^2). Equations A and B include adjustment for Seq.

A



B

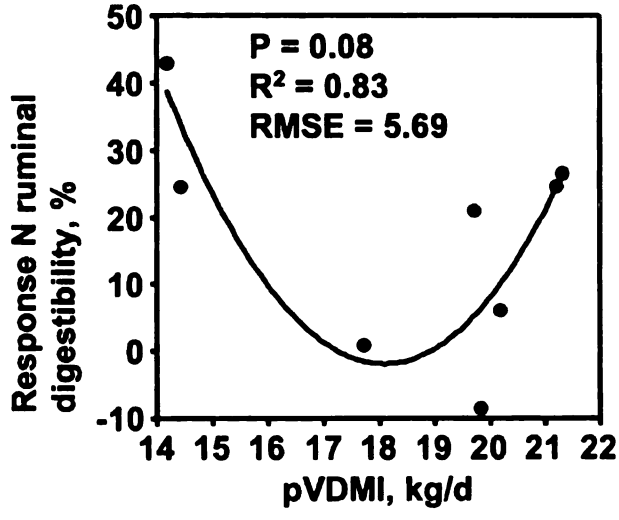
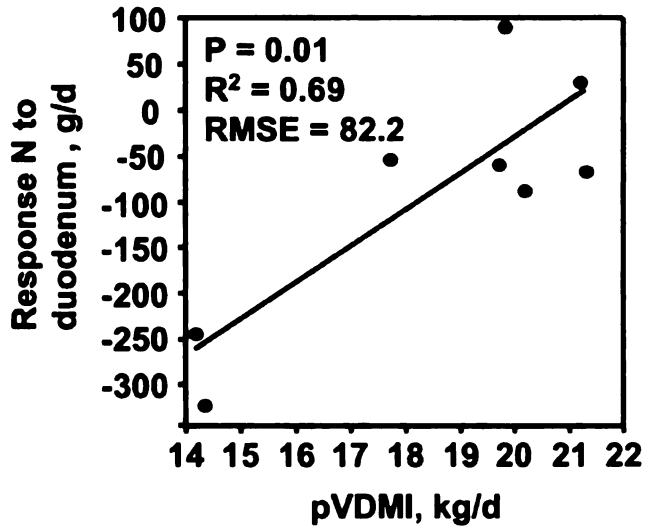


Figure 4. Relationship between mean DMI during the final 4 d of the preliminary period (pVDMI) and the response to the alfalfa diet (AL) over the orchardgrass diet (OG) in (A) N flow to the duodenum (N $flow_{AL}$ - N $flow_{OG}$ = -823 + 39.5 pVDMI); and (B) NAN flow to the duodenum (NAN $flow_{AL}$ - NAN_{OG} = -815 + 38.8 pVDMI).

A



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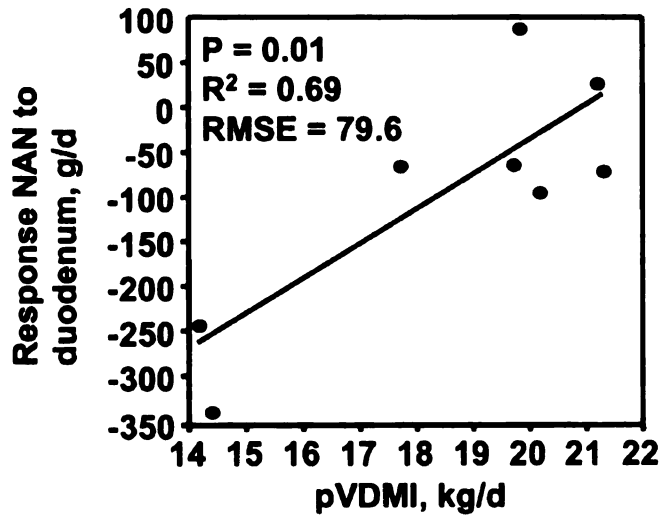
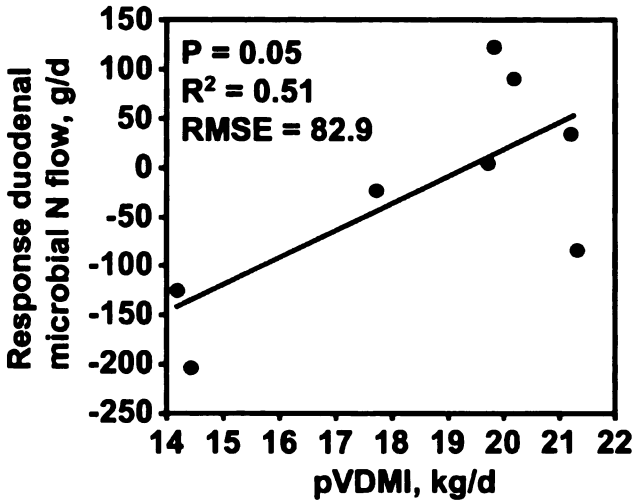


Figure 5. Relationship between mean DMI during the final 4 d of the preliminary period (pVDMI) and the response to the alfalfa diet (AL) over the orchardgrass diet (OG) in (A) microbial N flow to the duodenum ($MN_{AL} - MN_{OG} = -583 + 30.2$ pVDMI) and (B) microbial N (% truly rumen degraded OM (TRDOM)) ($MNE_{AL} - MNE_{OG} = -42.1 + 4.64$ pVDMI $- 0.13$ pVDMI^2).

A



B

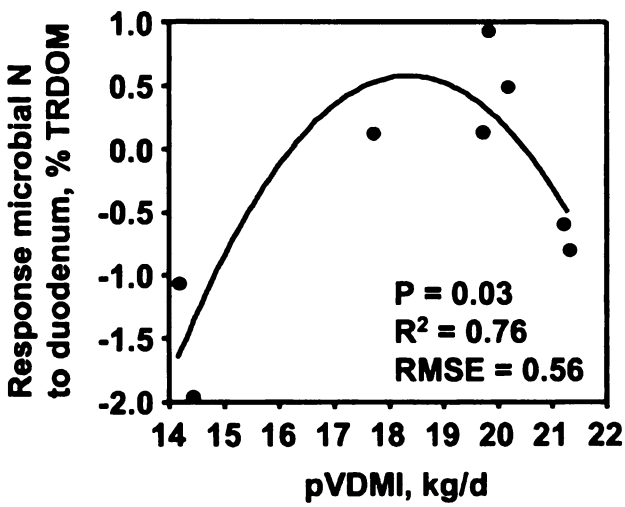
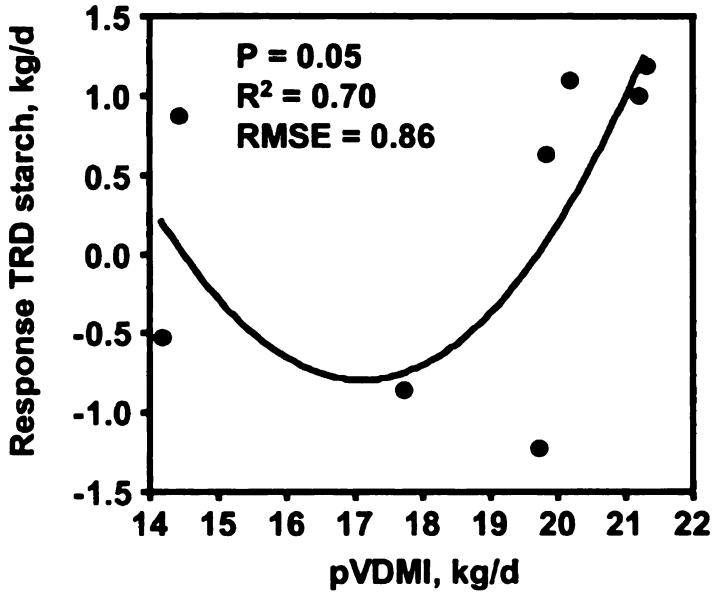


Figure 6. Relationship between mean DMI during the final 4 d of the preliminary period (pVDMI) and the response to the alfalfa diet (AL) over the orchardgrass diet (OG) in (A) Truly ruminally degraded starch (TRD starch), kg/d ($TRD\ starch_{AL} - TRD\ starch_{OG} = 66.1 - 7.88\ pVDMI + 0.23\ pVDMI^2$) and (B) True ruminal starch digestibility (TRSD), % ($TRSD_{AL} - TRSD_{OG} = 900 - 103\ pVDMI + 2.9\ pVDMI^2$). Equations A and B are corrected for Seq.

A



B

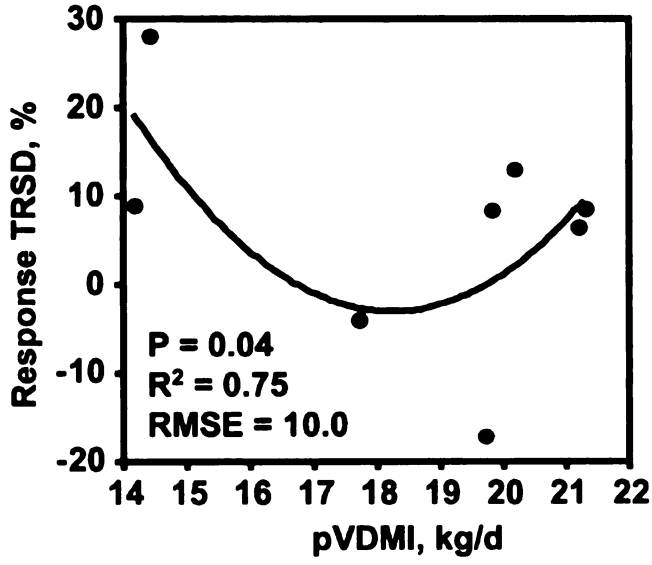
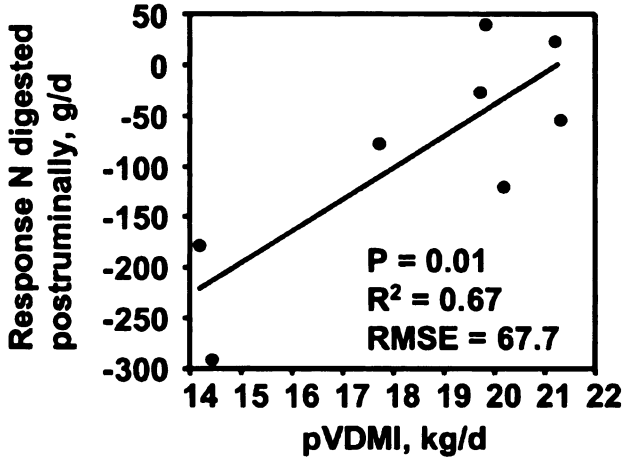


Figure 7. Relationship between mean DMI during the final 4 d of the preliminary period (pVDMI) and the response to the alfalfa diet (AL) over the orchardgrass diet (OG) in (A) N digested postruminally (N digested postruminally$_{AL}$ – N digested postruminally$_{OG}$ = -670 + 31 pVDMI) and (B) NAN digested postruminally (g/d) (NAN digested postruminally$_{AL}$ – NAN digested postruminally$_{OG}$ = -660 + 30 pVDMI).

A



B

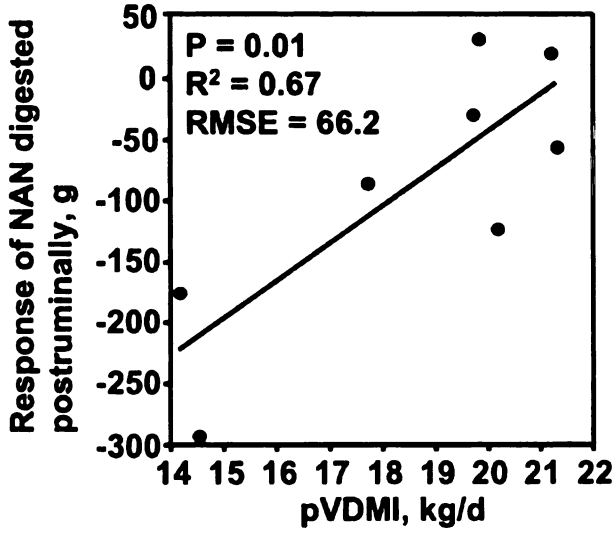
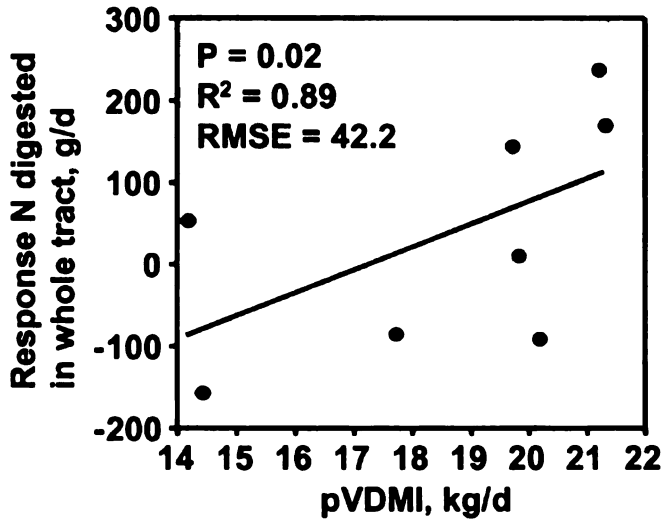


Figure 8. Relationship between mean DMI during the final 4 d of the preliminary period (pVDMI) and the response to the alfalfa diet (AL) over the orchardgrass diet (OG) in (A) N digested in the whole tract (g N digested$_{AL}$ – g N digested$_{OG}$ = -375 + 20.2 pVDMI) and (B) whole-tract digestibility of N (% of N intake) (N digestibility$_{AL}$ – N digestibility$_{OG}$ = 8.15 - 0.41 pVDMI). Equations A and B include adjustment for Seq.

A



B

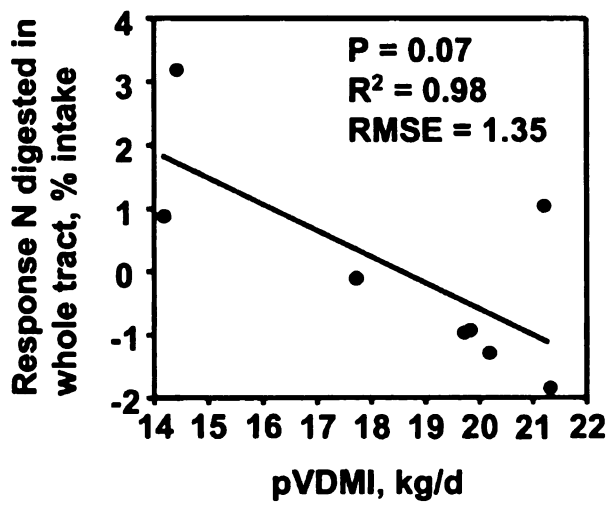
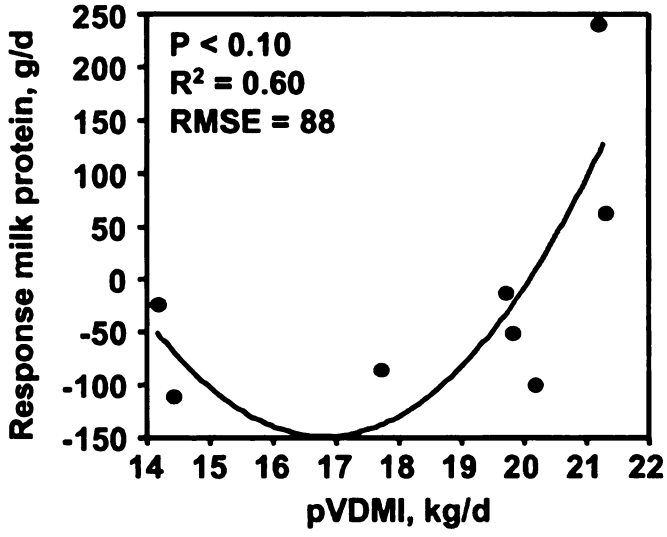


Figure 9. Relationship between mean DMI during the final 4 d of the preliminary period (pVDMI) and the response to the alfalfa diet (AL) over the orchardgrass diet (OG) in (A) milk true protein yield (Milk true protein $yield_{AL}$ – Milk true protein $yield_{OG}$ = 3.80– 0.47 x pVDMI + 0.014 x $pVDMI^2$); (B) total milk N (% N intake) (Milk True Protein N (% N intake)$_{AL}$ – Milk True Protein N (% N intake)$_{OG}$ = 11.6– 0.63 x pVDMI); (C) BCS change (BCS $change_{AL}$ – BCS $change_{OG}$ = -15.9 + 1.87 pVDMI – 0.0537 $pVDMI^2$); and (D) NE_L balance (NE_L $balance_{AL}$ – NE_L $balance_{OG}$ = -26.9 +1.44 pVDMI). Equation B includes adjustment for Sequence effect.

A



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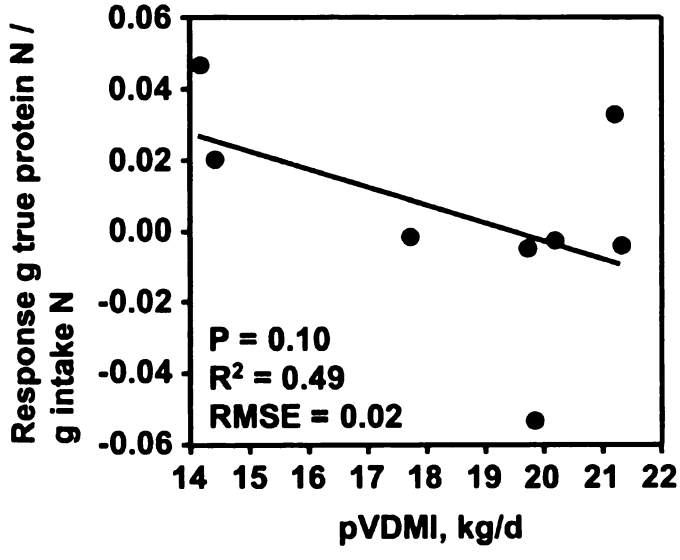
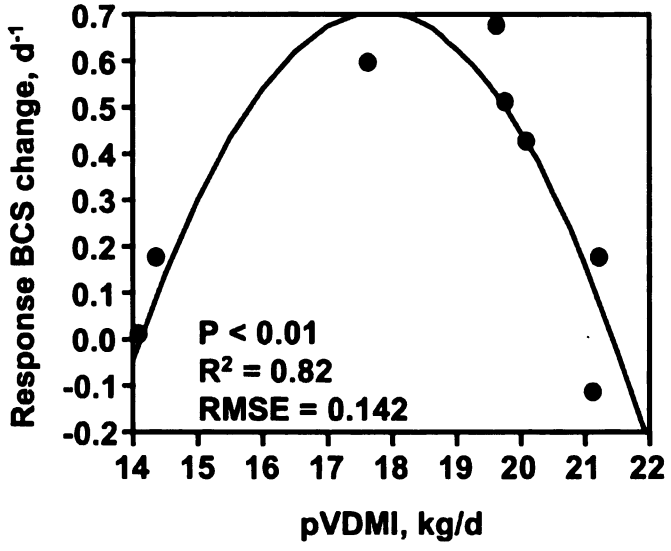
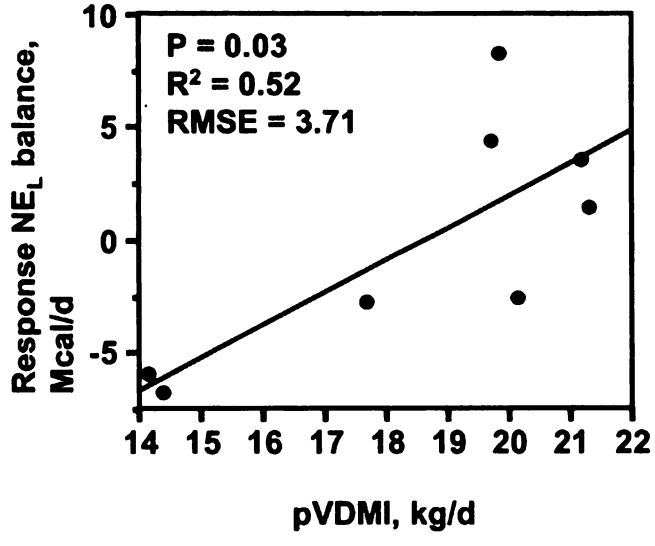


C


Response BCS change, d-1
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
-0.1
-0.2
P < 0.01
R2 = 0.82
RMSE = 0.142
14 15 16 17 18 19 20 21 22
pVDMI, kg/d

D


Response NEL balance, Mcal/d
10
5
0
-5
P = 0.03
R2 = 0.52
RMSE = 3.71
14 15 16 17 18 19 20 21 22
pVDMI, kg/d

CHAPTER 4

Nutrient Demand of Lactating Dairy Cows Affects Ruminal Digestion Responses to a Change in Dietary Forage Concentration

ABSTRACT

Previous research in our laboratory indicates that physical filling effects of high-forage diets become increasingly dominant in determining feed intake and milk production as nutrient demand increases. This effect was tested further using 14 ruminally and duodenally cannulated Holstein cows in a crossover design experiment with a 14 d preliminary period and two 15 d experimental periods. During the preliminary period, 3.5% fat-corrected milk yield (FCMY) was 15 to 60 kg/d (mean = 40 kg/d), and preliminary voluntary DMI (pVDMI) was 20.6 to 30.5 kg/d (mean = 25.0 kg/d). Treatments were a low-forage diet (LF), containing 20% (DM basis) forage neutral detergent fiber (NDF), and a high-forage diet (HF), containing 27% forage NDF. The ability of linear and quadratic factors of pVDMI to predict the difference in responses of individual cows to treatments ($Y_{LF} - Y_{HF}$) was tested by analysis of variance, with treatment sequence as a covariate. In contrast to results of previous research, differences in DMI and FCMY responses to LF and HF did not depend on pVDMI. This might be because of combined physical fill and metabolic satiety effects of LF, especially in cows with greatest pVDMI. Digestion and(or) passage of NDF might have been inhibited on LF among high-pVDMI cows. As pVDMI increased, NDF turnover time increased more on LF than on HF. Among high-pVDMI cows,

NDF turnover time was unexpectedly greater on LF than on HF. With increasing pVDMI, digestion rate of pdNDF decreased at a similar rate on both diets. Passage rates of potentially digestible NDF and indigestible NDF were not related to pVDMI, regardless of treatment. Because mean and minimum ruminal pH were lower for LF than for HF, a slight numerical reduction in pH with increasing pVDMI observed for both diets likely would inhibit NDF digestion more for LF than for HF. Inhibition of NDF digestion might cause low-forage and high-forage diets to have similar effects on DMI, depending on the VDMI of individual cows.

INTRODUCTION

Diet forage NDF concentration affects feeding and digestion in dairy cows through both physical and chemical mechanisms. Physical controls include gut distension (Lehman, 1941) and limitations to time spent eating and ruminating (Allen, 2000). Mechanisms through which diet affects the physical control of feed intake include retention time of digesta fractions (Campling et al., 1961), potential digestibility of fiber (Oba and Allen, 1999), diet particle size and rate of particle size reduction (Poppi et al., 1980), particle specific gravity (Balch and Kelly, 1951), and diet effects on frequency and duration of reticulorumen contractions (Okine and Mathison, 1991; Dado and Allen, 1995). Altered fermentation acid production in the rumen resulting from changes in diet forage NDF concentration may also affect intake and digestion responses to diet (Sheperd and Combs, 1998). Excess production of fermentations acids with low-forage-fiber diets

results in lower ruminal pH, which can decrease fiber digestibility (Hoover, 1986). Excess propionate production can result in lower feed intake, independent of pH effects (Allen, 2000).

However, energy balance influences both feed intake responses to diet characteristics and the extent to which physical or metabolic factors limit VDMI (Mertens, 1994; Allen, 1996). The effects on feed intake of diet characteristics (such as diet forage NDF concentration) that influence ruminal passage rate of digesta will depend on the extent to which physical filling effects limit feed intake in an individual animal. As a result, 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 the effects of nutrient demand on response to diet are even more necessary. We developed and successfully used an experimental model to evaluate effects of indices of nutrient demand, such as preliminary milk yield, on animal responses to dietary treatments (Oba and Allen, 1999a; Burato et al., 2001; Voelker et al., 2002; Harvatine and Allen, 2002; Bradford and Allen, 2004). This model was utilized to test our hypothesis that preliminary VDMI (pVDMI) affects individual responses of VDMI and digesta passage rate to diets containing high and low concentrations of forage NDF. A previous experiment (Voelker et al., 2002) investigated this hypothesis using intact and ruminally-cannulated cows; based on results from that experiment, we expect passage rates of digesta fractions to become increasingly greater for the low-forage diet compared to the high-forage diet as preliminary VDMI increases. The present

experiment was conducted using ruminally- and duodenally-cannulated cows with a wide range of pVDMI to investigate the mechanisms underlying the responses to changes in dietary forage-fiber concentration.

MATERIALS AND METHODS

Cows and Treatments

Experimental procedures were approved by the All University Committee on Animal Use and Care at Michigan State University. Fourteen 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 a 14 d preliminary period and two 15 d experimental periods. These fourteen cows were 178 ± 120 (mean ± SD) DIM at the beginning of the preliminary period (Table 1) and were selected deliberately to provide a wide distribution of milk yield and DMI (Figure 1). During the 14 d preliminary period, milk yield ranged from 16.1 to 59.1 kg/d (mean = 38.7 kg/d) and pVDMI ranged from 10.6 to 30.5 kg/d (mean = 25.0 kg/d). Cows were cannulated ruminally and duodenally prior to calving. Surgery was performed at the Department of Large Animal Clinical Science, College of Veterinary Medicine, Michigan State University. Cows were housed in tie-stalls and fed once daily (1100 h) at 110% of expected intake.

Treatments (Table 2) were a low-forage diet (LF) and a high-forage diet (HF) fed once daily as totally mixed rations. Diet LF was formulated to contain 20% of DM as forage neutral detergent fiber (NDF) and 24% as total dietary

NDF, and HF was formulated to contain 27% forage NDF and 31% total dietary NDF. Forage-to-concentrate ratios (% of DM) were 45:55 for LF and 61:39 for HF. Diets also contained dry ground corn, soybean meal, an expeller-processed soybean meal, and a vitamin-mineral premix; urea, and soybean meal were used to achieve similar CP and estimated RUP fractions in the two diets. Diets were formulated for 18% dietary CP but the actual diet CP contents were 16.2 and 16.6% (Table 2). The diet fed during the preliminary period was formulated, using the same ingredients, to contain 24% forage NDF.

Sample and Data Collection

Amounts of feed offered and orts were weighed for each cow daily. Samples of all dietary ingredients (0.5 kg) and orts from each cow (12.5% of orts) were collected daily on d 11 to 13 and combined into one sample per period. Cows were milked twice daily in a milking parlor (0300 and 1500 h); milk yield was measured, and milk was sampled, at each milking on d 11 to 13. Rumen-empty BW was measured after evacuation of ruminal digesta on d 14 of the preliminary period, and on d 15 of each experimental period. Body condition score was determined on the same days by three trained investigators blinded to treatments (Wildman et al., 1982; five-point scale where 1 = thin and 5 = fat).

Duodenal samples for digestion measurements (700 mL) and for particle-size analysis (700 mL), rumen fluid samples for microbial isolation (350 mL), and rumen fluid samples for pH (100 mL) were collected every 9 h from d 11 to d 13 so that eight samples were taken for each cow in each period, representing every 3 h of a 24-hour period to account for diurnal variation. Rumen fluid for microbial

isolation was collected from the reticulum, near the reticular-omasal orifice, and strained. Rumen fluid was obtained by combining digesta from five different sites in the rumen and straining it through a layer of nylon mesh (~1 mm pore size); fluid pH was recorded immediately. Samples were stored immediately at -20°C.

Ruminal contents were evacuated manually through the ruminal cannula at 1600 h (5 h after feeding) on d 14 and at 0700 h (4 h before feeding) on d 15 of each period. Total ruminal content mass and volume were determined. During evacuation, 10% aliquots of digesta were separated to allow accurate sampling. Aliquots were squeezed through a nylon screen (1 mm pore size) to separate into primarily solid and liquid phases. Both phases were weighed and sampled (two, 350 mL samples of each phase) for determination of nutrient pool size and particle size analysis. Samples were stored at -20°C.

Sample and Statistical Analyses

Diet ingredients and orts were dried in a 55°C forced-air oven for 72 h and analyzed for DM concentration. All samples were ground with a Wiley mill (1mm screen; Authur H. Thomas, Philadelphia, PA). One set of frozen duodenal samples for each cow period (n = 8) were chopped into "snow" using a commercial food processor (84142 Food cutter, Hobart Manufacturing Co., Troy, OH) and sub-sampled in the frozen state to obtain representative samples. These duodenal subsamples and one set of 350 mL ruminal solid and liquid samples for each rumen-emptying time were lyophilized (Tri-Philizer™ MP, FTS Systems, Stone Ridge, NY) and ground as 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), CP, and starch. Ash concentration was determined after 5 h oxidation at 500°C in a muffle furnace. Concentrations of NDF were determined according to Van Soest et al. (1991, method A). Indigestible NDF was estimated as NDF residue after 120-h in vitro fermentation (Goering and Van Soest, 1970). Rumen fluid for the in vitro incubations was collected from a non-pregnant dry cow fed only alfalfa hay. 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 a forced-air oven for more than 8 h.

Milk samples were analyzed for fat, true protein, MUN, and lactose with infrared spectroscopy by Michigan DHIA (East Lansing). Duodenal samples were analyzed for purines and ammonia to estimate microbial N flow and non-ammonia non-microbial N flow to the duodenum. Purine concentration was used as a microbial marker, and purine to microbial N ratio was estimated by analysis of microbial pellets obtained by differential centrifugation of the rumen fluid collected in the reticulum. Total purines were measured by spectrophotometer (Beckman Instruments, Inc., Fullerton, CA) at 260 nm (Zinn and Owens, 1986).

Ammonia concentration was determined for centrifuged duodenal and rumen fluid samples according to Broderick and Kang (1980).

Indigestible NDF was used as an internal marker to estimate nutrient digestibility in the rumen (Cochran et al., 1986), and to estimate rates of passage for iNDF, pdNDF, and starch, and rates of digestion for pdNDF and starch. Nutrient intake was calculated using the composition of feed offered and refused. Duodenal flow of microbial OM was determined as described by Oba and Allen (2003b), and true ruminally degraded OM (TRDOM) was calculated by subtracting duodenal flow of non-microbial OM from OM intake. Ruminal pool sizes (kg) of OM, NDF, iNDF, pdNDF, and starch were determined by multiplying the concentration of each component by the ruminal digesta DM mass (kg). Turnover rate in the rumen, passage rate from the rumen, and ruminal digestion rate of each component (%/h) were calculated as reported by Voelker and Allen (2003b).

Rates of particle size reduction in and particle passage from the rumen also were determined using iNDF as a marker (Figure 2). Triplicate 20-g feed and orts samples were sieved. Thawed subsamples of ruminal solid and liquid phases (the second set from each of two rumen evacuations per period) were recombined into duplicate 60-g samples based on the original (wet) ratio of solid and liquid phases. The second set of whole duodenal samples were thawed and combined (eight per cow-period), then separated into liquid and solid and stored frozen. The two phases were thawed and recombined in duplicate 200-g samples for sieving. Feed, orts, rumen, and duodenal samples were individually

wet-sieved sequentially through 4.75 mm, 2.36 mm and 38 μm screen (W.S. Tyler Inc., Gastonia, NC). Particles retained on each screen were removed and dried at 55° C for 48 h, then weighed. Material retained on each screen from replicate sievings were combined (keeping after-feeding and before-feeding rumen empty samples separate). Because DM in duodenal digesta retained on the 4.75 mm screen was < 5% of total DM on screens, 2.36 mm was selected as the threshold. Residue ≥ 2.36 mm, including residue on 4.75 and 2.36 mm screens, averaged 13.4% of total DM. Therefore, particles retained on the 2.36 and 4.75 mm screens were combined and the resulting fractions were designated ≥ 2.36 mm (less likely to escape the rumen) and < 2.36 mm (more likely able to escape the rumen). These two fractions were ground (1 mm, Wiley mill). Ground sieving residues were analyzed for DM, iNDF, and NDF concentrations. Indigestible NDF was used to calculate rate of particle size reduction in the rumen (≥ 2.36 to < 2.36), because (1) kinetics must be calculated for a homogeneous pool, and (2) pdNDF can leave pool by digestion as well as particle-size reduction and passage but iNDF can leave the pool only by breakdown or by passage. Passage rates of iNDF in large (≥2.36 mm) and small (<2.36 mm) particles, rate of flux of iNDF from the ≥2.36 mm pool to the <2.36 mm pool, and relative size threshold for escape from the rumen were calculated as follows:

Passage rate (k_p):

$$iNDFk_{p\geq 2.36} = iNDF_{Duod\geq 2.36}\ (kg/d) \,/\, iNDF_{RumenPool\geq 2.36}\ (kg)$$

$iNDFk_{p<2.36} = iNDF_{Duod<2.36}$ (kg/d) / $iNDF_{RumenPool<2.36}$ (kg)

where

$iNDFk_p$ = passage rate of iNDF in particles ≥ or < 2.36 mm,

$iNDF_{Duod}$ = duodenal flow of iNDF in particles ≥ or < 2.36 mm, and

$iNDF_{RumenPool}$ = rumen pool of iNDF in particles ≥ or < 2.36 mm.

Reduction rate (k_r) from ≥2.36 to <2.36:

$iNDFk_{r2.36} = [iNDF_{In\geq 2.36}$ (kg/d) - $iNDF_{Duod\geq 2.36}$ (kg/d)] / $iNDF_{RumenPool\geq 2.36}$ (kg)

where

$iNDFk_{r2.36}$ = rate of transfer of iNDF from pool of particles ≥ 2.36 to the pool of particles < 2.36 mm,

$iNDF_{In\geq 2.36}$ = intake of iNDF in particles ≥ 2.36,

$iNDF_{Duod\geq 2.36}$ =duodenal flux of iNDF in particles ≥ 2.36 mm, and

$iNDF_{RumenPool\geq 2.36}$ = rumen pool of iNDF in particles ≥ 2.36 mm.

Relative size threshold:

$iNDF_{Duod\geq 2.36}$ (kg/d) / $iNDF_{DuodTotal}$ (kg/d)

where

$iNDF_{Duod\geq 2.36}$ = duodenal flux of iNDF in particles ≥ 2.36 mm, and

$iNDF_{DuodTotal}$ = duodenal flux of iNDF in all particles.

Passage rates and relative size threshold were also calculated for pdNDF.

To determine differences between treatments, all data were analyzed using the fit model procedure of JMP® (Version 5.1.2, SAS Institute, Cary, NC) according to the following model:

$$Y_{ijk} = \mu + C_i + P_j + T_k + PT_{jk} + e_{ijk}$$

where

μ = overall mean,

C_i = random effect of cow (i = 1 to 8),

P_j = fixed effect of period (j = 1 to 2),

T_k = fixed effect of treatment (k = 1 to 2),

PT_{jk} = interaction of period and treatment, and

e_{ijk} = residual, assumed to be normally distributed.

The period by treatment interaction effect was removed when its *P*-value was greater than 0.30.

To correlate response to treatment with pVDMI, the response (Y) was calculated as follows:

$$Y = y_{LF} - y_{HF} .$$

where

y_{LF} = response for LF diet

y_{HF} = response for the HF diet

Preliminary VDMI was calculated as the mean of DMI values on d 11 to 14 of the 14-d preliminary period. Relationships between response to treatment and pVDMI were analyzed according to the following model:

$$Y_i = \mu + S_i + V + V^2 + e_i$$

Where

$Y_i = y_{LF} - y_{HF}$

μ = overall mean,

S_i = effect of sequence (i = 1 to 2),

V = pVDMI

V^2 = $pVDMI^2$

e_i = residual, assumed to be normally distributed.

Significance was declared at or below $P = 0.05$, and tendencies were declared at or below $P = 0.10$.

RESULTS AND DISCUSSION

Mean Responses to Dietary Forage NDF Concentration

Consistent with the majority of previously reported experiments comparing low-forage and high-forage diets (Allen, 2000), mean DMI was greater for cows fed LF compared to HF (Table 3). Intake of both NDF and forage NDF was greater for HF than for LF ($P < 0.001$), but intake of iNDF was similar between treatments ($P \geq 0.85$). Rumen pools of DM, NDF, and iNDF were greater for HF, but cows still consumed less DM when fed HF. The intake and ruminal pool responses suggest that physical fill was more limiting to intake for HF than for LF for most cows. Digestion rate of pdNDF was greater for HF than for LF (Table 3; $P = 0.001$), probably because greater starch fermentation on LF, the result of greater starch intake, caused greater inhibition of NDF digestion on LF than on HF (Grant, 1997).

Rate of reduction of feed particles from ≥ 2.36 to < 2.36 mm (measured using iNDF as a marker) was much greater on HF (6.86 %/h) than on LF (3.87 %/h; Table 4). This could be the result of greater fragility of large (forage)

particles on HF due to the faster rate of pdNDF digestion. It also could be the result of more chews, or more effective ruminating chews, per kg ruminal NDF. Wilson and Kennedy (1996) suggested that physical mastication of forage particles, rather than increased fragility caused by digestion, was the most important mechanism for particle size reduction. Feeding behavior was not measured in this experiment. In a similar experiment (Voelker et al., 2002), time spent ruminating and total chewing time were greater for the high-forage diet than for the low-forage diet, but time chewing per kg intake of NDF intake and forage NDF were greater for the low-forage diet.

Rates of passage of particles of various sizes and of particle size reduction are seldom reported for high-producing dairy cows, and this experiment was the first use of this particular method. As expected, intake of iNDF in particles < 2.36 mm was greater for LF ($P < 0.0001$), and intake of iNDF in particles ≥ 2.36 mm was greater for HF ($P < 0.0001$; Table 4). Intake of pdNDF responded similarly. Ruminal pool of iNDF in particles < 2.36 mm was greater for HF ($P < 0.0001$), but pool of iNDF in particles ≥ 2.36 mm was similar between treatments ($P \geq 0.50$). Ruminal pools of pdNDF in large and small particles were similar across treatments ($P > 0.25$). The NDF in small particles (<2.36 mm) contained a larger proportion of iNDF than did the NDF in large particles (≥ 2.36 mm; Table 4). Because a greater lignin concentration results in greater fragility (McLeod and Minson, 1988), particles with greater iNDF concentration likely break down to smaller particles more quickly. Therefore, small particles should contain greater concentrations of iNDF than should larger particles.

Furthermore, the proportion of iNDF in total NDF in small particles increased between intake pool (54.5 and 55.4% of NDF for LF and HF, respectively) and rumen pool (61.6 and 62.1% of NDF for LF and HF, respectively). This is expected, because some pdNDF was digested and iNDF was not. However, in large particles (≥ 2.36 mm), the proportion of iNDF was similar in intake pool (44.1 and 45.7% of NDF for LF and HF, respectively) and rumen pool (44.6 and 44.8% of NDF for LF and HF, respectively). This suggests that the digestion rate of pdNDF in particles ≥ 2.36 mm was very low, even negligible. Digestion rate of pdNDF in small and large particles could not be calculated, because pdNDF can disappear from the pools by digestion as well as by passage or particle size reduction. Larger forage particles may indeed undergo negligible NDF digestion because of the small surface area available for bacterial digestion relative to the total surface area or volume of the particle (Wilson and Hatfield, 1997). The similar proportions of iNDF in feed and rumen particles ≥ 2.36 mm suggest that almost no NDF digestion takes place in particles until they are broken down (by chewing) to < 2.36 mm.

Fractional passage rates of iNDF in particles < 2.36 mm and in particles ≥ 2.36 mm were greater on LF than on HF ($P < 0.03$, $P < 0.02$, respectively), and ranged from 2.10 %/h (particles ≥ 2.36 mm on HF) to 6.10 %/h (particles < 2.36 mm on LF), spanning the passage rates observed for total iNDF as would be expected. Passage rate of pdNDF tended to be greater on LF than HF in particles < 2.36 mm ($P = 0.06$), but pdNDF passage rate in particles ≥ 2.36 mm was similar between treatments ($P \geq 0.75$) and was numerically much slower

than the passage rate of iNDF in particles of similar size (Table 4). The range of passage rate of pdNDF in small and large particles (0.59 to 2.34 %/h) also spanned the passage rates observed for total pdNDF. The proportion of duodenal iNDF or pdNDF flux contained in particles ≥ 2.36 mm was quite small (13 to 21% of total; Table 4). A slightly greater proportion of iNDF was found in large duodenal particles on LF than on HF ($P = 0.03$), and a greater proportion of pdNDF was found in large duodenal particles on HF than on LF ($P < 0.05$).

Passage rate of total pdNDF tended to be greater for LF than for HF (Table 3). A period by treatment interaction existed for passage rate of total iNDF ($P = 0.06$); iNDF passage rate was similar between treatments during period 1 (mean = 4.8; $P \geq 0.65$) but was greater for LF (4.8 %/h) than for HF (3.9 %/h) during period 2 ($P < 0.01$). Starch passage rate responded to treatment in a manner similar to iNDF passage rate. The tendency for greater passage rate of digesta fractions for LF than for HF suggests that passage rate could not be increased on HF to permit greater DMI in response to a more physically filling, more slowly digested diet. The slower passage rates of pdNDF and iNDF for HF apparently outweighed both the greater digestion rate of pdNDF and the greater rate of particle size reduction for HF in determining the physical filling effects of the diet.

As a result of greater DMI for LF, yields of raw and 3.5% fat-corrected milk also were greater for LF (Table 3). Milk fat concentration was lower for LF ($P = 0.04$), possibly because of tendencies for faster passage rates of iNDF, pdNDF, and starch for LF. Faster passage rate of digesta on LF might have resulted in

greater escape of rumen biohydrogenation intermediates (Harvatine and Allen, 2006). Some partially biohydrogenated FA may inhibit milk fat synthesis and thus lower milk fat concentration (Bauman and Griinari, 2003).

Effect of pVDMI on Response to Diet

Many of the treatment effects observed here have been demonstrated previously; the primary hypothesis for this experiment was that the *differences* in responses of these parameters to treatment would change with increasing pVDMI, used as an index of nutrient demand. Contrary to the hypothesis, individual responses of DMI, digesta passage rates, and 3.5% FCMY did not depend on preliminary intake (data not shown). Only the response to treatment of ruminal NDF turnover time depended on pVDMI (Figure 3). Mean NDF turnover time demonstrated little difference between the two treatments (Table 3), but as pVDMI increased, NDF turnover time increased more greatly for LF than for HF (Figure 3). This is likely why DMI of cows with the greatest pVDMI did not respond as positively to the LF diet as expected; a longer ruminal NDF turnover time suggests that LF may have had more physical filling effects than HF among cows with high pVDMI. Neither digestion rate nor passage rate explain this turnover time effect, because with increasing pVDMI, digestion rate of pdNDF and passage rates of iNDF and pdNDF changed similarly for both diets. Responses of passage rates of iNDF and pdNDF in particles < 2.36 mm and ≥ 2.36, and response of particle size reduction rate, did not depend on pVDMI (data not shown). It is possible that undetectable interactions between

diet and pVDMI in affecting both digestion and passage rates combined to create the detectable NDF turnover time effect.

Inhibition of NDF digestion or passage on a low-forage diet at high DMI could be caused by direct and indirect effects of increased starch intake and fermentation. Although DMI response did not depend on pVDMI, the greater starch concentration in the LF diet still led to a greater increase in starch intake for LF than for HF, with increased pVDMI ($P = 0.03$). An increased rate of starch fermentation can reduce ruminal pH, and lower pH can depress rumination, rumen motility, and NDF fermentation, which would affect NDF turnover time. On both treatments, ruminal pH tended to decrease as pVDMI increased ($P = 0.11$), and there was no difference in the slopes of the two lines ($P \geq 0.60$). However, mean ruminal pH was lower for LF than for HF ($P < 0.0001$; Table 3), so any effect of decreasing pH with increasing pVDMI on NDF digestion, rumination, or rumen motility, was likely more severe for LF than for HF. Therefore, it is possible that a lower ruminal pH on LF among cows with high pVDMI caused a longer NDF turnover time on LF with increasing pVDMI.

Relationships reported in a previous similar experiment (Voelker et al., 2002) were between response and preliminary milk yield (or milk energy output), but the same relationships also existed with preliminary DMI, so similar responses were expected, but not observed, in the current experiment. Several factors might have contributed to the observation of different responses in the present experiment and the previous experiment. First, the previous experiment utilized 32 cows and the present experiment used only 14 cows. However, the

ranges of preliminary DMI and FCMY were similar for the two sample groups, and a 12-animal subgroup of ruminally-cannulated cows in the 32-cow study detected dependencies of DMI and ruminal kinetics responses on pVDMI. Second, although high-forage and low-forage diets in the two experiments contained very similar proportions of NDF (24 and 31% of diet DM for both experiments) and starch (33 and 23% of diet DM for both experiments), differences existed between treatment diets in the two experiments. Particle size distributions of diets might have differed between experiments, but this cannot be determined because diet particle size was not measured for the first experiment. Non-forage fiber sources were included in diets for the previous experiment, so diet forage NDF concentration was lower for both diets in that experiment (16 and 24% of diet DM) than for the diets fed in the present experiment (20 and 27% of diet DM). The NDF in the non-forage fiber sources used in the previous experiment (dried corn distillers grains and whole cottonseed) likely had higher rates of NDF digestion and(or) passage, so those diets may have been less physically filling. The non-forage fiber sources were also sources of fat, and diets in the previous experiment contained a commercial fat supplement. Therefore, the caloric densities of those diets were likely greater compared to diets in the present experiment. Also, the rumen-available fats may have altered ruminal fermentation.

Finally, the corn grain used in the previous experiment was rolled high-moisture corn, so its ruminal fermentation characteristics were likely different from fermentation characteristics of the ground dry corn used in the present

experiment. Ruminal starch digestibility and digestion rate are factors involved in determining the effects of dietary starch concentration on NDF fermentation and DMI. Oba and Allen (2003a) reported that increasing dietary starch concentration increased DMI when grain was more slowly fermented (dry corn) but not when it was more rapidly fermented (high-moisture corn). The more rapidly fermented high-moisture corn in the first experiment likely would have contributed to greater intake depression on LF among cows with low pVDMI, but it also would have been more likely to lower ruminal pH and interfere with NDF digestion on LF among cows with higher pVDMI. It is likely that, because of dietary differences, digesta in the previous experiment were more rapidly fermented and(or) escaped more quickly from the rumen compared to digesta in the present experiment. This might have caused cows with lower pVDMI on the previous experiment to respond more negatively to the low-forage diet, which would contribute to an increasingly positive response to that diet as pVDMI increased.

SUMMARY AND CONCLUSIONS

A longer NDF turnover time on LF with increasing pVDMI led to responses of DMI and milk production to high-forage and low-forage diets that were independent of pVDMI. This response might have been mediated by diet effects on ruminal pH. The results of this experiment suggest that models that predict intake need to account for not only the effects of nutrient demand, but also the

effects of the interactions of feed fractions (such as starch and NDF) on the intake responses of individual cows to high- and low-forage diets.

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

Parameter	Mean	SD
Parity	2.9	0.7
BW, kg	597	55
BCS	2.6	0.7
DIM	178	120
Milk yield, kg/d	38.7	12.3
DMI, kg/d	25.0	2.7

Figure 1. Distribution of voluntary DMI (VDMI) and 3.5% fat-corrected milk yield (FCMY) of 14 cows during the final 4 d of the preliminary period, when cows were fed a common diet.

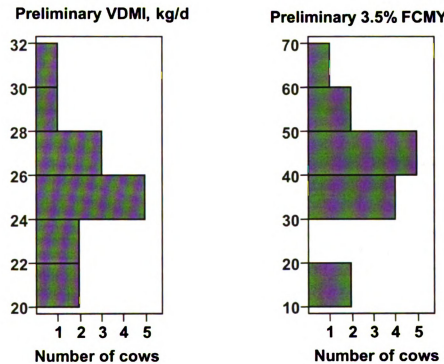


Table 2. Ingredient and nutrient composition of treatment diets, a low-forage diet (LF) and a high-forage diet (HF).

	LF	HF
Ingredient	% of DM	
Corn silage[1]	29.7	40.5
Alfalfa silage[2]	15.1	20.9
Dry ground corn	33.9	16.1
Soybean meal (48% CP)	11.0	9.1
Vitamin mineral mix[3]	3.2	4.3
Expeller-processed soybean meal[4]	5.9	9.1
Urea	0.2	----
Nutrient		
DM (% as fed)	47.0	39.6
	% of DM	
OM	93.0	92.1
NDF	24.4	30.7
Forage NDF	19.9	27.3
Indigestible NDF	13.2	15.1
Potentially digestible NDF	11.2	15.6
Starch	32.8	22.5
Crude protein	16.2	16.6
Rumen-undegraded CP[5]	7.2	7.3

[1] Corn silage contained 46.4% NDF, 16.9% iNDF, 18.6% starch, and 8.1 % CP. 30-h in vitro NDF digestibility was 47.5%.
[2] Alfalfa silage contained 40.6% NDF, 26.4% iNDF, 3.5% starch, and 18.3 % CP. 30-h in vitro NDF digestibility was 32.6%.
[3] Vitamin mineral mix contained (DM basis) 10.1 % dicalcium phosphate, 4.1% trace-mineral premix, 5.7% sodium bicarbonate, 1.2% magnesium oxide, 124.2 KIU/kg vitamin A, 40.3 KIU/kg vitamin D, 671.6 KIU/kg vitamin E, and 60.1% ground corn grain as a carrier.
[4] Nutrient composition: 86% DM, 7% ash, 16% NDF, 5% starch, 51% CP.
[5] Estimated using values from NRC (2001).

Figure 2. Model of ruminal particle size reduction and passage. Reduction of particle size during eating is included in rate of particle size reduction (k_r). Passage rates (k_p) are calculated for indigestible NDF (iNDF) and potentially digestible NDF (pdNDF); k_r is calculated for iNDF only.

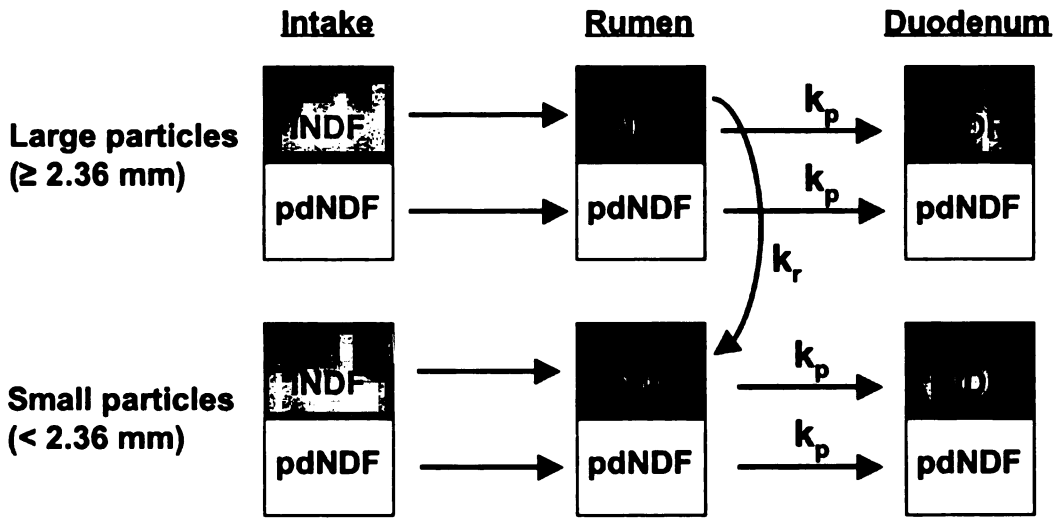


Table 3. Least-squares means of responses in feed intake, digestion, and production of 14 Holstein cows to low-forage (LF) and high-forage (HF) diets.

		Treatment LSM[1]				Trt x Per
		LF	HF	SEM[2]	*P*	*P*
Yield, kg/d						
Milk		39.6	36.1	3.5	0.0001	NS[3]
3.5% FCM		41.5	39.1	3.8	< 0.01	NS
Fat		1.51	1.46	0.15	0.13	NS
Milk composition, %						
Fat		3.79	3.93	0.11	0.04	NS
BW change, kg/15 d		8.2	-2.0	2.6	< 0.01	NS
BCS change, kg/15d		0.02	-0.03	0.06	0.47	NS
Intake, kg						
DM		27.8	24.7	0.9	< 0.0001	NS
NDF		6.8	7.5	0.2	< 0.001	NS
iNDF[4]		3.8	3.8	0.1	0.86	NS
Forage NDF, kg		6.7	5.6	0.2	< 0.0001	NS
Rumen Pool, kg						
DM		10.8	11.1	0.5	0.09	NS
NDF		5.7	6.2	0.3	< 0.01	NS
INDF		3.1	3.5	0.1	< 0.0001	NS
Rumen passage rate, /hr						
iNDF						0.06
	Per 1	4.9	4.8	0.16	0.68	
	Per 2	4.8	3.9	0.16	< 0.01	
pdNDF[5]		1.57	1.04	0.25	0.06	NS
Starch						0.08
	Per 1	21.9	23.1	3.1	0.79	
	Per 2	28.9	14.5	3.1	< 0.01	
pdNDF digestion rate, /hr		3.31	4.74	0.29	0.001	NS
Starch digestion rate, /hr						<0.01
	Per 1	23.4	33.1	2.1	< 0.01	
	Per 2	33.6	26.1	2.0	0.03	
Rumen turnover time of NDF, h						0.16
	Per 1	20.9	19.0	0.7	0.10	
	Per 2	20.4	21.6	0.7	0.32	
NDF digested in the rumen						
kg		2.0	3.0	0.1	< 0.0001	NS
%		29.3	40.1	2.0	< 0.001	NS
Mean pH		5.86	6.00	0.04	< 0.0001	NS

[1] Treatment least-squares means.
[2] Standard error of the mean.
[3] Not significant ($P \geq 0.30$), Trt x Per removed from model.
[4] Indigestible NDF.
[5] Potentially digestible NDF = NDF – iNDF.

Table 4. Least-squares means of particle size kinetics responses of 14 Holstein cows to low-forage (LF) and high-forage (HF) diets.

	Treatment LSM[1]			
	LF	HF	SEM[2]	*P*
Intake				
iNDF[3] < 2.36 mm, kg/d	2.35	1.87	0.04	< 0.0001
iNDF ≥ 2.36 mm, kg/d	1.46	1.96	0.03	< 0.0001
pdNDF[4] < 2.36 mm, kg/d	1.96	1.51	0.06	< 0.0001
pdNDF ≥ 2.36 mm, kg/d	1.85	2.32	0.07	< 0.0001
iNDF < 2.36 mm, % NDF	54.5	55.4	0.2	< 0.01
pdNDF < 2.36 mm, % NDF	45.5	44.6	0.2	< 0.01
iNDF ≥ 2.36 mm, % NDF	44.1	45.7	0.2	< 0.0001
pdNDF ≥ 2.36 mm, % NDF	55.9	54.3	0.2	< 0.0001
Rumen pool				
iNDF < 2.36 mm, kg	2.17	2.52	0.11	< 0.0001
iNDF ≥ 2.36 mm, kg	0.92	0.96	0.05	0.53
pdNDF < 2.36 mm, kg	1.43	1.50	0.11	0.25
pdNDF ≥ 2.36 mm, kg	1.16	1.21	0.08	0.48
iNDF <2.36 mm, % NDF	61.6	62.1	1.4	0.80
pdNDF < 2.36 mm, % NDF	38.4	37.9	1.4	0.80
iNDF ≥ 2.36 mm, % NDF	44.6	44.8	1.8	0.92
pdNDF ≥2.36 mm, % NDF	55.4	55.2	1.8	0.92
Passage rate, %/h				
iNDF < 2.36 mm	6.10	5.66	0.19	0.03
iNDF ≥ 2.36 mm	2.85	2.10	0.25	0.02
pdNDF < 2.36 mm	2.34	1.70	0.29	0.06
pdNDF ≥ 2.36 mm	0.64	0.59	0.16	0.78
Rate of reduction (iNDF pool ≥2.36 to iNDF pool <2.36 mm), /h	3.87	6.86	0.51	< 0.0001
Duodenal flux ≥ 2.36 /total duodenal flux				
iNDF	0.15	0.13	0.009	0.03
pdNDF	0.16	0.21	0.02	< 0.05

[1] Treatment least-squares means.
[2] Standard error of the mean.
[3] Indigestible NDF.
[4] Potentially digestible NDF = NDF – iNDF.

Figure 3. Relationship between mean DMI during the final 4 d of the preliminary period (pVDMI) and the response to a low-forage diet (LF) over a high-forage diet (HF) of ruminal NDF turnover time ($TOT_{LF} - TOT_{HF}$ = -5.6 – 0.24 pVDMI; P = 0.05). Equation is adjusted for Seq (Seq LF, HF = -1.08; Sequence HF, LF = 0).

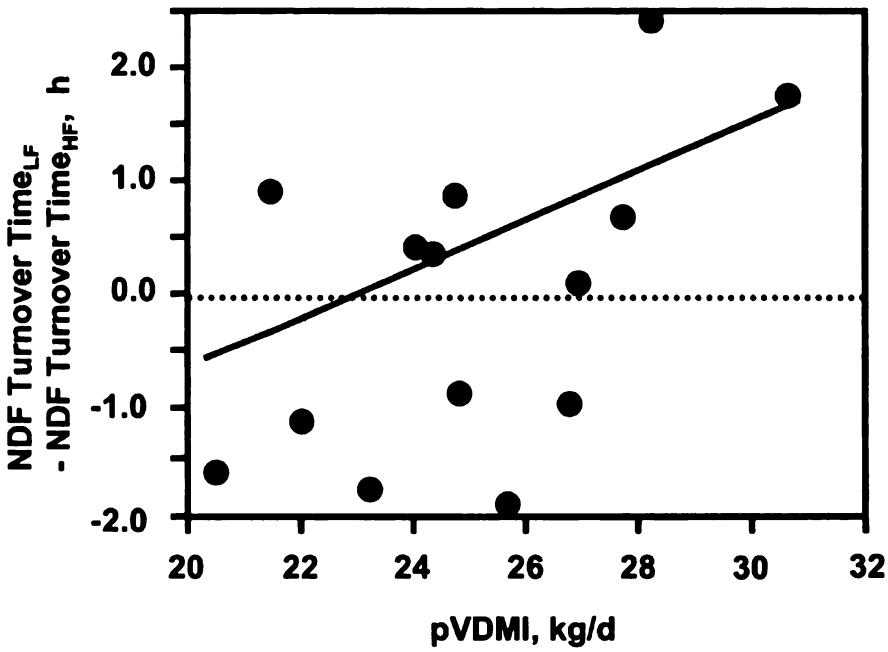
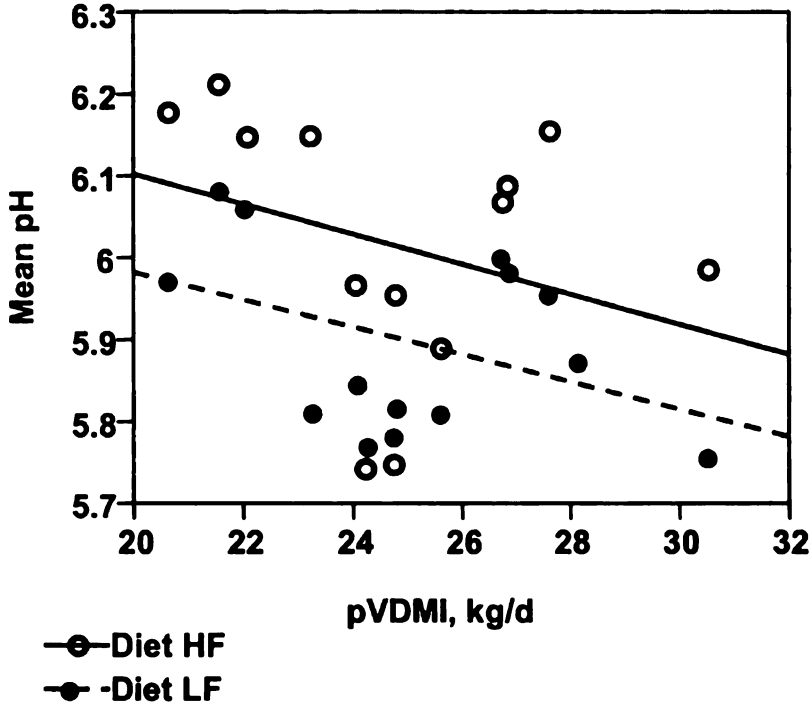


Figure 4. Relationship between mean DMI during the final 4 d of the preliminary period (pVDMI) and mean daily ruminal pH. Across treatments, ruminal pH = 6.4 – 0.02 pVDMI ($P = 0.11$, $R^2 = 0.10$, RMSE = 0.14). Mean pH was greater for HF than for LF ($P < 0.0001$), and relative response to treatment did not depend on pVDMI ($P \geq 0.60$).



CHAPTER 5

Predicting Ruminal Passage Rates of Fiber Fractions in Dairy Cattle

ABSTRACT

Passage rates of fiber fractions are important factors determining ruminal nutrient digestion, microbial protein production, efficiency, and flow to the duodenum, and the filling effects of a diet. Previous equations predicting passage rate have relied on measurements of ruminal disappearance or fecal appearance of external markers, which leads to inaccurate predictions. Data obtained in our laboratory from experiments utilizing the pool and flux method for estimating passage rates of digesta fractions were compiled and used to develop new regression equations predicting passage rates of indigestible NDF (iNDF) and potentially digestible NDF (pdNDF). Predictors used to develop the regression equations included dietary concentrations of NDF, forage NDF (forNDF), and starch; 30-h in vitro NDF digestibility of forages in the diet (forNDFD); DIM and BW; intake of DM, NDF, starch, and digested OM; MY, milk fat concentration, and 3.5% fat-corrected MY; ruminal pools of DM, NDF, and wet digesta. Equations were developed using both data that can be obtained by commercial dairy farms (e.g., DMI and diet composition) and data obtained in ruminal metabolism experiments (e.g., rumen pools). Predictions using data that can be obtained by dairy farms explained 68% and 53% of variation in passage rates of iNDF and pdNDF, respectively. The equations developed indicate that important predictors of passage rate that can be obtained by commercial dairy

farms include proportion of starch in the diet, DMI, forNDFD, proportion of forNDF in the diet, and FCMY. Improving predictions of passage rates will permit more efficient utilization of nitrogen and other nutrients and reduce their excretion as waste.

INTRODUCTION

To aid in formulating diets for dairy cows, numerous mathematical models of dairy cow digestion have been developed (Baldwin et al., 1987; Russell et al., 1992; NRC, 2001). These models estimate the availability of nutrients for milk production and other needs, given a particular set of feed composition characteristics, cow characteristics, and environmental factors. However, one of the factors most limiting to the accuracy of these models is their inability to account for the effects of dietary characteristics on voluntary feed intake and on the passage rate of digesta fractions from the rumen (Illius and Allen, 1994; Firkins et al., 1998).

Without an accurate prediction of passage rate, models cannot account for the effects of particle passage rate on feed intake or true protein flow to the duodenum. Most models overestimate both digestion rate and passage rate, and underestimate rumen pool size, because they rely on *in vitro* digestion of ground feeds and rare-earth or chromium marker passage data (Allen, 1996). Models of dairy cow digestion can be improved greatly by accurate predictions of passage rate. Therefore, the objective of this study was to develop new equations to predict passage rate of iNDF and pdNDF. Emphasis was placed on predictive

parameters that can be obtained by commercial dairy farms. The hypothesis was that important predictors would include DMI and those parameters that describe the potential of a diet to induce physical filling effects or metabolic factors to affect passage rate.

MATERIALS AND METHODS

Data sets from 11 studies conducted in our laboratory at Michigan State University between 1995 and 2003 were combined and used for estimations of ruminal passage rate of iNDF in dairy cattle (Table 1). Experimental procedures were approved by the Institutional Animal Care and Use Committee at Michigan State University. The data set included 254 animal-periods from multiparous lactating cows (nine studies), 29 animal-periods from primiparous lactating cows (one study), and 32 animal-periods from pregnant heifers (one study). All animals were ruminally and duodenally cannulated (gutter-style T cannulas); surgery was performed at the Department of Large Animal Clinical Science, College of Veterinary Medicine, Michigan State University. Nine studies followed Latin square designs and two followed crossover designs. Studies were designed originally to test hypotheses related to feed intake and ruminal and whole-tract digestion, and they were not designed for development of passage rate equations. Results of five of the 11 studies have been published in previous articles (Oba and Allen, 2000, 2003; Voelker and Allen, 2003; Taylor and Allen, 2005; Harvatine and Allen, 2006). Results of the other studies have been

reported in abstract form (Ying and Allen, 1998, 2005; Ying et. al, 1998; Mooney and Allen, 2004; Voelker Linton and Allen, 2005, 2006).

Forages fed in the totally mixed rations during the studies were primarily alfalfa silage and(or) corn silage; one study utilized orchardgrass silage. Diets also included ground or cracked dry or high-moisture corn grain and a variety of protein and fat supplements, byproducts, and mineral supplements. All animals were fed ad libitum, at 110% of expected intake.

Independent variables included in the data set (Table 2) were cow characteristics, chemical characteristics of diets and forages, parameters of intake and milk production that can be obtained by farms, and parameters of intake and digestion that can be measured in studies of ruminal and whole-tract digestion. All intake values were determined using weights and analysis of feed offered and orts. Rumen-empty BW was measured after evacuation of ruminal digesta on the day immediately before the start of the first period and on the final day of each period. Body condition score was determined on the same days by three trained investigators blinded to treatments (Wildman et al., 1982; 1=thin and 5 = fat). Changes in BW and BCS are reported per day to correct for different period lengths across studies; the value of cow BW is from the day before the start of period 1. For 9 of the 11 studies, ruminal pH was monitored over a 96-h period using a computerized data acquisition system via an indwelling probe inserted through the ruminal fistula (Dado and Allen, 1993). For the other two studies, ruminal pH was measured in fresh ruminal fluid samples removed through the ruminal fistula every 9 h in a 72-h period.

Sample Collection and Analyses

Passage rates of iNDF and pdNDF were measured using the pool and flux method (Oba and Allen, 2000). Most published equations predicting passage rate are determined by analysis of fecal excretion curves of external markers applied to intact forages and(or) concentrates and pulse dosed. By contrast, the pool and flux method requires a marker only to determine duodenal flux of digesta, so the marker does not need to flow with a specific digesta phase or fraction. Digesta markers differed among the 11 studies in the database and included a double-marker method (Cr-mordanted wheat straw and Co-EDTA), chromic oxide as an external marker, or iNDF as an internal marker. Dosing of external markers was spaced appropriately throughout the day to account for possible diurnal effects. Duodenal digesta were sampled every 9 h over a 72-h period. Ruminal contents were evacuated manually through the ruminal cannula approximately 4 h after feeding and 2 h before feeding on the second-to-last and last experimental days, respectively, of each period. Total ruminal content mass and volume were determined, and 10% aliquots were separated to allow accurate sampling of liquid and solid phases. Diet ingredients, orts, ruminal digesta, and duodenal digesta were analyzed for NDF according to Van Soest et al. (1991, method A), for iNDF after 120 or 240-h in vitro fermentation (Goering and Van Soest, 1970), and for starch by an enzymatic method (Karkalas, 1985) after samples were gelatinized with sodium hydroxide. Concentration of pdNDF was calculated as NDF (% DM) – iNDF (% DM). Original forage samples from 9 of the 11 studies were analyzed together for 30-h in vitro digestibility (forNDFD;

Goering and Van Soest, 1970). Samples were unavailable for the remaining two studies; one of those studies had published values for forNDFD, so those were included in the data set. When both samples and original (published) forNDFD values were available, the new and old values were compared. They were similar, so the new values were used in the data set.

Data were divided into two sets by randomly selecting two-thirds of the animal-periods from each study (a total of 210 records) to be assigned to a database that was used to develop models (BUILD), and then assigning the remaining one-third of the animal-periods from each study (a total of 105 records) to a database that was used to validate the models (VALIDATE). This horizontal division of the database was selected rather than a vertical division (using data from 2/3 of the studies for BUILD and 1/3 of the studies for VALIDATE), because differences in markers and slight differences in methods would have reduced the predictive power of the regressions developed in BUILD when applied to the VALIDATE set. Distributions of iNDF k_p, pdNDF k_p, and several predictor variables in BUILD and VALIDATE are reported in Table 4. Ranges of several variables were smaller in VALIDATE than in BUILD. This is expected because VALIDATE is a smaller subsample of the original data set, and it is acceptable because the subsample was selected randomly.

Statistical Analyses

Regression analyses were performed for iNDF k_p and for pdNDF k_p using backward stepwise regression of JMP (Version 5.1.2, SAS Institute, Cary, NC) for the BUILD data set. Predictor variables were included in linear, quadratic, and

cubic terms; if a higher-order term was significant, then all lower-order terms were also kept, regardless of their significance. Two-way interactions of main effects were included; three-way interactions were not included in order to avoid over-parameterization. Equations were developed by entering all potential predictors and removing predictors with the greatest *P*-value until all variables had $P < 0.10$. Overly influential animal-period records were identified by visual analysis of a distribution of Cook's D influence statistic after initial backward regression. Records were excluded when Cook's D was greater than 0.05 or when points were determined to be separate from the main cluster of values by visual examination. No more than 5% of records were removed during any regression operation. Backward regression was carried out again after removal of overly influential observations.

One set of potential predictors (Model 1) included all of the available parameters that typically could be obtained on or by a dairy farm: DIM, BW, diet % NDF, % forNDF, % starch, forNDFD, DMI, DMI(%BW), MY, FCMY, FCMY/DMI, milk fat %, BW change, and BCS change. A second set of potential predictors (Model 2) reduced the number of redundant parameters (e.g., included FCMY alone instead of FCMY, MY, and % milk fat). That predictor set included diet % NDF, diet % forNDF, forNDFD, diet % starch, DMI, BCS change, and either FCMY (iNDF Model) or both MY and milk % fat (pdNDF Model). These variables were selected not only to avoid over-parameterization, but also to attempt to create prediction equations for iNDF and pdNDF k_p that could be

incorporated into models of dairy cow digestion used for diet formulation or evaluation.

A third set of potential predictors (Model 3) included parameters that would be measured in a study designed to estimate k_p using the pool and flux method and that could be expected to be correlated with k_p and to have some reasonably causal influence on k_p (rather than being determined directly or primarily by k_p). These predictors included Model 1 parameters, plus intake of NDF, forNDF, and starch as a percent of BW, rumen pools of wet digesta, DM and NDF, rumen digesta volume, daily mean, variance and standard deviation of ruminal pH, and OM digested in the whole tract.

Initial evaluation of equations was performed in the BUILD data set by visual inspection of plots of residuals (observed – predicted) against predicted values (Neter et al., 1996). Patterns suggesting systematic tendencies for residuals to be positive or negative were considered indicators that the model under consideration did not sufficiently account for variation. Percentage of variation accounted for by an equation (R^2) and Akaike's Information Criterion (AIC; Akaike, 1974) also were used to determine the predictive value of a candidate equation. The AIC is calculated using the equation $AIC = 2p + n \ln(SSE / n)$, where p is the number of parameters, n is the number of observations, and SSE is the sum of squares of error (residuals). It examines the complexity of a model together with goodness of fit to the sample data, in order to find the minimal model that correctly explains the data. A lower AIC value indicates a more appropriate model.

After equations were developed using the BUILD data set and selected as candidate models, they were evaluated using the VALIDATE data set according to recommendations of Neter et al. (1996) and St-Pierre (2003). Each prediction equation for iNDF k_p developed in BUILD as used to calculate a set of predicted values for k_p in VALIDATE. Residuals (observed – predicted) were calculated, and predicted values were centered by subtracting the mean of all predicted values from each predicted value (St-Pierre, 2003). Residuals were regressed against centered predicted values and evaluated as recommended by St-Pierre (2003). Centering the predicted values places the intercept of the regression of residuals against predicted at the mean predicted value rather than at zero. This permits a t-test of the regression intercept to determine the statistical significance of mean bias, and a t-test of the regression slope to determine the statistical significance of linear bias. When the linear bias was statistically significant ($P < 0.05$), the magnitude of the bias was calculated for the maximum and minimum predicted values, using the following equation:

$$e_i = b_0 + b_1 (X_i - \bar{X})$$

where

e_i is the linear bias at i (maximum or minimum),

b_0 is the intercept of the regression of residuals on centered predicted values,

b_1 is the slope of the regression of residuals on centered predicted values,

X_i is the maximum or minimum predicted value, and

$\bar{X}$ is the mean of all predicted values.

The bias at the maximum and minimum predicted values was then judged relative to the size of the standard error of the regression. If either of the two calculated biases was greater than the standard error of the residual regression, then the linear bias was considered mathematically significant.

The effect of study was not included in the regressions performed in this analysis. Modern statistical software allows the Study effect to be included as a random effect in order to account for differences between studies such as experimental design, methods, and physiological status of animals, as well as to account for the mean and linear bias caused by Study (St-Pierre, 2001). However, the experiments from which these data were obtained used similar methods, which reduces the need to account for Study effect. Including Study effect in the iNDF models resulted in higher R^2 and lower AIC values for Model 2 and completely eliminated mean and linear bias from Models 2 and 3. However, the proportion of the range in actual predictor values that is accounted for by the range in mean (by study) predictor values was greater for diet-related parameters (e.g., dietary starch concentration) than for response-related parameters (e.g., MY). Thus, including Study as a random effect would remove more variation caused by diet factors than variation caused by other factors. Indeed, including Study resulted in the removal of one diet-related predictor, forNDFD, but no response-related predictor, from Model 2. Therefore, to avoid biasing the predictive power away from diet parameters and toward response parameters, the Study effect was not included in the models.

RESULTS AND DISCUSSION

Measuring Passage Rates of iNDF and pdNDF

This is the only large-scale summary of data known for passage rate of digesta NDF fractions calculated using the pool and flux method. Nearly all passage data available in the literature were measured by analysis of fecal excretion curves of external markers applied to intact forages and(or) concentrates and pulse dosed. Problems with external markers are well documented (Firkins et al., 1998). While these data might be useful to evaluate relative differences among treatments within experiments, they are not useful to predict digestibility of fractions within feeds across a wide range of conditions. Absolute measurements are required for passage rates when they are used with digestion rates to predict digestibility.

Other problems with external markers limit their usefulness even as a measure of passage rate of entire feeds. Problems include extensive migration from the labeled feeds (Teeter et al. 1984; Combs et al., 1992), preferential binding to small particles (Erdman and Smith, 1985), and a reduction in digestion rate which can increase density and passage rate (Firkins et al., 1998). Feeds intrinsically labeled with ^{14}C have been used (Holden et al., 1994) but this method is time consuming, expensive, and biased if the ^{14}C is not evenly distributed in the feed (Firkins et al., 1998). An additional problem is that passage rates usually are calculated by analysis of fecal excretion curves, the results of which are difficult to interpret. Two or more significant pools and rates can be determined, but it is not clear which rate represents passage from the rumen or

even that assignment of the resulting mathematically-defined pools to specific biological pools is valid. Finally, current predictions of ruminal digestibility of digesta fractions (e.g., starch, neutral detergent fiber (NDF), and protein fractions) are calculated using the digestion rates of those fractions and the passage rates of the individual feed ingredients that contain those fractions. Using the passage rates of feed ingredients produces inaccurate predictions for ruminal digestibility of digesta fractions, because the different fractions within a feed ingredient escape the rumen at different rates. Ruminal digestibility is determined for digesta chemical fractions, not for feed ingredients, so both the digestion rate and the passage used to predict digestibility ideally should be for fractions, not for ingredients. Passage rate data for the various chemical fractions have been either completely unavailable or limited until recently, when the development and increasing use of the pool and flux method resulted in the production of more data for passage rate of digesta fractions. To directly measure passage rates of digesta fractions, the fractional passage rates of individual, uniform digesta fractions can be calculated by dividing duodenal flux of the fraction by its ruminal pool size. This pool and flux method was used to obtain the data for this analysis.

Mean iNDF k_p in the BUILD data set was 3.17 h^{-1} and ranged from 1.04 to 5.81 (Table 4). In a recent summary, Seo et al. (2006) reported passage rates for dry forages, wet forages, and concentrates, estimated using rare earth markers, of 4.53, 5.17, and 6.69 h^{-1}, respectively, with ranges of 3.42 to 5.70, 3.9 to 6.29, and 3.61 to 9.22 h^{-1}, respectively. All three feed types contain some

iNDF; as a proportion of DM, forages contain more iNDF than do concentrates because they contain more total NDF. Mean iNDF k_p reported here was lower than k_p reported for forages by Seo et al (2006). In addition to iNDF, the NDF fraction of whole forages also contains a significant amount of potentially digestible NDF (pdNDF), which has a slower k_p (2.35 $\pm$1.05 h^{-1} in BUILD, Table 4) likely due to a greater concentration of fermentation gasses in pdNDF-rich particles (Allen, 1996). However, smaller particles likely contain a greater fraction of iNDF in total NDF than do large particles (see Chapter 3), and the pool and flux method measures passage rate of all iNDF, not only forage iNDF. As a result, iNDF passage rate is likely more representative of smaller, denser particles than are measured in marked forages and should therefore be faster, not slower, than is measured by marked forages. Furthermore, markers frequently increase the density of particles to which they are attached (Ehle et al., 1984), and rare earths migrate into the small particle and liquid pools (Erdman and Smith, 1985), so the actual passage rates of marked forages are artificially inflated compared to the passage rates of unmarked forages. This is why the ruminal passage rate of iNDF estimated using the pool and flux method is lower than the passage rates estimated for whole, marked forages. The overprediction of digesta passage rate with rare earths and other external markers, combined with actual digestion rate, leads to inaccurate estimates of ruminal digestibility and duodenal passage of nutrients in models used to formulate or evaluate dairy cow diets.

Passage rate of pdNDF demonstrated a width of range similar to that of iNDF, and the mean and range were approximately one unit lower than the mean and range of iNDF k_p (Table 4). More true and method-associated error are expected with pdNDF than with iNDF, because pdNDF can be removed from the rumen through digestion as well as through passage, and because pdNDF concentration is calculated using measured iNDF concentration. However, the data from these eleven experiments suggest similar variation in measurements of passage for both NDF fractions.

Predicting iNDF k_p Using Farm Data

A large number of parameters that can be obtained by commercial dairy operations demonstrated the potential to predict iNDF k_p (Table 2). Backward stepwise regression considering all of these parameters (iNDF Model 1) resulted in very strong predictive power within BUILD (R^2 = 0.94, AIC = -156; Table 5) but also in significant over-parameterization and a very weak capability to predict k_p in VALIDATE, as evidenced by significant mean ($P < 0.0001$) and linear ($P < 0.0001$) biases (Table 5, Figure 1). A description of the predictors included in iNDF Model 1 is presented in Table 6a. Beginning backward regression with a much smaller pool of potential predictors (iNDF Model 2), which were selected for mechanistic importance and to avoid redundancy, resulted in lower predictive power within BUILD (R^2 = 0.67) and a less favorable (higher) AIC value (-96; Table 5). However, when the resulting equation was evaluated in VALIDATE, no mean bias existed ($P = 0.86$; Figure 2). Although linear bias was statistically significant ($P < 0.001$; Table 5), bias at both the minimum and maximum

predicted values was 0.68, which is smaller than standard error of the residual regression (0.87) and therefore biologically insignificant. The model was able to account for a surprisingly large proportion (68%) of variation in iNDF k_p (Table 5).

Therefore, for applications in models used for diet formulation or evaluation on dairy farms, the equation created in iNDF Model 2 is likely the most appropriate equation for predicting iNDF k_p using the parameters available in this data set. A description of the predictors included in iNDF Model 2 is in Table 6b. The iNDF Model 2 accounts for the effects of: (1) proportion of starch in the diet, (2) DMI, (3) forNDFD, (4) proportion of forNDF in the diet, and (5) FCMY. Two-way interactions between diet % starch and forNDFD, DMI, and FCMY, and between DMI and FCMY, also contributed significantly to the prediction of iNDF k_p (Table 6b). Direct mechanistic interpretation of the equation is not practical because two quadratic, two cubic terms, and four interactions were included, but biological evidence exists for effects on k_p of the parameters selected.

Proportion of concentrate in the diet was determined to be the most significant predictor in the NRC (2001) calculations for passage rate of dry forage and concentrate, but not for wet forage, which is the source of the majority of the iNDF in the diets in the present data set. The relationship observed here between diet % starch and iNDF k_p was cubic; Seo et al. (2006) reported varying effects of increased diet concentrate on passage rates of concentrate and dry forages. Increasing grain content of the diet can increase passage rate (Grovum, 1986), but starch fermentation might reduce passage rate of digesta in general, or of iNDF in particular, by interfering with fiber digestion (Grant and Mertens,

1992) or through effects of ruminal pH on digestion and ruminal motility (Allen et al., 2006). Observed effects of increasing dietary grain concentration on passage rate likely depend on several factors. These include the relative proportions of forage and grain in the diet, the fermentability of the grain (i.e., conservation method, moisture content, vitreousness, and particle size), forage particle size, and the rate of NDF digestion and particle size reduction of the forage.

A positive correlation between DMI and k_p has often been assumed, but until recently, data were lacking to confirm this assumption (Illius and Allen, 1994). Predictions of passage rate in the 2001 NRC protein model (NRC, 2001), and the recent re-evaluation of those equations (Seo et al., 2006) both included DMI (as a percentage of BW) as a very important factor in predicting k_p; as DMI increases, k_p increases (NRC, 2001). In Model 2, DMI was used alone, rather than as DMI (%BW), because commercial dairy farms may not be equipped to obtain actual BW. Interestingly, effects of FCMY on k_p were not completely accounted for through DMI but needed to be included separately. Mechanisms by which nutrient demand affects passage rate might include increased ruminoreticular contraction rate, strength, or duration.

Digestibility of forNDF, estimated by 30-h in vitro fermentation, also contributed to the prediction of iNDF k_p (Table 6b). Within forage family (i.e., grasses or legumes), greater in vitro digestibility of forNDF usually results in greater DMI (Oba and Allen, 1999b), which suggests that ruminal passage rate is also increased with greater NDF digestibility. However, passage rate is slower

for grass despite greater NDFD (Chapter 2). The relationship between in vitro digestibility and passage rate is complicated not only by forage family but also by the fact that pdNDF usually exhibits a slower k_p than does iNDF, as mentioned above. Therefore, NDF digestibility is not a proxy for rate of NDF digestion; a clear example of this is the generally slow rate, but high extent, of NDF digestion in perennial grasses (Wilson and Hatfield, 1997). Rate of NDF digestion is not commonly measured for forages used on commercial dairy farms, but the measurement of in vitro NDF digestibility for forages is becoming increasingly common.

Actual NDF concentrations of forages (as opposed to values obtained from tables) also are increasingly available to dairy farms, so dietary forNDF concentration can be calculated. Furthermore, recommendations for NDF and non-forage carbohydrate concentrations in the most recent NRC (2001) include a minimum dietary forNDF concentration, in addition to minimum total dietary NDF and maximum total dietary non-forage carbohydrate. Generally, greater forNDF concentration would be expected to result in slower iNDF k_p. Effects of dietary forNDF concentration will depend on the digestibility of that forNDF and also on forage particle size. In the studies from which the present data set was obtained, particle size was seldom measured and so could not be used in prediction equations. Because forage type did not vary widely (diets contained primarily corn silages and(or) alfalfa silages) and chop lengths were similar across experiments, variation in forage particle size was likely much smaller than variation present in diets fed across the U.S. Quantifying the effects of particle

size on feed intake or nutrient digestibility continues to be a significant challenge, and sensitivity to particle size would be low in a data set with relatively small particle size variation.

Predicting iNDF k_p in Research Studies

A third model was tested to predict iNDF k_p using data that routinely are obtained in studies designed to estimate k_p using the pool and flux method. To the "farm" parameters (iNDF Model 2) were added forNDF intake, rumen pools of wet digesta, DM, and NDF, rumen digesta volume, daily mean and variance of ruminal pH, and digested OM intake. Results of backward regression using these parameters (iNDF Model 3) are presented in Tables 4 and 5c. Surprisingly, although a large number of terms were included in the model, no mean or linear bias existed when the model was evaluated in VALIDATE (Figure 3). The only cow descriptor included was BW; diet characteristics included dietary concentration (% DM) of total NDF, forNDF, and starch, and forNDFD. Instead of DMI, NDF intake, NDF intake (%BW), starch intake (% BW), and OM digested in the whole tract were significant predictors of iNDF k_p. These parameters likely account for effects of DMI on k_p. Intake of NDF likely accounts for some variation in k_p due to physical filling effects. No parameters of milk production were included, but BCS change (unit/d) contributed significantly to the model. This suggests that energy balance might affect passage rate, possibly through physiological controls such as rumen motility and reticular contraction frequency or duration. Rumen DM pool was the only significant predictor that could not be measured in intact animals. Creating acceptable passage rate

prediction equations that include only parameters that can be measured in intact animals would be preferable to creating equations that require cannulation surgery for continued use in predicting passage rate.

Predicting pdNDF k_p Using Farm Data

Fewer parameters that can be obtained by commercial dairy operations demonstrated the potential to predict pdNDF k_p (Table 3) compared to iNDF k_p (Table 2). Backward stepwise regression considering all of these parameters (pdNDF Model 1) resulted in strong predictive power within BUILD (R^2 = 0.85, AIC = -127; Table 5) but also in significant over-parameterization and a very weak capability to predict k_p in VALIDATE, as evidenced by significant mean (P = 0.05) and linear (P < 0.0001) biases (Table 5, Figure 4). A description of the predictors included in pdNDF Model 1 is presented in Table 7a. Beginning backward regression with a smaller pool of potential predictors (pdNDF Model 2), which were selected for mechanistic importance and to avoid redundancy, resulted in lower predictive power within BUILD (R^2 = 0.53) and a less favorable (higher) AIC value (-85; Table 5). However, when the resulting equation was evaluated in VALIDATE, no mean bias existed (P = 0.43; Figure 5). Although linear bias was statistically significant (P = 0.02; Table 5), the maximum bias (0.65) was smaller than standard error of the residual regression (0.95) and therefore mathematically insignificant.

Therefore, for applications in models used for diet formulation or evaluation for commercial dairy farms, the equation created in pdNDF Model 2 is likely the most appropriate equation for predicting iNDF k_p using the parameters

available in this data set. Because pdNDF can be removed from the rumen by digestion as well as by passage, the accuracy of prediction of pdNDF k_p might be lower than the accuracy of prediction of iNDF k_p, as demonstrated by the lower R^2 value for pdNDF Model 2 compared to iNDF Model 2 (Table 5). A description of the predictors included in pdNDF Model 2 is presented in Table 7b. The pdNDF Model 2 accounts for the effects of: (1) proportion of starch in the diet, (2) MY, (3) proportion of forNDF in the diet, (4) proportion of total NDF in the diet, (5) DMI, (6) BCS change, and (7) forNDFD. Two-way interactions between diet % NDF and diet % forNDF, MY, and forNDFD, between diet % forNDF and forNDFD and BCS change, and between diet % starch and BCS change, also contributed significantly to the prediction of iNDF k_p (Table 7b). The biological importance of diet % starch, diet % forNDF, diet % NDF, and forNDFD in affecting k_p were discussed previously. Two different predictors were included in pdNDF Model 2 compared to iNDF Model 2. The pdNDF Model 2 (Table 7b) included MY instead of FCMY, and BCS change (unit/d), which iNDF Model 2 did not include (Table 6b). However, MY, BCS change, and FCMY are all indicators of nutrient demand and utilization. The mechanisms for these effects are multifactorial and many are yet to be demonstrated empirically; however, in general, greater nutrient demand likely results in greater passage rate in order to permit greater DMI. A negative linear relationship between pdNDF k_p and BCS change (data not shown) indicates that more positive energy balance (gaining more body tissue) was associated with slower pdNDF k_p.

Predicting pdNDF k_p in Research Studies

A third model was tested to predict pdNDF k_p using the same predictors used for iNDF Model 3 (see above). Results of backward regression using these parameters (pNDF Model 3) are presented in Tables 4 and 6c. As with iNDF Model 3, no mean or linear bias existed when the pdNDF Model 3 was evaluated in VALIDATE (Table 5, Figure 6). However, the predictive power of pdNDF Model 3 was much lower (R^2 = 0.53) than the predictive power of iNDF Model 3 (R^2 = 0.91; Table 5). Predictors included in pdNDF Model 3 (Table 7c) were different from predictors included in iNDF Model 3 (Table 6c). Model 3 for pdNDF added rumen pools of DM, NDF, and wet digesta, and the standard deviation of ruminal pH, to diet forNDF concentration, DMI, FCMY, and BW change. The iNDF model included more diet and intake descriptors, and the pdNDF model included more rumen pool descriptors. Even though forNDFD is related directly to the concentration of pdNDF in the forages included in the diets, forNDFD did not add to the prediction of pdNDF k_p when ruminal pool and pH data were available. Only the pdNDF model, not the iNDF model, included a measure of ruminal pH; increasing pH standard deviation was related negatively to pdNDF k_p (Table 7c). More variation in ruminal pH throughout the day might reduce ruminal motility, which can reduce k_p of digesta, and more variation in pH likely slows the rate of pdNDF digestion, which can decrease the specific gravity of particles containing pdNDF and thus reduce the k_p of pdNDF.

Limitations to Application of Developed Models

The application of any model is limited, in particular, by the range of data from which it was developed and the segment of the population from which that data was obtained. Data for these models were obtained from experiments conducted by a single using animals from a single farm. Therefore, although the database demonstrated wide variation in diet characteristics (e.g., dietary NDF concentration range was 22.6 – 38.0), ingredients (particularly, forages and grains) used in the diets varied less than would be observed in a random sample of U.S. dairy farms. Furthermore, the animals utilized in the original experiments were samples from one farm over the course of 10 years and therefore represent far less genetic variation than exists across farms throughout the U.S. Therefore, the application of the current stage of these models to predict passage rate is very limited. Future prediction equations for passage rate utilizing data obtained through the pool and flux method should represent, at least, a wider range in dietary ingredients. Thus, the significance of these models lies not in their ability to predict passage rate across farms but rather in the biological significance of the variables that contributed to the prediction of passage rate.

CONCLUSIONS AND IMPLICATIONS

Prediction of passage rate of digesta is dependent on diet characteristics and nutrient demand of the individual animal. A model including diet % starch, DMI, forNDFD, diet % forNDF, and 3.5% FCMY accounted for 68% of variation in iNDF k_p. A model including diet % starch, MY, diet % forNDF, diet % NDF, DMI,

BCS change, and forNDFD accounted for 53% of variation in pdNDF k_p. Passage rate of digesta fractions is seldom measured directly, but strategic production of data sets containing more easily-measured parameters along with passage rates can increase the accuracy of the prediction of passage rates in models intended for use on commercial dairy farms. Improving predictions of passage rates will permit more efficient utilization of N and other nutrients and reduce their excretion as waste.

Table 1. Studies included in the data set used for development of passage rate equations in dairy cattle.

Study	Description
1	Treatments were coarsely or finely ground dry or high-moisture corn grain, fed to pregnant heifers (Ying and Allen, 1998)
2	Treatments were coarsely or finely ground dry or high-moisture corn grain, fed to primiparous lactating cows (Ying and Allen, 1998)
3	Treatments were high- or low-NDF diets containing normal or brown-midrib corn silage (Oba and Allen, 2000)
4	Treatments were high- or low-starch diets containing ground high-moisture or dry corn grain (Oba and Allen, 2003)
5	Treatments were dried, pelleted beet pulp substituted for high-moisture corn grain at 0 (control), 6, 12, and 24% of diet DM (Voelker and Allen, 2003)
6	Treatments were sodium bicarbonate, sodium chloride, or no added ions (Mooney and Allen, 2004)
7	Treatments were brown-midrib or control corn silage and floury or vitreous varieties of corn grain (Taylor and Allen, 2005)
8	Treatments were no added fat (control), saturated fat supplement, unsaturated fat supplement, or 50% of each fat supplement (Harvatine and Allen, 2006)
9	Treatments were dry or high-moisture preserved of floury or vitreous varieties of corn (Ying and Allen, 2005)
10	Treatments were alfalfa silage or orchardgrass silage (Voelker Linton and Allen, 2005)
11	Treatments were diets containing high or low concentrations of forage NDF (Voelker and Allen, 2006)

Table 2. Independent variables included in the data set used for development of iNDF passage rate equations in dairy cattle.

	Correlation to iNDF k_p					
	Linear		Quadratic		Cubic	
	P	r	*P*	r	*P*	r
Days in Milk	< 0.01	0.22	0.09	-0.26	< 0.01	0.35
BW[1], kg	< 0.0001	0.32	NS	-----	NS	-----
Diet % NDF	NS	-----	< 0.0001	0.32	0.13	-0.34
Diet % forNDF[2]	NS	-----	< 0.01	0.22	0.02	0.28
Diet % starch	< 0.0001	0.57	< 0.01	0.59	NS	-----
ForNDFD[3] %	< 0.0001	0.29	< 0.0001	-0.45	NS	-----
DMI, kg/d	< 0.0001	0.47	NS	-----	0.05	-0.49
DMI,%BW	< 0.0001	0.39	NS	-----	NS	-----
NDF intake, kg/d	< 0.0001	0.51	NS	-----	0.02	-0.53
ForNDF intake, kg/d	< 0.0001	0.54	NS	-----	0.02	-0.55
Starch intake, kg/d	< 0.01	0.24	NS	-----	0.03	0.29
MY, kg/d	NS	-----	NS	-----	0.12	0.15
FCMY[4], kg/d	NS	-----	NS	-----	0.11	-0.13
FCMY/DMI	< 0.0001	-0.31	NS	-----	NS	-----
Milk fat %	NS	-----	< 0.001	-0.29	NS	-----
BW change, kg/d	NS	-----	NS	-----	NS	-----
BCS change, /d	NS	-----	NS	-----	NS	-----
Ruminal DM pool, kg	NS	-----	< 0.0001	-0.29	NS	-----
Ruminal NDF pool, kg	NS	-----	NS	-----	0.03	-0.17
Ruminal digesta, kg	NS	-----	< 0.01	0.21	NS	-----
Ruminal digesta, L	NS	-----	0.01	-0.20	NS	-----
Digested OM, kg/d	< 0.0001	0.32	< 0.001	-0.40	0.04	-0.43
Ruminal pH mean	< 0.001	-0.26	0.08	0.29	0.02	0.30
pH variance	< 0.01	0.25	0.01	-0.32	0.01	0.38
pH standard deviation	0.02	0.17	0.04	-0.23	NS	-----

[1] Rumen-empty body weight.
[2] Forage NDF.
[3] 30-h in vitro NDF digestibility.
[4] 3.5% fat-corrected milk yield.

Table 3. Independent variables included in the data set used for development of pdNDF passage rate equations in dairy cattle.

	Correlation to pdNDF k_p					
	Linear		Quadratic		Cubic	
	P	r	*P*	r	*P*	r
Days in Milk	< 0.01	-0.22	0.18	0.25	NS	-----
BW[1], kg	NS	-----	NS	-----	NS	-----
Diet % NDF	NS	-----	NS	-----	NS	-----
Diet % forNDF [2]	NS	-----	NS	-----	NS	-----
Diet % starch	NS	-----	< 0.01	0.23	NS	-----
ForNDFD[3] %	NS	-----	0.02	-0.19	NS	-----
DMI, kg/d	NS	-----	< 0.01	-0.23	NS	-----
DMI,%BW	NS	-----	0.11	-0.16	0.03	0.16
NDF intake, kg/d	NS	-----	NS	-----	< 0.01	0.22
ForNDF intake, kg/d	NS	-----	NS	-----	0.001	0.24
Starch intake, kg/d	NS	-----	NS	-----	0.05	0.17
MY, kg/d	0.01	-0.19	< 0.01	-0.28	0.01	0.11
FCMY[4], kg/d	0.04	-0.16	< 0.01	-0.14	0.02	0.33
FCMY/DMI	0.19	-0.10	< 0.01	-0.26	NS	-----
Milk fat %	0.12	0.12	0.19	0.16	< 0.10	-0.20
BW change, kg/d	NS	-----	NS	-----	NS	-----
BCS change, d^{-1}	0.17	-0.10	0.19	0.14	NS	-----
Rumen DM pool, kg	0.02	-0.17	< 0.001	-0.30	< 0.01	0.37
Rumen NDF pool, kg	0.11	-0.12	< 0.01	-0.23	0.01	0.29
Ruminal contents, kg	0.05	-0.14	< 0.0001	-0.32	NS	-----
Ruminal contents, L	NS	-----	0.02	-0.18	0.001	0.29
Digested OM, kg/d	NS	-----	< 0.01	-0.20	NS	-----
Ruminal pH mean	NS	-----	NS	-----	0.07	-0.15
pH variance	NS	-----	NS	-----	NS	-----
pH standard deviation	< 0.01	-0.23	0.01	-0.30	NS	-----

[1] Rumen-empty body weight.
[2] Forage NDF.
[3] 30-h in vitro NDF digestibility.
[4] 3.5% fat-corrected milk yield.

Table 4. Comparison of distributions of iNDF and pdNDF k_p, descriptors, and potential predictor variables in BUILD and VALIDATE data sets used for development of passage rate equations in dairy cattle.

	BUILD				VALIDATE			
Variable	N	Range	Mean	S.D.	N	Range	Mean	S.D.
DIM	173	32 - 388	94	69.7	90	32 - 388	91.9	72.5
BW[1], kg	193	396 - 760	557	67	100	414 - 753	557	64
Diet % NDF	194	22.9 - 38.0	28.0	3.1	100	22.6 - 36.4	27.6	2.9
Diet % forNDF[2]	194	16.6 - 29.5	21.1	4.1	100	16.6 - 29.5	20.7	4.1
ForNDFD,[3] %	175	29.7 - 65.9	42.9	8.9	99	29.7 - 65.9	43.0	8.7
DMI, kg/d	194	3.2 - 34.1	21.1	6.0	99	2.8 - 29.9	21.7	6.0
DMI, %BW	193	0.74 - 5.84	3.79	1.01	99	0.68 - 5.91	3.88	1.00
MY, kg/d	173	9.9 - 59.8	36.9	9.4	89	20.5 - 56.3	38.9	8.3
FCMY[4], kg/d	189	8.5 - 60.7	37.4	8.8	89	21.5 - 62.8	39.2	8.1
Milk fat %	173	1.62 - 6.42	6.66	0.68	89	2.24 - 5.17	3.61	0.58
iNDF k_p,[5] h^{-1}	173	1.04 - 5.81	3.17	1.16	89	1.06 - 5.60	3.05	1.03
pdNDF k_p,[6] h^{-1}	188	-1.41 - 4.72	2.35	1.05	99	0.07 - 4.84	2.25	1.07

[1] Rumen-empty body weight.
[2] Forage NDF.
[3] 30-h in vitro NDF digestibility.
[4] 3.5% fat-corrected milk yield.
[5] Indigestible NDF passage rate.
[6] Potentially digestible NDF passage rate.

Table 5. Diagnostic statistics for building and validating models for prediction of passage rate of iNDF and pdNDF.

	Diagnostics in BUILD				Mean and linear bias in VALIDATE			
Model[a]	n	R^2	RMSE	AIC[b]	Mean bias P[c]	Linear bias P[d]	s[d]	Linear bias?[d]
iNDF								
1	146	0.943	0.473	-156	<0.0001	< 0.0001	1.06	Yes
2	148	0.676	0.687	-96	0.86	< 0.001	0.88	No
3	167	0.910	0.382	-296	0.70	0.11	0.55	No
pdNDF								
1	148	0.854	0.561	-127	0.05	< 0.0001	1.06	Yes
2	148	0.527	0.710	-85	0.43	0.02	0.95	No
3	143	0.529	0.741	-68	0.72	0.35	0.85	No

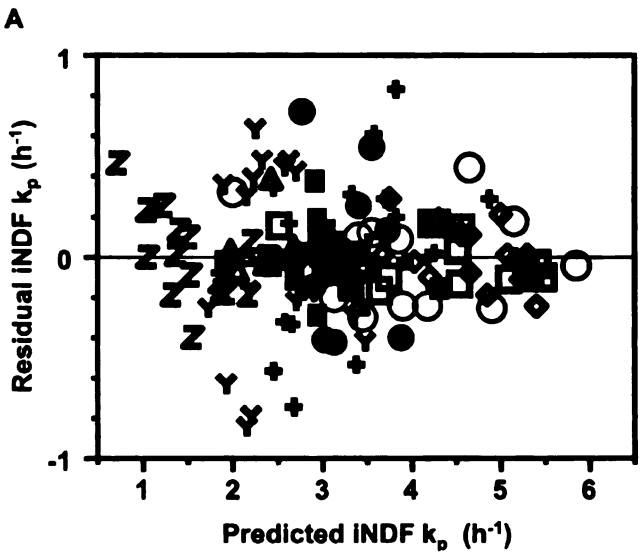
[a] See text for description of statistical analyses and see Tables 5(a-c) and 6(a-c) for descriptor statistics.

[b] Akaike's Information Criterion (AIC) = 2p + n ln(SSE / n), where p is the number of parameters, n is the number of observations, and SS is the sum of squares of the error (residuals).

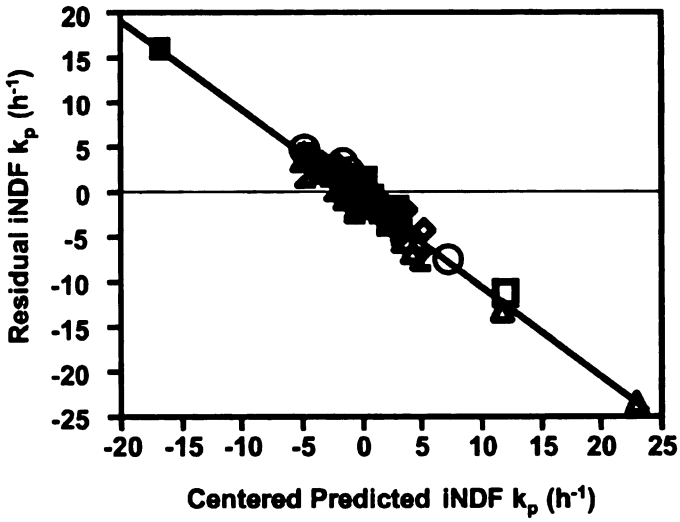
[c] Mean bias is the intercept of the regression of residuals against centered predicted values (predicted values – mean predicted value). $P < 0.05$ indicates significant mean bias (St-Pierre, 2003).

[d] Linear bias is the slope of the regression of residuals against centered predicted values (predicted values – mean predicted value). $P < 0.05$ indicates statistically significant linear bias. When the linear bias was statistically significant ($P < 0.05$), the magnitude of the bias was calculated for the maximum and minimum predicted values, using the equation $e_i = b_0 + b_1 (X_i - \bar{X})$ and then judged relative to the size of the standard error (s) of the regression of residuals on centered predicted values. See text for more details.

Figure 1. (A) Plot of residual (observed minus predicted) iNDF k_p vs. predicted iNDF k_p resulting from Model 1 in BUILD data set. (B) Plot of residual iNDF k_p vs. predicted iNDF k_p resulting from Model 1 applied to VALIDATE data set. Predicted iNDF k_p was centered around the mean predicted value. Both mean bias (-0.736403) and linear bias (-0.99) were significant ($P < 0.0001$). Different symbols represent data from individual studies.

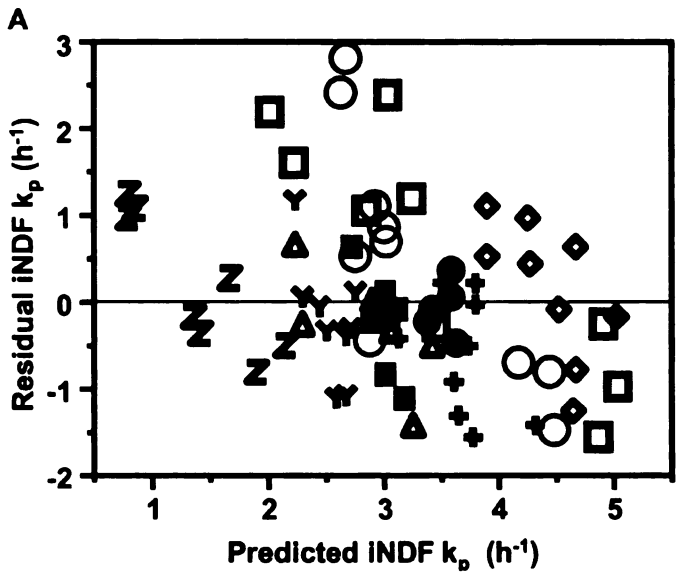


B



iNDF k_p = -0.736 (±0.122) - 0.991 (±0.027) (X - 3.88)
R^2 = 0.95, s = 1.06, P < 0.0001
Bias at min. predicted (-13.00) = 16.0
Bias at max. predicted (26.84) = 23.5

Figure 2. (A) Plot of residual (observed minus predicted) iNDF k_p vs. predicted iNDF k_p resulting from Model 2 in BUILD data set. (B) Plot of residual iNDF k_p vs. predicted iNDF k_p resulting from Model 2 applied to VALIDATE data set. Predicted iNDF k_p was centered around the mean predicted value. Mean bias was not significant ($P > 0.85$). Although linear bias (-0.374) was significant ($P < 0.001$), maximum bias (0.68) was lower than standard error of residuals (0.88). Different symbols represent data from individual studies.



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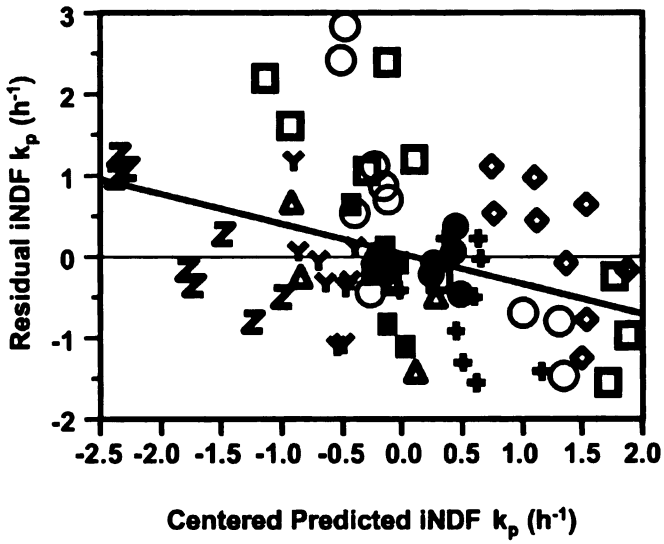
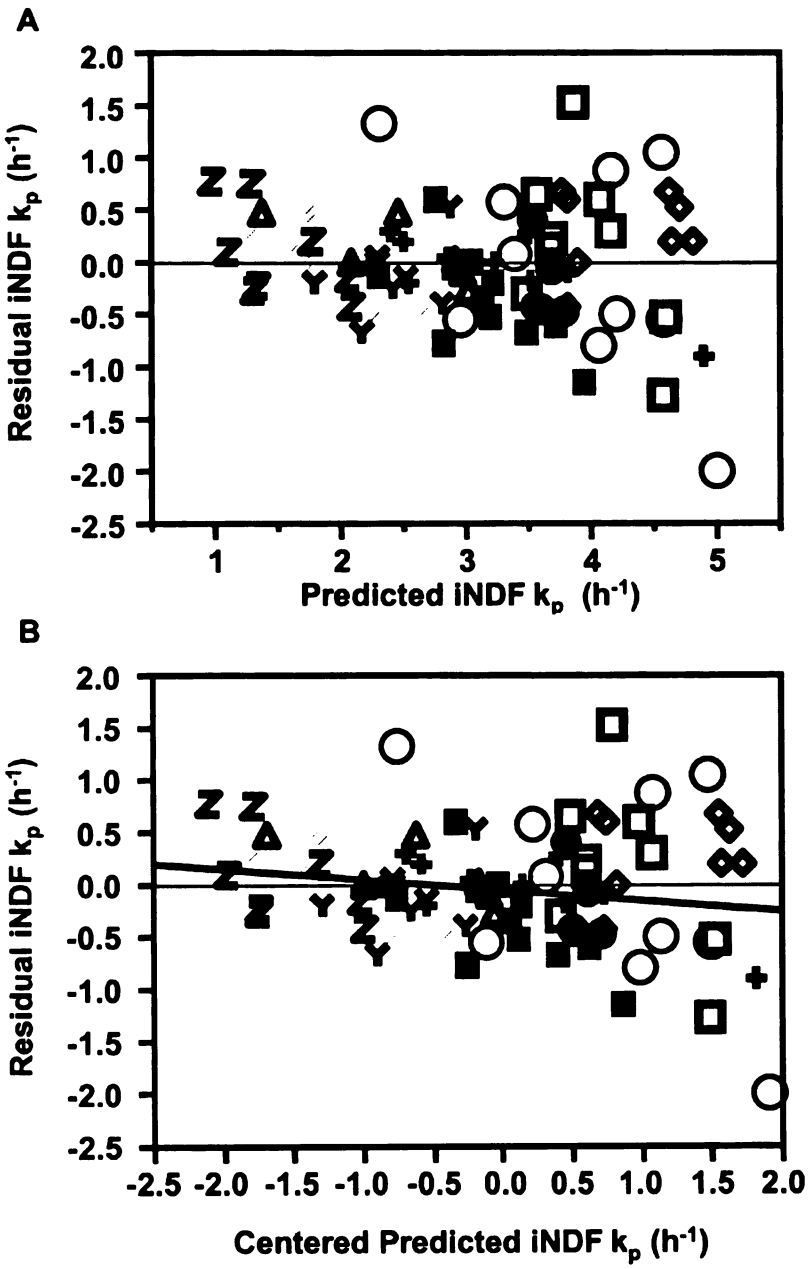


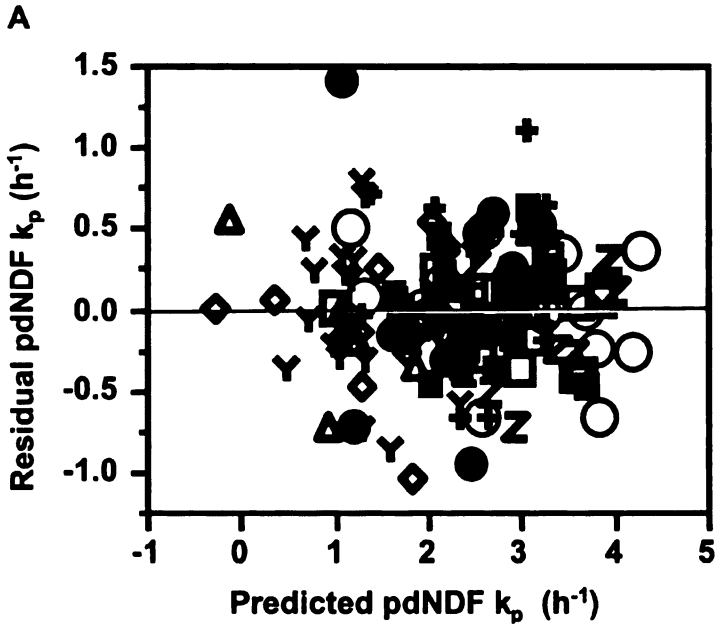
iNDF k_p = 0.0183 (±0.1000) - 0.374 (±0.105) (X - 3.15)
R^2 = 0.15, s = 0.878, P < 0.001
Bias at min. predicted (0.786) = 0.68
Bias at max. predicted (5.01) = 0.68

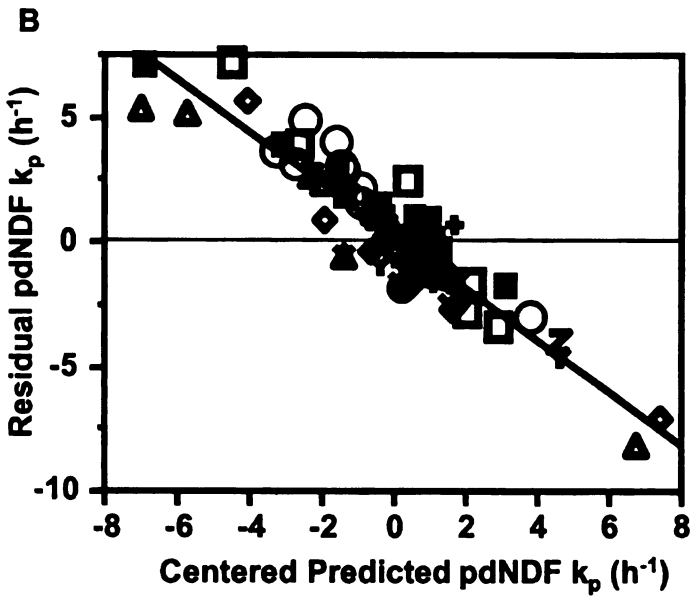
Figure 3. (A) Plot of residual (observed minus predicted) iNDF k_p vs. predicted iNDF k_p resulting from Model 3 in BUILD data set. (B) Plot of residual iNDF k_p vs. predicted iNDF k_p resulting from Model 3 applied to VALIDATE data set. Predicted iNDF k_p was centered around the mean predicted value. Mean bias was not significant ($P > 0.70$), nor was linear bias ($P > 0.10$). Different symbols represent data from individual studies.



iNDF k_p = -0.0227 (±0.0597) - 0.0970 (±0.0597) (X - 3.08)
R^2 = 0.030, s = 0.554, P = 0.11

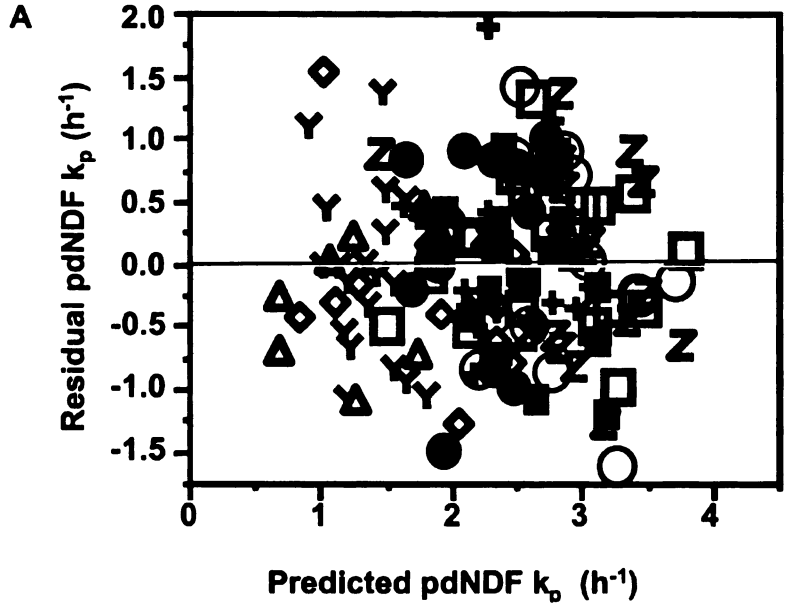
Figure 4. (A) Plot of residual (observed minus predicted) pdNDF k_p vs. predicted pdNDF k_p resulting from Model 1 in BUILD data set. (B) Plot of residual pdNDF k_p vs. predicted pdNDF k_p resulting from Model 1 applied to VALIDATE data set. Predicted pdNDF k_p was centered around the mean predicted value. Both mean bias (0.24) and linear bias (-1.05) were significant ($P = 0.05$, $P < 0.0001$, respectively). Different symbols represent data from individual studies.



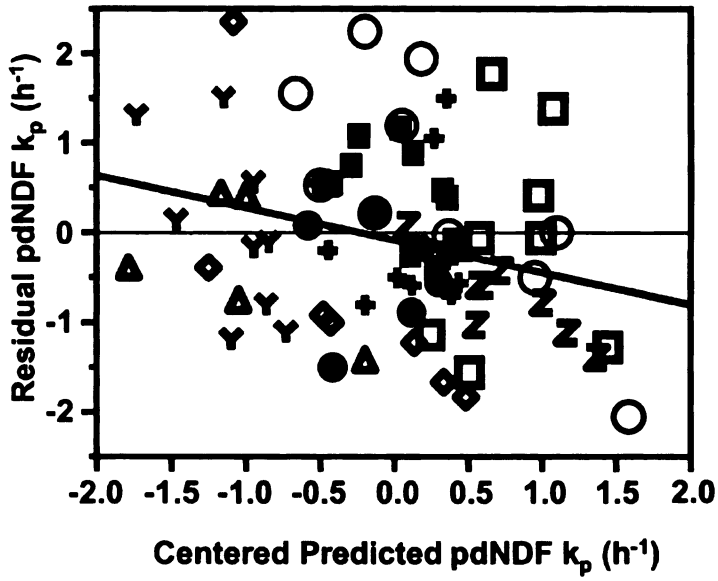


pdNDF k_p = 0.239 (±0.122) - 1.05 (±0.0496) (X - 2.05)
R^2 = 0.86, s = 1.06, P < 0.0001
Bias at min. predicted (-4.97) = 7.6
Bias at max. predicted (9.47) = 7.5

Figure 5. (A) Plot of residual (observed minus predicted) pdNDF k_p vs. predicted pdNDF k_p resulting from Model 2 in BUILD data set. (B) Plot of residual pdNDF k_p vs. predicted pdNDF k_p resulting from Model 2 applied to VALIDATE data set. Predicted pdNDF k_p was centered around the mean predicted value. Mean bias was not significant ($P > 0.43$). Although linear bias (-0.36) was significant ($P = 0.02$), absolute value of the maximum bias (0.65 at maximum predicted value of 3.95) was lower than standard error of residuals (0.95). Different symbols represent data from individual studies.



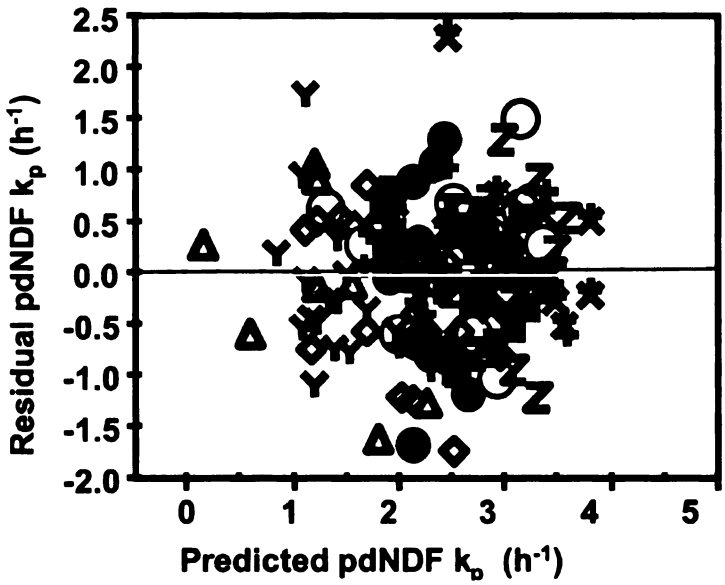
B



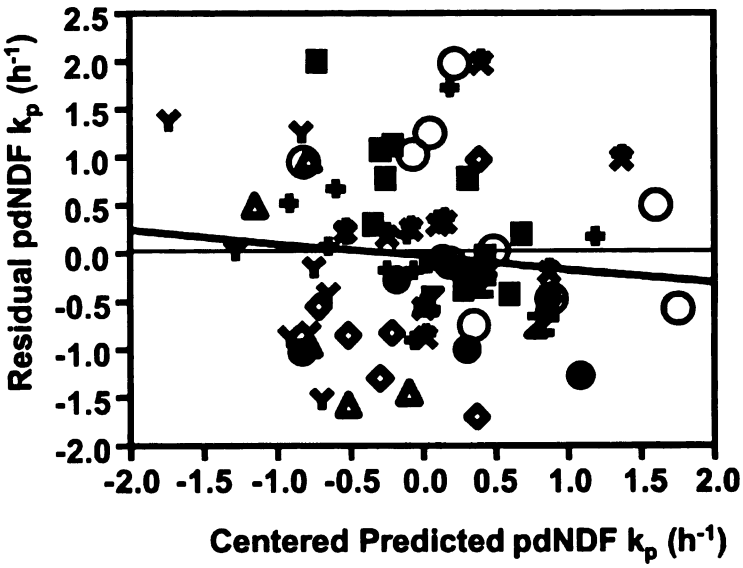
pdNDF k_p = -0.0865 (±0.109) - 0.357 (±0.147) (X - 2.36)
R^2 = 0.07, s = 0.954, P = 0.02
Bias at min. predicted (0.58) = 0.55
Bias at max. predicted (3.95) = -0.65

Figure 6. (A) Plot of residual (observed minus predicted) pdNDF k_p vs. predicted pdNDF k_p resulting from Model 3 in BUILD data set. (B) Plot of residual pdNDF k_p vs. predicted pdNDF k_p resulting from Model 3 applied to VALIDATE data set. Predicted pdNDF k_p was centered around the mean predicted value. Mean bias was not significant ($P > 0.72$), nor was linear bias ($P > 0.35$). Different symbols represent data from individual studies.

A



B



pdNDF k_p = -0.0348 (±0.0970) - 0.137 (±0.146) (X - 2.30)
R^2 = 0.01, s = 0.851, P = 0.35

Table 6a. Descriptive statistics of Model 1 for passage rate of indigestible NDF (iNDF k_p), created using backward stepwise regression, ranked by total contribution to the prediction (Sum of Squares) of each parameter (sums of linear, quadratic and cubic terms). The regression utilized 146 animal-period observations. Because of the large size and significant mean and linear bias of Model 1, the full model is not included here.

Parameter	Estimate	Sum of Squares	*P*
Intercept	6870	----	<0.0001
FCMY[a]	-61.7	6.09	<0.0001
$FCMY^2$	-0.619	4.30	<0.0001
$FCMY^3$	0.00289	3.97	0.0001
BW[b]	-16.4	5.90	<0.0001
BW^2	0.0136	5.51	<0.0001
BW^3	0.0000024	2.65	0.001
DMI, %BW	-2290	5.89	<0.0001
$(DMI, \%BW)^2$	298	6.25	<0.0001
$(DMI, \%BW)^3$	4.95	1.83	<0.01
FCMY/DMI	1440	6.24	<0.0001
$FCMY/DMI^2$	74.9	4.10	<0.0001
$FCMY/DMI^3$	-19.0	2.99	<0.001
DMI	510.	6.41	<0.0001
DMI^2	5.93	2.73	0.001
DMI^3	-0.0287	2.58	<0.01
DIM	0.0180	2.81	<0.001
DIM^2	0.000500	3.74	<0.001
DIM^3	-0.000001	3.36	<0.001
Diet % NDF	0.704	5.10	<0.0001
Diet % NDF^2	0.0943	4.75	<0.0001
BCS change	2.62	0.18	0.38
BCS $change^2$	52.3	4.17	<0.0001
BCS $change^3$	-261	4.37	<0.0001
Diet % forNDF[c]	0.183	0.63	<0.10
Diet % $forNDF^2$	-0.256	6.06	<0.0001
Milk fat %	-12.0	1.57	0.01
Milk fat $\%^2$	-13.0	5.23	<0.0001
BW change	0.663	2.13	<0.01
BW $change^2$	-0.286	2.41	<0.01
BW $change^3$	-0.101	1.33	0.02
ForNDF Digestibility[d]	0.115	2.05	<0.01
ForNDF $Digestibility^2$	0.0114	1.12	0.03
ForNDF $Digestibility^3$	-0.000755	1.89	<0.01
Diet % starch	-0.0550	0.32	0.24
MY	-1.66	1.11	0.03
MY^2	-0.248	2.08	<0.01
MY^3	-0.00129	1.92	<0.01
ForNDF Digestibility * Diet % starch	-0.105	9.47	<0.0001

Table 6a, continued

Parameter	Estimate	Sum of Squares	*P*
DMI, %BW * FCMY	-13.1	8.43	<0.0001
DMI * FCMY	2.94	8.21	<0.0001
Diet % forNDF * FCMY/DMI	13.5	7.46	<0.0001
Diet % forNDF * FCMY	-0.538	7.34	<0.0001
DMI, %BW * FCMY/DMI	383	7.10	<0.0001
Diet % forNDF * DMI, %BW	4.96	7.05	<0.0001
BW * FCMY/DMI	2.51	7.02	<0.0001
BW * Diet % forNDF	0.0381	6.87	<0.0001
FCMY/DMI * BCS change	35.7	5.76	<0.0001
BW * FCMY	-0.0652	5.43	<0.0001
FCMY * BW change	-1.70	5.43	<0.0001
MY * BW change	0.824	5.11	<0.0001
Diet % starch * FCMY	-0.388	5.07	<0.0001
Diet % NDF * FCMY	-0.528	4.70	<0.0001
DMI * DMI, %BW	-86.6	4.54	<0.0001
BW * Milk fat %	-0.194	4.27	<0.0001
Diet % NDF * MY	0.486	4.24	<0.0001
Diet % starch * MY	0.278	4.23	<0.0001
Diet % forNDF * Diet % starch	-0.125	4.19	<0.0001
BW * BW change	0.0670	4.17	<0.0001
BW * DMI	-0.594	4.14	<0.0001
MY * Milk fat %	-4.02	3.94	0.0001
Milk fat % * BW change	4.48	3.86	0.0001
FCMY * Milk fat %	4.66	3.73	<0.001
Diet % forNDF * BCS change	3.79	3.66	<0.001
Diet % NDF * DMI, %BW	0.889	3.61	<0.001
DMI, %BW * BW change	8.80	3.58	<0.001
Diet % NDF * BW change	-0.352	3.52	<0.001
DIM * Diet % NDF	0.0129	3.23	<0.001
BW * MY	-0.0442	3.23	<0.001
Diet % NDF * Milk fat %	2.211	3.13	<0.001
Diet % forNDF * ForNDF Digestibility	0.0594	3.10	<0.001
MY * FCMY	0.721	2.98	<0.001
FCMY/DMI * BW change	17.5	2.92	<0.001
DIM * Diet % starch	0.00363	2.90	<0.001
DIM * ForNDF Digestibility	0.00161	2.70	0.001
BW * ForNDF Digestibility	-0.00104	2.69	0.001
Milk fat % * BCS change	-8.51	2.66	<0.01
BW * BCS change	0.181	2.52	<0.01
Diet % NDF * BCS change	-4.25	2.40	<0.01
DIM * BW change	-0.00893	2.35	<0.01
DIM * BCS change	-0.299	2.26	<0.01
Diet % starch * DMI, %BW	1.16	2.26	<0.01

Table 6a, continued

Parameter	Estimate	Sum of Squares	*P*
Diet % starch * Milk fat %	0.916	2.22	<0.01
DIM * BW	-0.00156	1.76	<0.01
BW * Diet % starch	0.00817	1.71	<0.01
Diet % starch * FCMY/DMI	2.56	1.68	<0.01
Diet % forNDF * BW change	0.247	1.66	<0.01
ForNDF Digestibility * BCS change	1.36	1.63	<0.01
ForNDF Digestibility * FCMY/DMI	0.138	1.58	0.01
DMI, %BW * MY	-3.39	1.56	0.01
DIM * Diet % forNDF	-0.00512	1.41	0.02
Diet % NDF * forNDF Digestibility	-0.0430	1.17	0.03
DIM * DMI	0.0303	1.08	0.03
DMI, %BW * Milk fat %	-7.29	1.01	0.04
DIM * DMI, %BW	-0.161	0.90	0.05
Diet % starch * BW change	0.107	0.85	0.06
DMI * MY	0.247	0.83	0.06
ForNDF Digestibility * Milk fat %	0.0305	0.66	0.09

[a] 3.5% fat-corrected milk yield.
[b] Rumen-empty body weight.
[c] Forage NDF.
[d] 30-h in vitro NDF digestibility.

Table 6b. Descriptive statistics of prediction Model 2 for iNDF k_p, created using backward stepwise regression, ranked by contribution to the prediction (Sum of Squares) of each parameter (sums of linear, quadratic and cubic terms). The regression utilized 148 animal-period observations.

Parameter	Estimate	Sum of Squares	*P*
Intercept	-0.285	----	0.88
Diet % starch	0.0985	1.73	0.06
Diet % starch^2	0.000483	0.0103	0.88
Diet % starch^3	-0.00415	13.4	< 0.0001
Diet % forNDF[a]	-0.000397	0.000021	0.99
Diet % forNDF^2	-0.0407	7.47	0.0001
Diet % forNDF^3	0.00506	3.39	< 0.01
DMI	0.0642	2.50	0.02
DMI^2	0.0158	4.92	< 0.01
ForNDF Digestibility[b]	0.0140	0.826	0.19
ForNDF Digestibility ^{2}	-0.00235	4.58	< 0.01
FCMY[c]	-0.0240	2.38	0.03
ForNDF Digestibility * Diet % starch	-0.0296	14.6	< 0.0001
DMI * FCMY	-0.00703	3.90	< 0.01
Diet % starch * DMI	0.0161	3.52	< 0.01
Diet % starch * FCMY	-0.00383	1.45	0.08

[a] Forage NDF.
[b] 30-h in vitro NDF digestibility.
[c] 3.5% fat-corrected milk yield.

Model 2: iNDF k_p = -0.285 – 0.000397 x %forNDF – 0.0407 x (%forNDF – 20.8)^2 + 0.00506 x (%forNDF – 20.8)^3 + 0.0140 x NDFD (%) – 0.00125 x (NDFD(%) – 43.7)^2 + 0.0985 x % starch + 0.000483 x (% starch – 30.2)^2 – 0.00415 x (% starch – 30.2)^3 + 0.0642 x DMI + 0.0158 x (DMI – 22.7)^2 – 0.0140 x 3.5% FCMY – 0.0296 x [(NDFD(%) – 43.7) x (% starch – 30.2)] + 0.0161 x [(% starch – 30.2) x (DMI – 22.7)] – 0.00383 x [(% starch – 30.2) x (FCMY – 37.2)] – 0.00703 x [(DMI – 22.7) x (FCMY – 37.2)].

Table 6c. Descriptive statistics of prediction Model 3 for iNDF k_p, created using backward stepwise regression, ranked by contribution to the prediction (Sum of Squares) of each parameter (sum of linear, quadratic, and cubic terms). The regression utilized 167 animal-period observations.

Parameter	Estimate	Sum of Squares	*P*
Intercept	18.2	-----	<0.0001
Rumen DM pool	-0.354	35.5	<0.0001
Rumen DM pool^2	0.0333	4.22	<0.0001
Diet % forNDF[a]	-0.153	1.48	<0.01
Diet % forNDF^2	-0.0135	0.851	0.02
Diet % forNDF^3	0.00626	3.31	<0.0001
ForNDF Digestibility[b]	-0.0192	0.547	0.05
ForNDF Digestibility^2	-0.00323	1.50	<0.01
ForNDF Digestibility^3	0.000173	1.74	<0.001
NDF intake	1.45	2.80	<0.0001
NDF intake^2	-0.0585	0.720	0.03
OM digested in whole tract	-0.153	2.85	<0.0001
Diet % starch	-0.0657	0.788	0.02
Diet % starch^2	-0.00321	0.411	<0.10
Diet % starch^3	-0.00103	1.18	<0.01
Diet % NDF	-0.140	2.29	0.0001
Starch intake, %BW	1.03	1.57	0.001
NDF intake, %BW	-4.71	0.993	0.01
BW	-0.00643	0.619	0.04
BCS change	0.940	0.388	0.11
Diet % Starch * Rumen DM pool	0.0260	4.32	<0.0001
Rumen DM pool * OM digested in whole tract	-0.0343	1.81	<0.001
NDF intake * Rumen DM pool	0.0863	1.40	<0.01
BW Empty Rumen * Diet % forage NDF	-0.000448	0.983	0.01
Diet % Starch * BCS change	-0.274	0.875	0.02
Diet % forage NDF * OM digested in whole tract	0.0114	0.637	0.04
Diet % NDF * Starch intake, %BW	-0.173	0.593	<0.05
BCS change * OM digested in whole tract	0.3323	0.514	0.06

[a] Forage NDF.

[b] 30-h in vitro NDF digestibility.

Model 3: iNDF k_p = 18.2 – 0.00643 x BW – 0.140 x % NDF – 0.153 x % forNDF – 0.0135 x (% forNDF – 21.7)^2 + 0.00626 x (% forNDF – 21.7)^3 – 0.0192 x NDFD(%) – 0.00324 x (NDFD(%) – 42.6)^2 + 0.000173 x (NDFD(%) – 42.6)^3 – 0.0657 x % starch – 0.00321 x (% starch -31.2)^2 – 0.00103 x (% starch – 31.2)^3 + 1.45 x NDF intake – 0.0585 x (NDF intake – 5.64)^2 – 4.71 x NDF intake(%BW) + 1.03 x starch intake(%BW) – 0.354 x rumen DM pool + 0.0333 x (rumen DM pool – 10.4)^2 + 0.940 x BCS change – 0.153 x digested OM intake – 0.000448 x [(BW – 559) x (% forNDF – 21.7)] – 0.173 x [(%NDF – 28.2) x (starch intake(%BW) –

Table 6c, continued

1.13)] + 0.0114 x [(% forNDF – 21.7) x (digested OM intake – 13.4)] + 0.0260 x [(% starch – 31.2) x (rumen DM pool – 10.4)] – 0.274 x [(% starch – 31.2) x (BCS change – 0.00813)] + 0.0863 x [(NDF intake – 5.64) x (rumen DM pool – 10.4)] – 0.0343 x [(rumen DM pool – 10.4) x (digested OM intake – 13.4)] + 0.333 x [(BCS change – 0.00813) x (digested OM intake – 13.4)].

Table 7a. Descriptive statistics of prediction equation 1 for pdNDF k_p, created using backward stepwise regression, ranked by contribution to the prediction (Sum of Squares) of each parameter (sums of linear, quadratic and cubic terms). The regression utilized 149 animal-period observations. Because of the size and significant mean and linear bias of Model 1, the full model is not included here.

Parameter	Estimate	Sum of Squares	*P*
Intercept	-158	---	0.37
DIM	-0.00115	0.0381	0.73
BW[a], kg	-1.02	2.05	0.01
BW^2	0.000888	2.10	0.01
BW^3	-8.1e-7	5.53	< 0.0001
Diet % NDF	0.179	2.45	< 0.01
Diet % forNDF[b]	-0.552	13.9	< 0.0001
Diet % $forNDF^2$	-0.121	8.15	< 0.0001
Diet % $forNDF^3$	0.0160	10.3	< 0.0001
ForNDF Digestibility[c] %	-0.0155	0.910	0.09
Diet % starch	-0.00215	0.000972	0.96
DMI, kg/d	57.4	4.24	< 0.001
DMI, %BW	-145	2.07	0.01
$(DMI, \%BW)^2$	23.4	2.81	< 0.01
MY, kg	1.08	0.606	0.17
MY^2	-0.213	3.83	< 0.001
FCMY[d], kg	-20.4	6.17	< 0.0001
$FCMY^2$	-0.637	11.5	< 0.0001
$FCMY^3$	0.000427	2.46	< 0.01
FCMY/DMI	438	6.02	< 0.0001
$FCMY/DMI^2$	-97.7	4.96	< 0.001
Milk fat %	7.11	0.769	0.12
Milk fat $\%^2$	-6.06	4.67	< 0.001
BW change, kg/d	-0.216	1.61	0.03
$BW\ change^2$	0.247	3.22	< 0.01
BCS change /d	2.22	0.293	0.34
$BCS\ change^2$	17.3	1.59	0.03
DIM * BW	0.00155	8.20	< 0.0001
DIM * Diet % NDF	-0.00541	1.61	0.03
DIM * Diet % forage NDF	0.00313	0.899	< 0.10
DIM * DMI	-0.0344	5.69	< 0.0001
DIM * DMI, %BW	0.215	6.33	< 0.0001
DIM * MY	0.0222	8.77	< 0.0001
DIM * FCM	-0.0218	8.17	< 0.0001
DIM * Milk fat %	0.130	9.52	< 0.0001
DIM * BW change	-0.00416	0.908	< 0.10
DIM * BCS change	0.235	5.66	< 0.0001
BW * Diet % forage NDF	-0.00182	1.53	0.03
BW * Diet % starch	-0.0119	8.03	< 0.0001
BW * DMI	-0.0543	4.53	< 0.001

Table 7a, continued

Parameter	Estimate	Sum of Squares	*P*
BW * MY	0.0738	6.92	< 0.0001
BW * FCMY	-0.0684	5.71	< 0.0001
BW * Milk fat %	0.594	10.5	< 0.0001
Diet % NDF * DMI	0.211	2.47	< 0.01
Diet % NDF * FCMY	-0.116	1.96	0.01
Diet % NDF * FCMY/DMI	2.17	1.66	0.02
Diet % NDF * BW change	-0.134	2.18	0.01
Diet % forage NDF * Diet % starch	-0.0440	4.43	< 0.001
Diet % forage NDF * DMI, %BW	-0.389	2.58	< 0.01
Diet % forage NDF * MY	0.0232	2.24	< 0.01
Forage NDF Digestibility * DMI	0.140	5.84	< 0.0001
Forage NDF Digestibility * MY	0.00548	1.71	0.02
Forage NDF Digestibility *FCMY	-0.0841	5.70	< 0.0001
Forage NDF Digestibility * FCMY/DMI	1.87	6.05	< 0.0001
Diet % starch * DMI	0.313	8.62	< 0.0001
Diet % starch * DMI, %BW	-1.52	7.08	< 0.0001
Diet % starch * MY	0.375	17.0	< 0.0001
Diet % starch * FCMY	-0.383	18.3	< 0.0001
Diet % starch * Milk fat %	1.88	15.8	< 0.0001
Diet % starch * BW change	-0.147	6.91	< 0.0001
Diet % starch * BCS change	0.536	1.05	0.07
DMI * DMI, %BW	-8.66	5.12	0.0001
DMI * MY	-2.11	7.66	< 0.0001
DMI *FCMY	2.92	11.3	< 0.0001
DMI * Milk fat %	-16.5	10.6	< 0.0001
DMI * BCS change	5.97	2.31	< 0.01
DMI, %BW * MY	10.9	6.32	< 0.0001
DMI, %BW * FCMY	-10.3	5.38	< 0.0001
DMI, %BW * Milk fat %	87.8	9.68	< 0.0001
DMI, %BW * BW change	0.639	1.12	0.06
MY * FCMY	0.588	6.18	< 0.0001
MY * Milk fat %	-2.30	3.97	< 0.001
MY * BCS change	-2.74	1.61	0.03
FCMY * FCMY/DMI	10.5	5.60	< 0.0001
FCMY * Milk fat %	3.37	7.49	< 0.0001
FCMY * BW change	-0.0680	1.13	0.06
FCMY/DMI * BW change	2.87	2.72	< 0.01
FCMY/DMI * BCS change	68.9	1.64	0.03
Milk fat % * BCS change	-15.8	1.15	0.06
BW change * BCS change	-6.07	5.45	< 0.0001

[a] Rumen-empty body weight.
[b] Forage NDF.
[c] 30-h in vitro NDF digestibility.
[d] 3.5% fat-corrected milk yield.

Table 7b. Descriptive statistics of prediction equation 2 for pdNDF k_p, created using backward stepwise regression, ranked by contribution to the prediction (Sum of Squares) of each parameter (sums of linear, quadratic and cubic terms). The regression utilized 148 animal-period observations.

Parameter	Estimate	Sum of Squares	*P*
Intercept	7.99	---	< 0.0001
Diet % starch	-0.0585	24.8	< 0.01
Diet % starch^2	0.0216	22.1	< 0.0001
MY, kg	-0.0939	25.6	< 0.0001
MY^2	0.000299	8.36	< 0.001
MY^3	0.000135	7.58	< 0.001
Diet % forNDF[a]	-0.277	29.8	< 0.0001
Diet % NDF	0.0192	29.1	< 0.0001
DMI, kg/d	0.111	10.6	< 0.0001
BCS change /d	-7.70	6.16	< 0.01
ForNDF Digestibility[b]	0.0331	5.42	0.001
Interactions			
Diet % NDF * Diet % forNDF	0.0341	10.1	< 0.0001
Diet % NDF * MY	0.00932	5.60	0.001
Diet % NDF * ForNDF Digestibility	0.0223	4.05	< 0.01
Diet % forNDF * ForNDF Digestibility	-0.0149	3.99	< 0.01
Diet % starch * BCS change	-1.54	1.98	< 0.05
Diet % forNDF * BCS change	-1.76	1.96	0.05

[a] Forage NDF.

[b] 30-h in vitro NDF digestibility.

Model 2: pdNDF k_p = 9.81 + 0.124 x DMI -0.258 x Diet % forage NDF + (Diet % forage NDF - 20.7)^2 x 0.0515 - 0.0839 x Diet % starch + (Diet % starch - 30.4)^2 x 0.0229 + 0.0160 x Forage NDF Digestibility -0.109 x MY + (MY - 37.1)^2 x 0.000628 + (MY - 37.1)^3 x 0.000142 - 2.37 x BCS change + (Diet % forage NDF - 20.7) x (Diet % starch - 30.4) x 0.0313 + (Diet % forage NDF - 20.7) x (MY - 37.1) x 0.00825

Table 7c. Descriptive statistics of prediction equation 3 for pdNDF k_p, created using backward stepwise regression, ranked by contribution to the prediction (Sum of Squares) of each parameter (sums of linear, quadratic and cubic terms). The regression utilized 143 animal-period observations.

Parameter	Estimate	Sum of Squares	*P*
Intercept	6.11	----	< 0.0001
Diet % forNDF	-0.0964	26.8	< 0.00001
Rumen DM pool, kg	-0.273	17.7	< 0.0001
FCMY, kg	-0.0310	16.5	< 0.0001
DMI, kg/d	0.0678	15.7	< 0.00001
Rumen NDF pool, kg	0.00939	14.7	0.0001
BW[c] change, kg/d	-0.0978	11.7	< 0.001
Rumen wet weight, kg	0.0186	6.74	<0.01
pH standard deviation	-2.76	6.67	< 0.01
Interactions			
Diet % forage NDF * DMI	-0.0361	4.88	< 0.01
Diet % forage NDF * Rumen DM pool	-0.0793	10.1	< 0.0001
Diet % forage NDF * Rumen NDF pool	0.145	8.21	< 0.001
Diet % forage NDF * FCMY	0.0134	9.15	< 0.0001
DMI * Rumen NDF pool	-0.0367	3.56	0.01
DMI * BW change	-0.0757	6.99	< 0.001
Rum DM pool * Rumen NDF pool	0.104	5.14	< 0.01
Rum DM pool * Rumen wet weight	-0.0068	3.65	0.01
FCMY * BW change	0.0326	8.26	< 0.001
FCMY * pH standard deviation	0.153	1.60	0.09

[a] Forage NDF.

[b] 3.5% fat-corrected milk yield.

[c] Rumen-empty body weight.

Model 3: pdNDF k_p = 6.11 - 0.0963 x Diet % forNDF + 0.0678 x DMI -0.273 x Ruminal DM pool kg + 0.00939 x Ruminal NDF pool + 0.0186 x Ruminal wet weight - 0.0309 x FCMY -0.0978 x BW change -2.76 x pH standard deviation + (Diet % forNDF - 19.9) x (DMI - 22.6) x (-0.0361) + (Diet % forNDF - 19.9) x (Ruminal DM pool - 11.3) x (-0.0793) + (Diet % forNDF - 19.9) x (Ruminal NDF pool - 5.70) x 0.145 + (Diet % forNDF - 19.9) x (FCMY - 37.4) x 0.0134 + (DMI - 22.6) x (Ruminal NDF pool - 5.70) x (-0.0367) + (DMI - 22.6) x (BW change - 0.290) x (-0.0757) + (Ruminal DM pool - 11.3) x (Ruminal NDF pool - 5.70) x 0.104 + (Ruminal DM pool - 11.3) x (Ruminal wet wt. - 80.0) x (-0.00680) + (FCMY - 37.4) x (BW change - 0.290) x 0.0326) + (FCMY - 37.4) x (pH standard deviation - 0.308) x 0.153

CHAPTER 6

Predicting Ruminal Passage Rate of Starch in Dairy Cattle

ABSTRACT

Passage rate of starch is an important factor determining ruminal starch digestibility, microbial protein production, efficiency, and flow to the duodenum, and metabolic satiety in response to a diet. Previous equations predicting passage rate have relied on measurements of ruminal disappearance or fecal appearance of external markers. Data obtained in our laboratory from experiments utilizing the pool and flux method for estimating passage rate of digesta fractions were compiled and used to develop new regression equations predicting passage rate of starch. Predictors used in the regression equations included dietary concentrations of NDF, forage NDF (forNDF), and starch; 30-h in vitro NDF digestibility of forages (forNDFD); DIM and BW; intake of DM, NDF, starch, and digested OM; MY, milk fat concentration, and 3.5% fat-corrected MY; ruminal pools of DM, NDF, and wet digesta. Equations were developed using both data that can be obtained by commercial dairy farms (e.g., DMI and diet composition) and data obtained in ruminal metabolism experiments (e.g., rumen pools). Predictions of starch passage rate indicate that important predictors include proportion of NDF in the diet, forNDFD, DMI, starch intake, MY, change in BCS (d^{-1}), and proportion of starch in the diet. The best prediction using data that can be obtained by dairy farms explained 42% of variation in starch passage rate. Improving the accuracy of prediction of ruminal starch passage rate will

increase the ability to optimize ruminal starch degradability of dairy cow diets, which can aid in optimizing DMI and ruminal fermentation, maximizing milk yield, and increasing the efficiency of nutrient utilization.

INTRODUCTION

To aid in formulating diets for dairy cows, numerous mathematical models of digestion have been developed (Baldwin et al., 1987; Russell et al., 1992; NRC, 2001). These models estimate the availability of nutrients for milk production and other needs, given a particular set of feed composition characteristics, cow characteristics, and environmental factors. However, one of the factors most limiting to the accuracy of these models is their inability to account for the effects of dietary characteristics on voluntary feed intake and on the passage rate of digesta fractions from the rumen (Illius and Allen, 1994; Firkins et al., 1998).

Without an accurate prediction of passage rate, models cannot account for the effects of particle passage rate on feed intake, ruminal nutrient digestion, or microbial protein production and flow to the duodenum. Most models overestimate both digestion rate and passage rate, and underestimate rumen pool size, because they rely on *in vitro* digestion of ground feeds and rare-earth or chromium marker passage data (Allen, 1996). Models of dairy cow digestion can be improved greatly by accurate predictions of passage rate. Therefore, the objective of this study was to develop new equations to predict passage rate of starch. Emphasis was placed on predictive parameters that can be obtained by

commercial dairy farms. The hypothesis was that important predictors would include DMI and those parameters that describe the potential of a diet to induce physical filling effects or metabolic factors to affect passage rate.

MATERIALS AND METHODS

Data sets, laboratory methods, and statistical analysis were described previously (Chapter 5). Briefly, data sets from 11 studies conducted in our laboratory at Michigan State University between 1995 and 2003 were combined and used for estimations of ruminal passage rate of starch in dairy cattle (Table 1). Experimental procedures were similar among experiments and were approved by the Institutional Animal Care and Use Committee at Michigan State University. The data set included 254 animal-periods from multiparous lactating cows (nine studies), 29 animal-periods from primiparous lactating cows (one study), and 32 animal-periods from pregnant heifers (one study). All animals were ruminally and duodenally cannulated (gutter-style T cannulas). Passage rate of starch was measured using the pool and flux method (Oba and Allen, 2000).

Forages fed during the studies were primarily alfalfa silage and(or) corn silage; one study utilized orchardgrass silage. Diets also included dry or high-moisture corn grain and a variety of protein and fat supplements, byproducts, and mineral supplements. All animals were fed ad libitum, at 110% of expected intake.

Data were divided into two sets by randomly selecting two-thirds of the animal-periods from each study (a total of 210 records) to be assigned to a database that was used to create models (BUILD), and then assigning the remaining one-third of the animal-periods from each study (at total of 105 records) to a database that was used to validate the models (VALIDATE). This horizontal division of the database was selected rather than a vertical division (using data from 2/3 of the studies for BUILD and 1/3 of the studies for VALIDATE), because differences in markers and slight differences in methods would have reduced the predictive power of the regressions created in BUILD when applied to the VALIDATE set. Regression analyses were performed for starch k_p using backward stepwise regression of JMP (Version 5.1.2, SAS Institute, Cary, NC) for the BUILD data set. Predictor variables were included in linear, quadratic, and cubic terms; if a higher-order term was significant, then all lower-order terms also were kept, regardless of their significance. Two-way interactions of main effects were included; three-way interactions were not included in order to avoid over-parameterization. Equations were developed by entering all potential predictors and removing predictors with the greatest *P*-value until all variables had $P < 0.10$.

One set of potential predictors (Model 1) included all of the available parameters that could be measured on or by a dairy farm: DIM, BW, diet % NDF, % forNDF, % starch, forNDFD, DMI, DMI(%BW), MY, FCMY, FCMY/DMI, Milk fat %, BW change, and BCS change. A second set of potential predictors (Model 2) reduced the number of redundant parameters (e.g., included FCMY

alone instead of FCMY, MY, and % milk fat). That predictor set included diet % NDF, diet % forNDF, forNDFD, diet % starch, DMI, FCMY, MY, milk % fat, and BCS change. It was selected not only to avoid over-parameterization, but also to attempt to create a prediction equation for starch k_p that could be incorporated into models of dairy cow digestion used for diet formulation or evaluation.

A third set of potential predictors (Model 3) included parameters that would be measured in a study designed to estimate k_p using the pool and flux method and that could be expected to be correlated with k_p and to have some reasonably causal influence on k_p (rather than being determined directly or primarily by k_p). These predictors included Model 1 parameters, plus intake of NDF, forNDF, and starch as a percent of BW, rumen pools of wet digesta, DM and NDF, rumen digesta volume, daily mean, variance and standard deviation of ruminal pH, and OM digested in the whole tract.

Initial evaluation of equations was performed in the BUILD data set by visual inspection of plots of residuals (observed – predicted) against predicted values (Neter et al., 1996). Patterns suggesting systematic tendencies for residuals to be positive or negative were considered indicators that the model under consideration did not account sufficiently for variation. Percentage of variation accounted for by an equation (R^2) and Akaike's Information Criterion (AIC; Akaike, 1974) were also used to determine the predictive value of a candidate equation. A lower AIC value indicates a more appropriate model. After equations were developed using the BUILD data set and selected as candidate models, they were evaluated using the VALIDATE data set according to

recommendations of Neter et al. (1996) and St-Pierre (2003), as described in Chapter 5.

RESULTS AND DISCUSSION

Measuring Starch Passage Rate

This is the only large-scale summary of data known for passage rate of total starch in digesta calculated using the pool and flux method. Nearly all passage data available in the literature were measured by analysis of fecal excretion curves of external markers applied to intact forages and(or) concentrates and pulse dosed. The biological and mathematical significance of the pool and flux method was discussed previously (Chapter 5).

Mean starch k_p in the BUILD data set was 15.3 h^{-1} and the 95% confidence interval of values was 1.6 to 40.9. In a recent summary, Seo et al. (2006) reported passage rates for dry forages, wet forages, and concentrates, estimated using rare earth markers, of 4.53, 5.17, and 6.69 h^{-1}, respectively, with ranges of 3.42 to 5.70, 3.9 to 6.29, and 3.61 to 9.22 h^{-1}, respectively. All three feed types usually contain some starch; as a proportion of DM, concentrates such as grains contain more starch than do forages. Mean starch k_p reported here was much greater than the k_p reported for concentrates by Seo et al. (2006). That analysis pooled fibrous byproduct data with concentrate data because the researchers found that fibrous byproduct k_p was predicted accurately by the prediction equation for concentrates, rather than by the equation for dry forages. The inclusion of fibrous byproducts in the concentrates

might have reduced the mean k_p of concentrate particles, lowering it below the mean k_p of starch in our database. In addition to starch, concentrates contain NDF, protein, and other components which can cause the passage rate of starch-containing particles to be different from k_p of the total pool of starch. Furthermore, markers frequently increase the density of particles to which they are attached (Ehle, 1984), and rare earth elements migrate into the small particle and liquid pools (Erdman and Smith, 1985), so the actual k_p of marked concentrates is inflated artificially compared to the k_p of unmarked concentrates. The difference between concentrate k_p estimated using external markers and starch k_p measured using the pool and flux method suggests that the k_p of individual high-starch feed ingredients certainly should not be substituted for the k_p of starch for the purpose of calculating ruminal starch digestibility. The predictions tested by Seo et al. (2006) were developed in order to calculate ruminal protein degradation (NRC, 2001). It is possible that the differences between actual k_p of the various protein fractions and k_p of the concentrates that contain them are as great as are the differences between k_p of starch measured using the pool and flux method and the k_p of whole concentrates. If so, then the use of k_p of an individual concentrate for the k_p of protein fractions likely does not accurately predict ruminal degradation values of protein fractions.

Predicting Starch k_p Using Farm Data

Several parameters that are can be obtained by commercial dairy operations demonstrated the potential to predict iNDF k_p (Table 2). Backward stepwise regression considering all of these parameters (Model 1) resulted in

strong predictive power within BUILD (R^2 = 0.65, AIC = 480; Table 3); Figure 1a is a regression of residuals against predicted starch k_p. Model 1 also resulted in significant over-parameterization and a very weak capability to predict k_p in VALIDATE, as evidenced by significant linear ($P < 0.0001$) bias (Table 3, Figure 1b). A description of the predictors included in Model 1 is presented in Table 4a. Beginning backward regression with a much smaller set of potential predictors (Model 2), which were selected for mechanistic importance and to avoid redundancy, resulted in lower predictive power within BUILD (R^2 = 0.42) and a less favorable (higher) AIC value (551; Table 3); Figure 2a is a regression of residuals against predicted starch k_p. However, when the resulting equation was evaluated in VALIDATE, although linear bias was statistically significant (P = 0.01; Table 3), the absolute values of bias at the minimum and maximum predicted values were 5.27 and 5.99 (Figure 2b), which were smaller than standard error of the residual regression (6.89) and therefore mathematically insignificant. The model was able to account for 42% of variation in starch k_p (Table 3).

Therefore, for applications in models used for diet formulation or evaluation on commercial dairy farms, the equation created in Model 2 is likely the most appropriate equation for predicting starch k_p using the parameters available in this data set. A description of the predictors included in Model 2 is in Table 4b. Model 2 accounts for the effects of: (1) proportion of NDF in the diet, (2) forNDFD, (3) DMI, (4) starch intake, (5) MY, (6) change in BCS (d^{-1}), and (7) proportion of starch in the diet. Two-way interactions contributing significantly to

the prediction of starch k_p were between diet % starch and BCS change; between diet % NDF and starch intake, forNDFD, BCS change, and DMI; between DMI and MY, BCS change and forNDFD, and between starch intake and forNDFD (Table 4b). Direct mechanistic interpretation of the equation is not practical because one quadratic term and nine interactions were included, but biological evidence exists for effects on k_p of the parameters selected.

Proportion of concentrate in the diet was determined to be a significant predictor in the NRC (2001) calculations for passage rate of concentrate. Concentrate proportion typically would be correlated with the dietary starch concentration reported for the present data set, and likely would be correlated inversely with dietary concentration of NDF, which was the strongest predictor in the present regression. In the present prediction of starch k_p, diet % NDF had a negative coefficient (-1.42), and diet % starch had a positive coefficient (+ 0.0760), which are in contrast to the negative coefficient for diet concentrate content reported by Seo et al. (2006) in the prediction of concentrate k_p. In this regression equation, it is impossible to assign biological significance to the signs of the coefficients because they are not independent of the other predictors. Starch passage rate likely is affected by grain particle size, which was measured for only four of the 11 studies in this data set (Ying and Allen, 1998; Ying et al., 1998; Ying and Allen, 2005; Taylor and Allen, 2005) and was a treatment for only one experiment (Ying et al., 1998). In that study, fine grinding of corn grain decreased starch passage rate in the rumen compared to coarsely ground grain.

Digestibility of forNDF, as estimated by 30-h in vitro fermentation, also contributed to the prediction of iNDF k_p (Table 4b). The quadratic relationship is difficult to interpret biologically, but in general, greater forNDFD should permit greater k_p of all digesta, including starch. An exception to this generality might occur when cows are fed diets with lower concentrations of forNDF; high forNDFD and low concentration of forNDF could lead to lower ruminal pH and reduced ruminal motility, which could reduce digesta k_p. However, one study (Oba and Allen, 2000) demonstrated that starch k_p was consistently greater when cows were fed forage with greater NDFD, regardless of diet forNDF concentration.

As DMI increases, k_p increases (NRC, 2001), and this relationship is reflected in the positive coefficient of DMI in Model 2 (Table 4b). Predictions of passage rate in the 2001 NRC protein model (NRC, 2001), and the recent re-evaluation of those equations (Seo et al., 2006), both included DMI (as a percentage of BW) as a very important factor in predicting k_p. Both MY and change in BCS (d^{-1}) also contributed to the prediction of starch k_p (Table 4b), suggesting that nutrient demand can affect passage rate. Mechanisms through which nutrient demand affects passage rate might include increased ruminoreticular contraction rate, strength, or duration.

Predicting Starch k_p in Research Studies

A third model was tested to predict starch k_p using data that are obtained routinely in studies designed to estimate k_p using the pool and flux method. To the parameters in Model 2 were added intake of starch, NDF, and forNDF, rumen

pools of wet digesta, DM, and NDF, rumen digesta volume, daily mean and variance of ruminal pH, and digested OM intake. Results of backward regression using these parameters (Model 3) are presented in Table 3, Table 4c, and Figure 2. No mean bias existed for this model when it was evaluated in the VALIDATE data set (Table 3, Figure 3b). Although the linear bias tended toward statistical significance (Table 3), the absolute values of the biases (see Figure 3b) at the minimum predicted value (3.15) and at the maximum predicted value (5.31) were smaller than the standard error of the residual regression (6.96).

In Model 3, milk fat concentration, but not MY or BCS, was included (Table 4c). Milk fat concentration might be an indicator of the effects of energy status and(or) ruminal pH on passage rate. Lower milk fat concentration is often associated with lower ruminal pH, and lower ruminal pH can decrease passage rate by reducing ruminal motility. DMI was not a significant predictor in Model 3; total OM digested in the whole tract and wet weight of ruminal digesta did contribute significantly to this prediction of starch k_p (Table 4c). The amount of OM digested in the whole tract might account for a large proportion of the variation in k_p due to DMI. Also, wet weight of ruminal digesta might be an indicator of the physical filling effects of the diet, which also can affect k_p. Standard deviation of ruminal pH also was a significant predictor (Table 4c); greater variation in ruminal pH throughout the day might reduce ruminal motility, which can reduce k_p of digesta, including starch.

Limitations to Application of Developed Models

The application of any model is limited, in particular, by the range of data from which it was developed and the segment of the population from which that data was obtained. As previously reported (Chapter 5), the samples of animals and dietary ingredients utilized in the experiments from which this database was developed were limited in variation relative to the respective populations in the U.S. Therefore, the significance of these models lies not in their ability to predict passage rate across farms but rather in the biological significance of the variables that contributed to the prediction of passage rate.

CONCLUSIONS AND IMPLICATIONS

Prediction of passage rate of digesta is dependent on diet characteristics and nutrient demand of the individual animal. A model including diet concentrations of NDF and starch, forNDFD, intake of DM and of starch, MY, and BCS change accounted for 42% of variation in starch k_p. Ruminal digestibility of starch has important implications for the regulation of feed intake, extent and efficiency of microbial protein production, and ruminal NDF digestibility. Accurate prediction of the passage rate of starch from the rumen is needed for accurate prediction of ruminal starch digestibility. Therefore, improving the accuracy of prediction of ruminal starch passage rate will increase the ability to optimize ruminal starch degradability of dairy cow diets, which can aid in optimizing DMI and ruminal fermentation, maximizing milk yield, and increasing the efficiency of nutrient utilization.

Table 1. Studies included in the data set used for development of passage rate equations in dairy cattle.

Study	Description
1	Treatments were coarsely or finely ground dry or high-moisture corn grain, fed to pregnant heifers (Ying and Allen, 1998)
2	Treatments were coarsely or finely ground dry or high-moisture corn grain, fed to primiparous lactating cows (Ying and Allen, 1998)
3	Treatments were high- or low-NDF diets containing normal or brown-midrib corn silage (Oba and Allen, 2000)
4	Treatments were high- or low-starch diets containing ground high-moisture or dry corn grain (Oba and Allen, 2003)
5	Treatments were dried, pelleted beet pulp substituted for high-moisture corn grain at 0 (control), 6, 12, and 24% of diet DM (Voelker and Allen, 2003)
6	Treatments were sodium bicarbonate, sodium chloride, or no added ions (Mooney and Allen, 2004)
7	Treatments were brown-midrib or control corn silage and floury or vitreous varieties of corn grain (Taylor and Allen, 2005)
8	Treatments were no added fat (control), saturated fat supplement, unsaturated fat supplement, or 50% of each fat supplement (Harvatine and Allen, 2006)
9	Treatments were dry or high-moisture preserved of floury or vitreous varieties of corn (Ying and Allen, 2005)
10	Treatments were diets containing alfalfa silage or orchardgrass silage (Voelker Linton and Allen, 2005)
11	Treatments were diets containing high or low concentrations of forage NDF (Voelker and Allen, 2006)

Table 2. Independent variables included in the data set used for development of passage rate equations of starch in dairy cattle.

	Correlation to Starch k_p					
	Linear		Quadratic		Cubic	
	P	r	*P*	r	*P*	r
Days in milk	0.08	0.14	NS	-----	NS	-----
BW[1], kg	NS	-----	NS	-----	NS	-----
Diet % NDF	< 0.01	-0.23	NS	-----	0.06	-0.26
Diet % forNDF[2]	0.03	-0.16	0.03	-0.22	NS	-----
ForNDFD[3], %	NS	-----	0.09	-0.14	0.19	0.17
Diet % starch	0.17	-0.10	NS	-----	0.09	-0.17
DMI, kg/d	0.01	0.19	NS	-----	NS	-----
NDF intake, kg/d	NS	-----	NS	-----	NS	-----
ForNDF intake, kg/d	0.12	0.11	< 0.01	-0.23	NS	-----
iNDF intake, kg/d	< 0.01	0.20	0.16	-0.23	NS	-----
Starch intake, kg/d	0.03	0.16	NS	-----	NS	-----
N intake, kg/d	0.01	0.19	NS	-----	NS	-----
MY, kg/d	NS	-----	0.15	-0.12	NS	-----
FCMY[4], kg/d	NS	-----	NS	-----	NS	-----
FCMY/DMI	NS	-----	NS	-----	NS	-----
Milk fat %	NS	-----	0.02	-0.20	NS	-----
BW change, kg/d	NS	-----	NS	-----	NS	-----
BCS change, unit/d	0.10	-0.12	NS	-----	NS	-----
Rumen DM pool, kg	NS	-----	< 0.001	-0.27	0.06	0.30
Rumen DM, % BW	NS	-----	< 0.01	-0.23	NS	-----
Rumen NDF pool, kg	NS	-----	< 0.001	-0.26	NS	-----
Rumen iNDF pool, kg	0.09	-0.12	0.13	-0.16	0.11	0.20
Rumen digesta weight, kg	NS	-----	< 0.0001	-0.31	NS	-----
Rumen volume, L	NS	-----	< 0.001	-0.25	NS	-----
Digested OM, kg/d	0.05	0.14	0.07	-0.19	NS	-----
pH mean	0.12	-0.12	NS	-----	NS	-----
pH variance	NS	-----	NS	-----	NS	-----
pH standard deviation	0.07	-0.14	< 0.01	-0.25	NS	-----

[1] Rumen-empty body weight.
[2] Forage NDF.
[3] 30-h in vitro NDF digestibility.
[4] 3.5% fat-corrected milk yield.

Table 3. Diagnostic statistics for building and validating models for prediction of k_p of starch.

	Diagnostics in BUILD				Mean and linear bias in VALIDATE			
Model[a]	n	R^2	RMSE	AIC[b]	Mean bias P[c]	Linear bias P[d]	s[d]	Linear bias?[d]
1	146	0.647	4.67	480	0.32	< 0.0001	7.12	Yes
2	151	0.415	5.87	551	0.59	0.01	6.59	No
3	129	0.497	5.43	452	0.33	0.06	6.96	No

[a] See text and Chapter 4 for description of statistical analyses and see Table 4a-c for descriptor statistics.

[b] Akaike's Information Criterion (AIC) = 2p + n ln(SSE / n), where p is the number of parameters, n is the number of observations, and SS is the sum of squares of the error (residuals).

[c] Mean bias is the intercept of the regression of residuals against centered predicted values (predicted values – mean predicted value). $P < 0.05$ indicates significant mean bias (St-Pierre, 2003).

[d] Linear bias is the slope of the regression of residuals against centered predicted values (predicted values – mean predicted value). $P < 0.05$ indicates statistically significant linear bias. When the linear bias was statistically significant ($P < 0.05$), the magnitude of the bias was calculated for the maximum and minimum predicted values, using the equation $e_i = b_0 + b_1 (X_i - \bar{X})$ and then judged relative to the size of the standard error of the regression (s) of residuals on centered predicted values. See text for more details.

Table 4a. Descriptive statistics of prediction Model 1 for starch k_p, created using backward stepwise regression, ranked by total contribution to the prediction (Sum of Squares) of each parameter (sums of linear, quadratic and cubic terms). The regression utilized 146 animal-period observations.

Parameter	Estimate	Sum of Squares	*P*
Intercept	199	----	----
ForNDFD[a], %	-0.181	1340	< 0.0001
ForNDFD^2	0.0620	763	< 0.0001
ForNDFD^3	-0.00478	620	< 0.0001
Diet % forNDF[b]	-3.53	1050	< 0.0001
Diet % forNDF^2	0.647	708	< 0.0001
Diet % forNDF^3	0.107	489	< 0.0001
Diet % NDF	-2.98	1590	< 0.0001
Diet % NDF^2	0.751	270	< 0.001
N intake, g/d	0.0497	784	< 0.0001
N intake^2	-0.000903	361	< 0.001
N intake^3	0.000000367	134	0.01
Diet % starch	-0.831	912	< 0.0001
DMI, kg/d	-1.53	553	< 0.001
DMI^2	-0.774	315	< 0.001
DIM	0.0205	662	0.0001
BCS change d^{-1}	-34.8	344	< 0.01
BCS change^2	72.3	124	0.02
MY, kg/d	-0.0394	291	< 0.01
MY^2	-0.0167	175	< 0.01
Diet % NDF * ForNDFD	-1.03	985	< 0.0001
Diet % forNDF * ForNDFD	0.426	498	< 0.0001
ForNDFD * Diet % starch	-0.219	408	< 0.0001
Diet % NDF * Diet % forNDF	-1.57	398	< 0.0001
DIM * Diet % NDF	-0.0226	322	< 0.001
Diet % NDF * Diet % starch	-0.657	318	< 0.001
N intake * DMI	0.0515	293	< 0.001
Diet % forage NDF * DMI	-0.827	261	< 0.001
DIM * ForNDFD	0.00720	248	0.001
DIM * MY	-0.00324	221	< 0.01
Diet % NDF * DMI	0.605	163	< 0.01
N intake * Diet % forNDF	0.0221	136	0.01
Diet % starch * BCS change /d	-4.22	133	0.02
DIM * BCS change	-0.636	116	0.02
N intake * DIM	0.000303	112	0.03
N intake * Diet % NDF	-0.0168	94.5	0.04

[a] 30-h in vitro NDF digestibility.
[b] Forage NDF.

Table 4b. Descriptive statistics of prediction Model 2 for starch k_p, created using backward stepwise regression, ranked by total contribution to the prediction (Sum of Squares) of each parameter (sums of linear, quadratic and cubic terms). The regression utilized 151 animal-period observations.

Parameter	Estimate	Sum of Squares	*P*
Intercept	48.3	----	----
Diet % NDF	-1.42	2040	< 0.0001
ForNDF digestibility[a]	0.143	833	< 0.001
(ForNDF digestibility)^2	-0.0237	519	< 0.001
DMI, kg/d	0.463	1030	0.0001
Starch intake, kg/d	-0.730	668	< 0.001
MY, kg	-0.159	547	< 0.001
BCS change /d	-25.8	540	< 0.01
Diet % starch	0.0760	275	0.02
starch intake * Diet % NDF	-0.976	360	< 0.01
DMI * MY	-0.0441	338	< 0.01
Diet % starch * BCS change /d	-6.80	270	< 0.01
Diet % NDF * ForNDF digestibility	-0.101	265	< 0.01
DMI * BCS change /d	5.03	212	0.01
ForNDF digestibility* DMI	0.0672	200	0.02
Diet % NDF * BCS change /d	-7.36	143	0.04
Diet % NDF * DMI, kg/d	0.187	123	0.06
starch intake * ForNDF digestibility	-0.168	105	0.08

[a] 30-h in vitro digestibility of forage NDF.

Model 2: starch k_p = 48.3 -1.42 x Diet % NDF + 0.143 x ForNDF Digestibility + (Forage NDF Digestibility - 43.6)^2 x (-0.0237) + 0.0760 x Diet % starch + 0.463 x DMI - 0.159 x MY - 25.8 x BCS change + (Diet % NDF - 27.7) x (Forage NDF Digestibility - 43.6) x (-0.101) + (Diet % NDF - 27.7) x (DMI - 22.6) x 0.187 + (Diet % NDF - 27.7) x (BCS change - 0.00750) x (-7.36) + (Forage NDF Digestibility - 43.6) x (DMI - 22.6) x 0.0672 + (Diet % starch - 30.2) x (BCS change - 0.00750) x (-6.80) + (DMI - 22.6) x (MY - 36.9) x (-0.0441) + (DMI - 22.6) x (BCS change - 0.00750) x 5.03 - 0.730 x starch intake + (starch intake - 6.79) x (Diet % NDF - 27.7) x (-0.976) + (starch intake - 6.79) x (Forage NDF Digestibility - 43.6) x (-0.168)

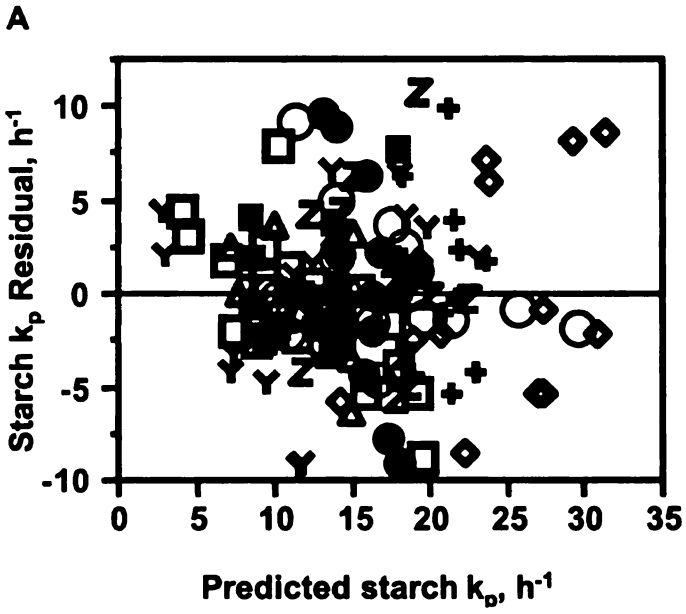
Table 4c. Descriptive statistics of prediction Model 3 for starch k_p, created using backward stepwise regression, ranked by total contribution to the prediction (Sum of Squares) of each parameter (sums of linear, quadratic and cubic terms). The regression utilized 129 animal-period observations.

Parameter	Estimate	Sum of Squares	*P*
Intercept	12.2	----	----
ForNDFD[a], %	-0.298	1080	< 0.0001
ForNDFD^2	-0.0660	860	< 0.0001
ForNDFD^3	0.00233	260	< 0.01
Diet % starch	-0.000521	1080	< 0.0001
Milk fat %	3.82	989	< 0.0001
Starch intake, kg/d	2.89	713	0.0001
OM digested in whole tract	-1.20	627	< 0.001
Diet % NDF	0.896	578	0.0001
Rumen digesta weight, kg	-0.130	284	< 0.01
pH standard deviation	-19.3	272	< 0.01
Diet % NDF * ForNDFD	-0.301	530	< 0.0001
Diet % starch * Milk fat %	-1.05	451	< 0.001
Starch intake * Milk fat %	3.47	405	< 0.001
Diet % starch * OM digested in whole tract	0.324	386	< 0.001
Diet % starch * starch intake	-0.634	362	< 0.001
Milk fat % * OM digested in whole tract	-1.18	163	0.02

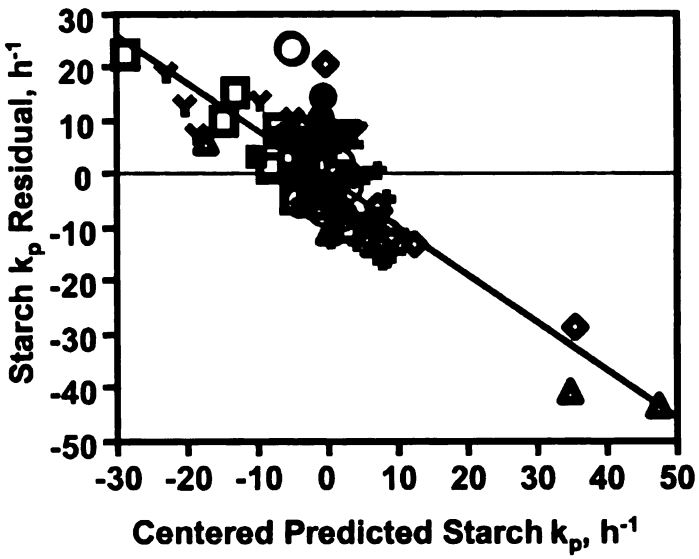
[a] 30-h in vitro digestibility of forage NDF.

Model 3: starch k_p = 12.2 + 0.896 x Diet % NDF -0.298 x Forage NDF Digestibility + (Forage NDF Digestibility - 42.8)^2 x (-0.0660) + (Forage NDF Digestibility - 42.8)^3 x 0.00233 -0.000521 x Diet % starch + 2.89 x starch intake + 3.82 x Milk fat % -0.130 x Ruminal wet weight -1.20 x total tract digested OM - 19.3 x pH standard deviation + (Diet % NDF - 26.9) x (Forage NDF Digestibility - 42.8) x (-0.300) + (Diet % starch - 30.3) x (starch intake - 6.71) x (-0.634) + (Diet % starch - 30.3) x (total tract digested OM - 14.5) x 0.324) + (Diet % starch - 30.3) x (Milk fat % - 3.62) x (-1.05) + (starch intake - 6.71) x (Milk fat % - 3.62) x 3.47 + (Milk fat % - 3.62) x (total tract digested OM - 14.5) x (-1.18)

Figure 1. (A) Plot of residual (observed minus predicted) starch k_p vs. predicted starch k_p resulting from Model 1 in BUILD data set. (B) Plot of residual starch k_p vs. predicted starch k_p resulting from Model 1 applied to VALIDATE data set. Predicted starch k_p was centered around the mean predicted value. Mean bias was not significant ($P = 0.32$). Linear bias was significant ($P < 0.0001$), and the absolute value of the maximum bias (43) was larger than the standard error of residuals (7.12). Different symbols represent data from individual studies.

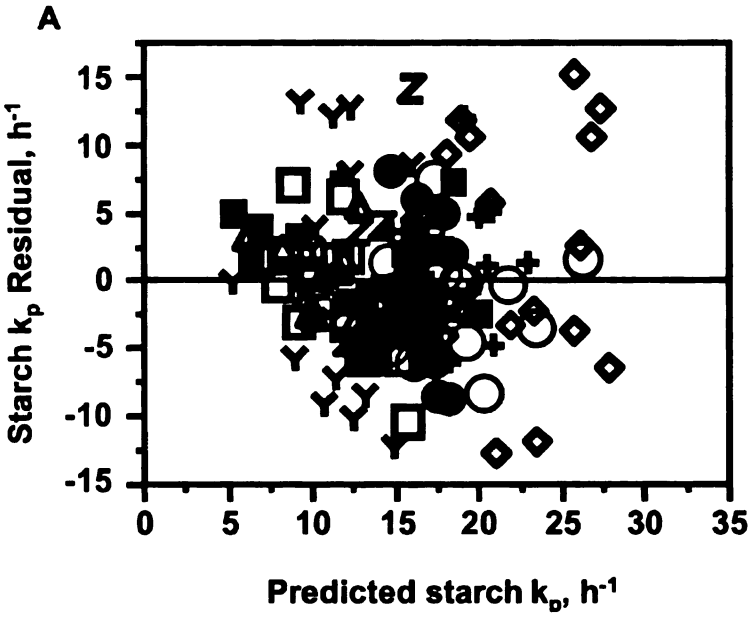


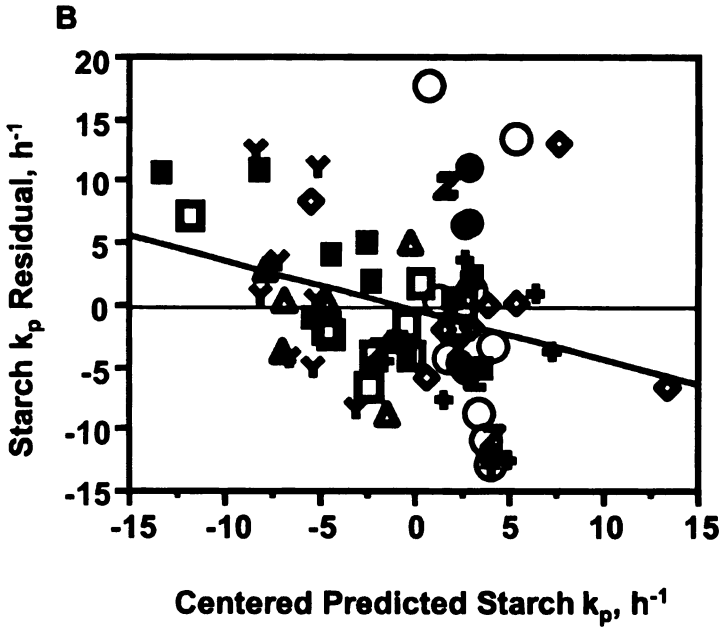
B



Starch k_p = -0.83 (± 0.82) - 0.90 (± 0.07) (X - 15.5)
R^2 = 0.67, s =7.12, P < 0.0001
Bias at min predicted (-14) = 25
Bias at max predicted (63) = -43

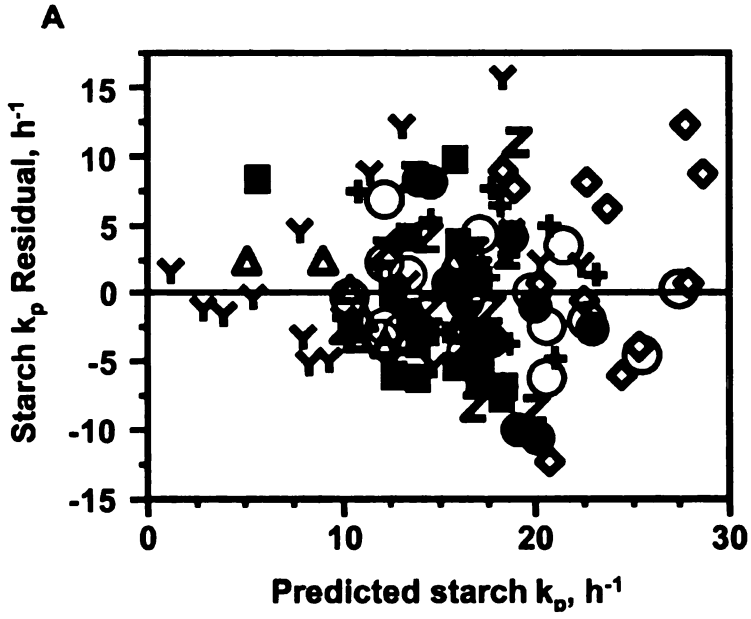
Figure 2. (A) Plot of residual (observed minus predicted) starch k_p vs. predicted starch k_p resulting from Model 2 in BUILD data set. (B) Plot of residual starch k_p vs. predicted starch k_p resulting from Model 2 applied to VALIDATE data set. Predicted starch k_p was centered around the mean predicted value. Mean bias was not significant ($P = 0.59$). Although linear bias was significant ($P = 0.01$), the absolute value of the maximum bias (5.72) was lower than standard error of residuals (6.59). Different symbols represent data from individual studies.



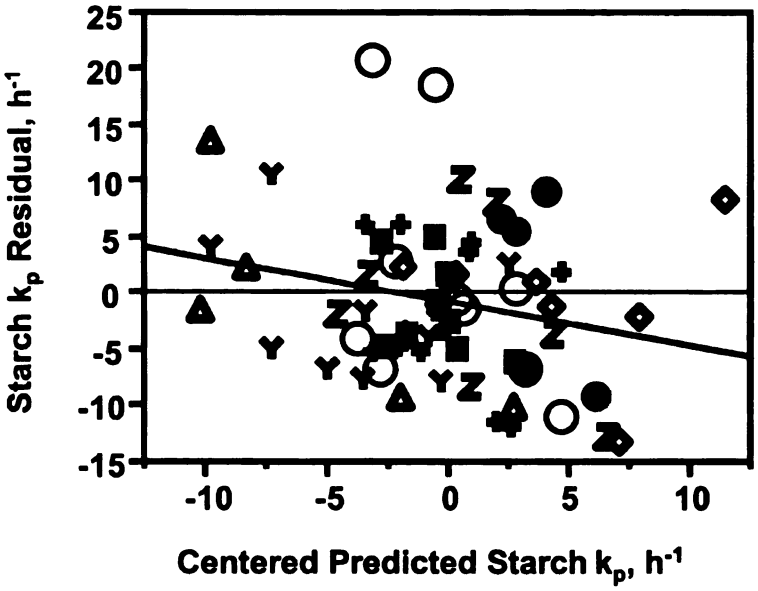


Starch k_p = -0.41 (± 0.76) - 0.40 (± 0.16) (X - 15.2)
R^2 = 0.08, s = 6.59, P = 0.01
Bias at min predicted (1.85) = 4.91
Bias at max predicted (28.5) = -5.72

Figure 3. (A) Plot of residual (observed minus predicted) starch k_p vs. predicted starch k_p resulting from Model 3 in BUILD data set. (B) Plot of residual starch k_p vs. predicted starch k_p resulting from Model 3 applied to VALIDATE data set. Predicted starch k_p was centered around the mean predicted value. Mean bias was not significant ($P = 0.33$). Although linear bias tended to be significant ($P = 0.06$), the absolute value of the maximum bias (5.31) was lower than standard error of residuals (6.96). Different symbols represent data from individual studies.



B



Starch k_p = -0.84 (± 0.85) - 0.39 (± 0.20) (X - 16.0)
R^2 = 0.05, s = 6.96, P = 0.06
Bias at min predicted (5.80) = 3.15
Bias at max predicted (27.5) = -5.31

CHAPTER 7

Summary and Implications

Passage Rate and Forage Family

As hypothesized, with greater pVDMI, DMI on AL was increasingly greater than DMI on OG. This occurred because NDF turnover time in the rumen decreased more for AL than for OG as pVDMI increased. The faster disappearance of NDF on AL, caused primarily by a greater increase in passage rate of iNDF on AL with increasing pVDMI, reduced the physical filling effects for AL more than was possible for NDF from diet OG. These results corroborate previous research suggesting that intake is more limited by physical fill effects with increasing nutrient demand and on grass forages compared to legume forages. Through its effect on passage rate responses, pVDMI also altered the extent to which diet affected the production of milk and its components; milk fat concentration, FCMY and BCS responses to AL over OG were related to pVDMI values. Cows with the greatest drive to eat, as estimated by pVDMI, responded the most positively in DMI and milk production responses to alfalfa versus orchardgrass as the primary dietary fiber source.

As expected, disappearance of N from ruminal digesta was greater when the dietary forage was alfalfa than when it was orchardgrass. The extent to which forage type affected N intake, digestion and utilization depended on the pVDMI of individual animals. Site of digestion and efficiency of utilization of dietary N for microbial protein and for milk true protein depended not only on

intake of N but also on responses of ruminal passage rate and ruminal starch digestion. The reduction of passage rate by OG, particularly among cows with high pVDMI, reduced the total amount of N consumed and utilized for microbial protein and milk true protein production. However, despite the expected dilution of maintenance N with increasingly greater MY on AL, a decreasing proportion of the additional N consumption that was allowed by the increased DMI on AL among cows with greater pVDMI was digested and used for increased milk production or body condition gain.

Increasing passage rate and DMI by feeding a perennial legume forage instead of a perennial grass forage can increase yields of milk and milk protein among cows with greater nutrient demand. However, increasing N intake at the same rate as DMI is increased likely will lead to less efficient utilization of dietary N for production of microbial protein, body tissue, or milk protein. When feeding less-filling diets, such as those containing large proportions of legume forage, to high-producing cows, reducing dietary N concentration could increase the efficiency of N utilization and reduce the extent to which greater DMI leads to greater N excretion. Furthermore, the effects of pVDMI on N digestion and utilization reinforce the need to group and feed animals according to some index of nutrient demand. Reducing the variation in energy and protein demand within the group for which a diet is formulated would allow diets to be formulated to more accurately meet each individual animal's demands and thus lead to more efficient utilization of N among all groups of animals on the farm. Increased N digestibility and utilization and more accurate diet formulation will reduce the

proportion and amount of N excreted in feces and urine. It should also reduce the likelihood of overfeeding N. Thus, a better understanding of the different effects of perennial grass and legume forages on N utilization, as they are influence by nutrient demand, will aid in field management decisions to minimize the turnover and loss of N on the whole farm.

Passage Rate and Dietary Forage NDF Concentration

When cows of varying pVDMI were fed a low-forage diet and a high-forage diet, DMI and passage rate responses differed from previously-observed responses (Voelker et al., 2002). Although DMI and passage rate responses previously were dependent on pVDMI, they were not in the present experiment. This apparently occurred because a longer NDF turnover time on LF with increasing pVDMI led to responses of DMI and milk production to HF and LF that were independent of pVDMI. A greater reduction of ruminal pH caused by greater starch intake on LF might have mediated this response. The results of this experiment suggest that models that predict intake need to account for not only the effects of nutrient demand, but also the effects of the interactions of feed fractions (such as starch and NDF) on the intake responses of individual cows to high- and low-forage diets.

Predicting Passage Rates of NDF Fractions and Starch

Prediction of passage rate of digesta is dependent on diet characteristics and nutrient demand of the individual animal. A model including diet % starch, DMI, forNDFD, diet % forNDF, and 3.5% FCMY accounted for 67% of variation in iNDF k_p. A model including diet % starch, MY, diet % forNDF, diet % NDF, DMI,

BCS change, and forNDFD accounted for 53% of variation in pdNDF k_p. A model including diet concentrations of NDF and starch, forNDFD, intake of DM and of starch, MY, and BCS change accounted for 42% of variation in starch k_p. The ability to account for more variation in iNDF k_p than in pdNDF k_p, and to account for more variation in pdNDF k_p than in starch k_p, suggests that iNDF passage kinetics are more homogeneous than kinetics of pdNDF or of starch. This is likely because of greater variation in the physical and chemical characteristics that affect k_p in pdNDF than in iNDF, and in starch than in pdNDF or in iNDF.

Accurate prediction of the passage rates of pdNDF and starch from the rumen is needed to accurately predict ruminal NDF, starch, and total OM digestibility. Ruminal digestibility of pdNDF and of starch have important implications for the regulation of feed intake and for the extent and efficiency of microbial protein production. Passage rate of digesta fractions is seldom measured directly, but strategic collection of data sets containing more easily measured parameters along with passage rates can increase the accuracy of the prediction of passage rates in models intended for use on commercial dairy farms. Improving predictions of passage rates will increase the ability to optimize DMI and ruminal diet digestibility, and maximize milk yield. It also will permit more efficient utilization of N and other nutrients, and will reduce the proportion of nutrients that are excreted as waste.

Many models of feed intake, digestion, and metabolism in dairy cows may be improved by incorporating the quantified effects of nutrient demand and feed

sources on feed intake and passage rate. Data describing these effects can be provided by the experiments reported here and by future experiments testing other important dietary characteristics. Forage-related treatments for which the dependence of response on pVDMI still need to be quantified include: forage particle size for corn silage, legume forage, and perennial grass forages; forage maturity for legume and perennial grass forages; and forage lignification (e.g., brown midrib strains). Predictions of passage rate also would be improved by experiments testing the effects on passage rate of treatments such as grain type, conservation method, and physical form, non-forage fiber sources, and supplementary fat amount and composition. Finally, an immediate implication of this research is its clear demonstration of the need to provide separate diets for cows with higher and lower nutrient demand, in order to maximize the efficiency of nutrient utilization within the entire herd.

REFERENCES

Ahvenjärvi, S., A. Vanhatalo, P. Huhtanen, T. Varvikko. 2000. Determination of reticulo-rumen and whole-stomach digestion in lactating cows by omasal canal or duodenal sampling. Br. J. Nutr. 83:67-77.

Ahvenjärvi, S., B. Skiba, and P. Huhtanen. 2001. Effect of heterogeneous digesta chemical composition on the accuracy of measurements of fiber flow in dairy cows. J. Anim. Sci. 79:1611-1620.

Ahvenjärvi, S., A. Vanhatalo, and P. Huhtanen. 2002. Supplementing barley or rapeseed meal to dairy cows fed grass-red clover silage: I. Rumen degradability and microbial flow. J. Anim. Sci. 80:2176-2187.

Ahvenjärvi, S., A. Vanhatalo, K.J. Shingfield, and P. Huhtanen. 2003. Determination of digesta flow entering the omasal canal of dairy cows using different marker systems. Br. J. Nutr. 90:41-52.

Ahvenjärvi, S., E. Joki-Tokola, A. Vanhatalo, S. Jaakkola, and P. Huhtanen. 2006. Effects of replacing grass silage with barley silage in dairy cow diets. J. Dairy Sci. 1678-1687.

Akaike, H. 1974. A new look at the statistical model identification. IEEE Transactions on Automatic Control 19(6): 716–723.

Akin, D.E. 1989. Histological and physical factors affecting digestibility of forages. Agron. J. 81:17-25.

Alhadhrami, G., and J.T. Huber. 1992. Effects of alfalfa hay of varying fiber fed at 35 or 50% of diet on lactation and nutrient utilization by dairy cows. J. Dairy Sci. 75:3091-3099.

Allen, M. S. 1982. Investigation into the use of rare earth elements as gastrointestinal markers. M.S. Thesis. Department of Animal Science, Cornell University.

Allen, M. S. 1996. Physical constraints on voluntary intake of forages by ruminants. J. Anim. Sci. 74:3063–3075.

Allen, M. S. 2000. Effects of diet on short-term regulation of feed intake by lactating dairy cattle. J. Dairy Sci. 83:1598–1624.

Allen, M.S., and D.R. Mertens. 1988. Evaluating constraints on fiber digestion by rumen microbes. J. Nutr. 118:261-270.

Allen, M. S., J. G. Coors, and G. W. Roth. 2003. Corn silage. Pp. 547-608 In: Silage Science and Technology, D. Buxton, R. Muck, and J. Harrison, eds. American Society of Agronomy, Crop Science Society of America, and Soil Science Society of America, Madison, WI.

Allen, M. S., J. A. Voelker, and M. Oba. 2006. Physically effective fiber and regulation of ruminal pH: it's more than just chewing. Pp. 270-278 In: Production Diseases in Farm Animals, Wageningen Academic Publishers, Wageningen, Netherlands.

Al-Mabruk, R.M., N.F.G. Beck, and R.J. Dewhurst. 2004. Effects of silage species and supplemental Vitamin E on the oxidative stability of milk. J. Dairy Sci. 87:406-412.

Anil, M.H., J.N. Mbanya, H.W. Symonds, and J.M. Forbes. 1993. Responses in the voluntary intake of hay or silage by lactating cows to intraruminal infusions of sodium acetate or sodium propionate, the tonicity of rumen fluid or rumen distension. Br. J. Nutr. 69:699-712.

Balch, C.C., and A. Kelly. 1951. Factors affecting the utilization of food by dairy cows. 3. Specific gravity of digesta from the reticulo-rumen of cows. Br. J. Nutr. 4:395-398.

Baldwin, R.L., J.H.M. Thornley, D.E. Beever. 1987. Metabolism of the lactating cow. II. Digestive elements of a mechanistic model. J. Dairy Research 54: 107-131.

Bauman, D.E., and J.M. Griinari. 2003. Nutritional regulation of milk fat synthesis. Annu. Rev. Nutr. 23: 203-227.

Beauchemin, K.A., and A.D. Iwaasa. 1993. Eating and ruminating activities of cattle fed alfalfa or orchardgrass harvested at two stages of maturity. Can. J. Anim. Sci. 73:79-88.

Bradford, B. J. and M. S. Allen. 2004. Milk fat responses to a change in diet fermentability vary by production level in dairy cattle. J. Dairy Sci. 2004 87: 3800-3807.

Broderick, G. A., and J. H. Kang. 1980. Automated simultaneous determination of ammonia and total amino acids in rumen fluid and *in vitro* media. J. Dairy Sci. 63:64-75.

Broderick, G.A., R.G. Koegel, R.P. Walgenbach, and T.J. Kraus. 2002. Ryegrass or alfalfa silage as the dietary forage for lactating dairy cows. J. Dairy Sci. 85:1894-1901.

Burato, G. M, J. A. Voelker, and M. S. Allen, 2001. Effects of pretrial milk yield on feed intake, production, and feeding behavior responses to forage particle size by lactating cows. J. Dairy Sci. 84 (Suppl. 1):199.

Campling, R.C., M. Freer, and C.C. Balch. 1961. Factors affecting the voluntary intake of food by cows. 2. The relationship of digesta in the reticulo-rumen and the rate of disappearance of digesta from the alimentary tract. Br. J. Nutr. 15:531-540.

Chai, K., P.M. Kennedy, and L.P. Milligan. 1984. Reduction in particle size during rumination in cattle. Can. J. Anim. Sci. 64 (Suppl.): 339-340.

Choi, C.W., S. Ahvenjarvi, A. Vanhatalo, V. Toivonen, and P. Huhtanen. 2002. Quantitation of the flow of soluble non-ammonoia nitrogen entering the omasal canal of dairy cows fed grass silage based diets. Anim. Feed Sci. Technol. 96:203-220.

Cochran, R. C., D. C. Adams, J. D. Wallace, and M. L. Galyean. 1986. Predicting digestibility of different diets with internal markers: evaluation of four potential markers. J. Anim. Sci. 63:1476–1483.

Combs, D.K., R.D. Shaver, and L.D. Satter. 1992. Retention of rare earths by hay particles following incubation in fresh or autoclaved rumen fluid. J. Dairy Sci. 75:132-139.

Dado, R. G., and M. S. Allen. 1993. Continuous computer acquisition of feed and water intake, chewing reticular motility, and ruminal pH of cattle. J. Dairy Sci. 76:1589-1600.

Dado, R.G., and M.S. Allen. 1995. Intake limitations, feeding behavior, and rumen function of cows challenged with rumen fill from dietary fiber or inert bulk. J. Dairy Sci. 78:118-133.

Dewhurst, R.J., W.J. Fisher, J.K.S. Tweed, and R.J. Wilkins. 2003a. Comparison of grass and legume silages for milk production. 1. Production responses with different levels of concentrate. J. Dairy Sci. 86:2598-2611.

Dewhurst, R.J., R.T. Evans, N.D. Scollan, J.M. Moorby, R.J. Merry, and R. J. Wilkins. 2003b. Comparison of grass and legume silages for milk production. 2. In vivo and in sacco evaluations of rumen function. J. Dairy Sci. 86:2612-2621.

Dixon, R.M., and L.P. Milligan. 1985. Removal of digesta components from the rumen of streers determined by sieving techniques and fluid, particulate and microbial markers. Br. J. Nutr. 53:347-362.

Ehle, F.R., F. Bas, B. Barno, R. Martin, and F. Leone. 1984. Particulate rumen turnover rate as influenced by density of passage marker. J. Dairy Sci. 67:2910-2913.

Erdman, R.A., and L.W. Smith. 1985. Ytterbium binding among particle size fractions of forage cell walls. J. Dairy Sci. 68:3071-3075.

Firkins, J.L., M.S. Allen, B.S. Oldick, N.R.St-Pierre. 1998. Modeling ruminal digestibility of carbohydrates and microbial protein flow to the duodenum. J. Dairy Sci. 81: 3350-3369.

Forbes, J. M. 1995. Voluntary Food Intake and Diet Selection in Farm Animals. CAB Internations, Oxon, UK.

Fuentes-Pila, J., M. Ibañez, J.M. DeMiguel, and D.K. Beede. 2003. Predicting average feed intake of lactating Holstein cows fed totally mixed rations. J. Dairy Sci. 86:309-323.

Goering, H. H., and P. J. Van Soest. 1970. Forage Fiber Analysis (Apparatus, Reagents, Procedures, and Some Applications). Agric. Handbook No. 379. ARS-USDA, Washington, DC.

Grant, R.J., and D.R. Mertens. 1992. Influence of buffer pH and raw corn starch addition on in vitro fiber digestion kinetics. J. Dairy Sci. 75:2762-2768.

Grant, R. J. 1997. Interactions among forages and nonforage fiber sources. J. Dairy Sci. 80:1438–1446.

Grovum, W.L. 1986. The control and motility of the Ruminoreticulum. Pages 18-40 in Control of digestion and metabolism in Ruminants: Proc. Sixth Int. Symp. Rumin. Physiol. L. P. Miligan, W.L. Grovum, and A. Dobson, eds. Prentice-Hall, Englewood Cliffs, NJ.

Hach, C. C., B. K. Bowden, A. B. Lopelove, and S. V. Brayton. 1987. More powerful peroxide Kjeldahl digestion method. J. AOAC 70:783–787.

Harvatine, K. J., and M. S. Allen. 2002. Saturation effects of rumen-inert fat sources on feed intake, milk production, and feeding behavior in lactating cows varying in milk yield. J. Dairy Sci. 85(Suppl. 1):141-142.

Harvatine, K. J., and M. S. Allen. 2005. The effect of production level on feed intake, milk yield, and endocrine responses to two fatty acid supplements in lactating cows. J. Dairy Sci. 88:4018-4027.

Harvatine, K.H., and M.S. Allen. 2006. Fat supplements affect fractional rates of ruminal fatty acid biohydrogenation and passage in dairy cows. J. Nutr. 136:677-685.

Hoffman, P.C., D.K. Combs, and M.D. Casler. 1998. Performance of lactating dairy cows fed alfalfa silage or perennial ryegrass silage. J. Dairy Sci. 81: 162-168.

Hoffman, P.C., S.J. Sievert, R.D. Shaver, D.A. Welch, and D.K. Combs. 1993. In situ dry matter, protein, and fiber digestion of perennial forages. J. Dairy Sci. 76:2632-2643.

Holden, L.A., B.P. Glenn, R.Al. Erdman, and W.E. Potts. 1994. Effects of alfalfa and orchardgrass on digestion by dairy cows. J. Dairy Sci. 77:2580-2594.

Hoover, W. H. 1986. Chemical factors involved in ruminal fiber digestion. J. Dairy Sci. 69:2755–2766.

Huhtanen, P., P. G. Brotz, and L.D. Satter. 1997. Omasal sampling technique for assessing fermentative digestion in the forestomach of dairy cows. J. Anim. Sci. 75:1380-1392.

Illius, A.W., and M.S. Allen. 1994. Assessing forage quality using integrated models of intake and digestion by ruminants. Pp. 869-890 *in* Forage Quality, Evaluation, and Utilization, G.C. Fahey, Jr., M. Collins, D.R. Mertens, and L.E. Moser, eds. American Society of Agronomy, Crop Science Society of America, and Soil Science Society of America, Madison, WI.

Isaacson, H. R., F. C. Hinds, M. P. Bryant, and F. N. Owens. 1975. Efficiency of energy utilization by mixed rumen bacteria in continuous culture. J. Dairy Sci. 58:1645-1659.

Jenness, R. 1985. Biochemical and Nutritional Aspects of Milk and Colostrum. Pp. 164-197 in: Lactation. B. L. Larson, ed. Iowa State University Press, Ames.

Jung, H. G., and M. S. Allen. 1995. Characteristics of plant cell walls affecting intake and digestibility of forages by ruminants. J. Anim. Sci. 73:2774-2790.

Karkalas, J. 1985. An improved enzymatic method for the determination of native and modified starch. J. Sci. Food Agric. 36:1019-1027.

Kaske, M., and A. Midasch. 1994. Effects of experimentally inhibited reticular contraction on digesta passage in sheep. In: D. Giesecke (ed.) Proc. Of the Society of Nutritional Physiology, DLG-Verlag, Frankfurt, Germany.

Kennedy, P. M., and L. P. Milligan. 1978. Effects of cold exposure on digestion, microbial synthesis and nitrogen transformations in sheep. Br. J. Nutr. 39:105-117.

Kohn, R.A., and M.S. Allen. 1995. Effect of plant maturity and preservation method on in vitro protein degradation of forages. J. Dairy Sci. 78:1544-1551.

Kwakkel, R.P., J. Van Bruchem, G. Hof, and H. Boer. 1986. The in sacco degradation of crude protein and cell wall constituents in grass, alfalfa and maize silages. Neth. J. Agric. Sci. 34:116.

Lehman, F. 1941. Die lehre vom ballast. Z. Tierphysiol. Tierernaehr. Futtermittelkd. 5:155-173.

Lundy, F.P. III, E. Block, W.C. Bridges Jr., J.A. Bertrand, and T.C. Jenkins. 2004. Ruminal biohydrogenation in Holstein cows fed soybean fatty acids as amides or calcium salts. J. Dairy Sci. 87:1038-1046.

Martin, C., and B. Michalet-Doreau. 1996. Influence of barley and buffer supplements on quantitative aspects of ruminal fiber digestion of cows. Arch. Tierernahr. 49:203-211.

Mathison, G.W., E.K. Okine, A.S. Vaage, M. Kaske, and L.P. Milligan. 1995. Current understanding of the contribution of the propulsive activities in the forestomach to the flow of digesta. P. 23 in: Ruminant Physiology: Digestion, metabolism, growth and reproduction, W.V. Engelhardt, S. Leonhard-Marek, G. Breves, and D. Giesecke, eds. Ferdinand Enke Verlag, Stuttgart, Germany.

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

McLeod, M.N., P.M. Kennedy, and D.J. Minson. 1990. Resistance of leaf and stem fractions of tropical forage to chewing and passage in cattle. Br. J. Nutr. 63:105-119.

Mertens, D. R. 1994. Regulation of forage intake. Pp. 450–493 in Forage Quality, Evaluation, and Utilization. G. C. Fahey, Jr., M. Colins, D. R. Mertens, and L. E. Moser, eds., American Society of Agronomy, Crop Science Society of America, and Soil Science Society of America, Madison, WI.

Mooney, C.S., and M.S. Allen. 2004. Effects of dietary sodium bicarbonate on ruminal and total tract digestibility of diet and diet components in dairy cows. J. Dairy Sci. 82 (Suppl. 1): 214.

National Research Council. 2001. Nutrient Requirements of Dairy Cattle, seventh revised edition. National Academy Press, Washington, D.C.

Neter, J., M.H. Kutner, C.J. Nachtsheim, and W. Wasserman. 1996. Applied Linear Statistical Models, Fourth Edition. WCB McGraw-Hill, Boston, Mass.

Nicholson, T., and S. A. Omer. 1983. The inhibitory effect of intestinal infusions of unsaturated long-chain fatty acids on forestomach motility of sheep. Br. J. Nutr. 50:141-149.

Noftsger, S., N.R. St-Pierre, and J.T. Sylvester. 2005. Determination of rumen degradability and ruminal effects of three sources of methionine in lactating cows. J. Dairy Sci. 88:223-237.

O'Connor, J. D., C. J. Sniffen, D. G. Fox, and W. Chalupa. 1993. A net carbohydrate and protein system for evaluating cattle diets: IV. Predicting amino acid adequacy. J. Anim. Sci. 71:1298-1311.

Oba, M., and M.S. Allen. 1999a. Effects of brown midrib 3 mutation in corn silage on dry matter intake and productivity of high yielding dairy cows. J. Dairy Sci. 82:135-142.

Oba, M., and M.S. Allen. 1999b. Evaluation of the importance of the digestibility of neutral detergent fiber from forage: effects on dry matter intake and milk yield of dairy cows. J. Dairy Sci. 82:589-596.

Oba, M., and M. S. Allen. 2000. Effects of Brown Midrib 3 Mutation in Corn Silage on Productivity of Dairy Cows Fed Two Concentrations of Dietary Neutral Detergent Fiber: 3. Digestibility and Microbial Efficiency. J. Dairy Sci. 83:1350–1358.

Oba, M. and M. S. Allen. 2003a. Effects of corn grain conservation method on feeding behavior and productivity of lactating dairy cows at two dietary starch concentrations. J. Dairy Sci. 86:174-183.

Oba, M., and M. S. Allen. 2003b. Effects of Corn Grain Conservation Method on Ruminal Digestion Kinetics for Lactating Dairy Cows at Two Dietary Starch Concentrations. J. Dairy Sci. 86: 184–194.

Oba, M., and M.S. Allen. 2003c. Effects of diet fermentability on efficiency of microbial nitrogen production in lactating dairy cows. J. Dairy Sci. 86:195-207.

Okeke, G.C., J.G. Buchanan-Smith, D.G. Grieve. 1983. Effect of sodium bicarbonate on rate of passage and degradation of soybean meal in postpartum dairy cows. J. Dairy Sci. 66:1023-1031.

Okine, E. K., and G. W. Mathison. 1991. Reticular contraction attributes and passage of digesta from the ruminoreticulum in cattle fed roughage diets. J. Anim. Sci. 69:2177-2186.

Onetti, S.G., S.M. Reynal, and R.R. Grummer. 2004. Effect of alfalfa forage preservation method and particle length on performance of dairy cows fed corn silage-based diets and tallow.

Owens, F.N., and R. Zinn. 1988. Protein metabolism of ruminant animals. Pp. 227-249 In The Ruminant Animal: Digestive Physiology and Nutrition. D.C. Church, ed. Waveland Press, Prospect Heights, Illinois.

Poppi, D. P., B. W. Norton, D. J. Minson, and R. E. Hendricksen. 1980. The validity of the critical size theory for particles leaving the rumen. J. Agric. Sci. 94:275-280.

Poppi, D. P., D. J. Minson, and J. H. Ternouth. 1981. Studies of cattle and sheep eating leaf and stem fractions of grasses. III. The retention time in the rumen of large feed particles. Aust. J. Agric. Res. 32:123.

Reynal, S.M., and G.A. Broderick. 2003. Effects of feeding dairy cows protein supplements of varying ruminal degradability. J. Dairy Sci. 86:835-843.

Reynal, S.M., G.A. Broderick, S. Ahvenjarvi, P. Huhtanen. 2003. Effect of feeding protein supplements of differing degradability on omasal flow of microbial and undegraded protein. J. Dairy Sci. 86:1292-1305.

Riewe, M. E., and H. Lippke. 1970. Considerations in determining the digestibility of harvested forages. In: Proc. Nat. Conf. on Forage Quality Evaluation and Utilization, p. F1. Univ. Nebraska, Lincoln.

Rodrigue, C.B., and N.N. Allen. 1960. The effect of fine grinding of hay on ration digestibility, rate of passage, and fat content of milk. Can. J. Anim. Sci. 40:23-29.

Russell, J.B., W.G. Bottje, and M.A. Cotta. 1981. Degradation of protein by mixed cultures of rumen bacteria: Identification of *Streptococcus bovis* as an actively proteolytic rumen bacterium. J. Anim. Sci. 53:242-252.

Russell, J.B., J.D. O'Connor, D.G. Fox, P.J. Van Soest, and C.J. Sniffen. 1992. A net carbohydrate and protein system for evaluating cattle diets: I. Ruminal fermentation. J. Anim. Sci. 70: 3551-3561.

St-Pierre, N.R. 2001. Integrating Quantitative Findings from Multiple Studies Using Mixed Model Methodology. J. Dairy Sci. 84: 741-755.

St-Pierre, N.R. 2003. Reassessment of biases in predicted nitrogen flows to the duodenum by NRC 2001. J. Dairy Sci. 86: 344-350.

Seo, S., L.O. Tedeschi, C. G. Schwab, B.D. Garthwaite, and D.G. Fox. 2006. Evaluation of the passage rate equations in the 2001 Dairy NRC model. J. Dairy Sci. 89:2327-2342.

Sheperd, A. C., and D. K. Combs. 1998. Long-term effects of acetate and propionate on voluntary feed intake by midlactation cows. J. Dairy Sci. 81:2240–2250.

Smith, L. W., H. K. Goering, and C. H. Gordon. 1972. Relationships of forage compositions with rates of cell wall digestion and indigestibility of cell walls. J. Dairy Sci. 55:1140.

Sniffen, C.J., J.D. O'Connor, P.J.Van Soest, D.G. Fox, and J.B. Russell. 1992. A net carbohydrate and protein system for evaluating cattle diets. II. Carbohydrate and protein availability. J. Animal Sci. 70:3562-3577.

Spandl, E., and O.B. Hesterman. 1997. Forage quality and alfalfa characteristics in binary mixtures of alfalfa and bromegrass or timothy. Crop Sci. 37:1581-1585.

Stouthamer, A. H., and C. Bettenhaussen. 1973. Utilization of energy for growth and maintenance in continuous and batch cultures of microorganisms. Biochim. Biophys. Acta, 301:53-70.

Strobel, H.J., and J.B. Russell. 1986. Effect of pH and energy spilling on bacterial protein synthesis by carbohydrate-limited cultures of mixed rumen bacteria. J. Dairy Sci. 69:2941-2947.

Sutherland, T.M. 1988. Particle separation in the forestomachs of sheep. In A. Dobson and M. J. Dobson, eds., Aspects of Digestive Physiology in Ruminants, Cornell Univ. Press, Ithaca, New York.

Taylor, C. C. and M. S. Allen. 2005. Corn grain endosperm type and brown midrib 3 corn silage: site of digestion and ruminal digestion kinetics in lactating cows. J. Dairy Sci. 88:1413-1424.

Teeter, R.G., F.N. Owens, and T.L. Mader. 1984. Ytterbium chloride as a marker for particulate matter in the rumen [Feeds, digestibility]. J. Animal Sci. 58:464-473.

Ulyatt, M. J. 1983. Plant fibre and regulation of digestion in the ruminant. In: G. Wallace and L. Bell (ed.) Fibre in Human and Animal Nutrition. p 103. The Royal Soc. of New Zealand, Wellington, N.Z.

Van Soest, P. J., C. J. Sniffen, and M. S. Allen. 1988. Rumen dynamics. In: A. Dobson and M. J. Dobson (ed.) Aspects of Digestive Physiology in Ruminants. Cornell Univ. Press, Ithaca, NY.

Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583–3597.

Van Soest, P. J., M. B. Rymph, and D. G. Fox. 1992. Discounts for net energy and protein – fifth revision. Pp. 40-68 in Proc. Cornell Nutr. Conf., Ithaca, N.Y.

Voelker, J. A., G. M. Burato, and M. S. Allen. 2002. Effects of pretrial milk yield on responses of feed intake, digestion, and production to dietary forage concentration. J. Dairy Sci. 85: 2650–2661.

Voelker, J.A., and M. S. Allen. 2003a. Pelleted beet pulp substituted for high-moisture corn: effects on digestion and rumen digestion kinetics in lactating dairy cows. J. Dairy Sci. 86:3553-3561.

Voelker, J. A., and M. S. Allen. 2003b. Pelleted beet pulp substituted for high-moisture corn: 3. Efffects on rumen fermentation, pH, and microbial protein efficiency in lactating dairy cows. J. Dairy Sci. 86:3562-3570.

Voelker Linton, J. A., and M.S. Allen. 2005. Pretrial intake affects relative intake, digestion, and production responses of lactating cows to alfalfa and grass silages. Journal of Dairy Science 88 (Suppl. 1): 252.

Voelker Linton, J.A., and M. S. Allen. 2006. Voluntary feed intake affects response to dietary forage content. Journal of Dairy Science 89 (Suppl. 1): 263.

Waghorn, G.C., I.D. Shelton, and V.J. Thomas. 1989. Particle breakdown and rumen digestion of fresh ryegrass (*Lolium perenne* L.) and lucerne (*Medicago sativa* L.) fed to cows during a restricted feeding period. Br. J. Nutr. 61:409-423.

Waldo, D. R., L. W. Smith, and E. L. Cox. 1972. Model of cellulose disappearance from the rumen. J. Dairy Sci. 55:125-129.

Wallace, R. J., and C. A. McPherson. 1987. Factors affecting the rate of breakdown of bacterial protein in rumen fluid. Br. J. Nutr. 58:313-323.

Weiss, W.P., and W.L. Shockey. 1991. Value of orchardgrass and alfalfa silages fed with varying amounts of concentrates to dairy cows. J. Dairy Sci. 74:1933-1943.

Wells, J. E., and J. B. Russell. 1996. Why do so many ruminal bacteria die and lyse so quickly? J. Dairy Sci. 79:1487-1495.

Wheeler, W.E., C.H. Noller, and C.E. Coppock. 1975. Effect of forage-to-concentrate ratio in complete feeds and feed intake on digestion of starch by dairy cows. J. Dairy Sci. 58:1902-1906.

Wildman, E. E., G. M. Jones, P. E. Wagner, R. L. Boman, H. F. Troutt Jr., and T. N. Lesch. 1982. A dairy cow body condition scoring system and its relationship to selected production characteristics. J. Dairy Sci. 65:495–501.

Wilson, J.R., D.E. Akin, M.N. McLeod, and D.J. Minson. 1989. Particle size reduction of the leaves of a tropical and a temperate grass by cattle. II. Relation of anatomical structure to the process of leaf breakdown through chewing and digestion. Grass and Forage Sci. 44:65-75.

Wilson, J.R., and P.M. Kennedy. 1996. Plant and animal constraints to voluntary feed intake associated with fibre characteristics and particle breakdown and passage in ruminants. Aust. J. Agric. Res. 47:199-225.

Wilson, J.R., and R.D. Hatfield. 1997. Structural and chemical changes of cell wall types during stem development: consequences for fibre degradation by rumen microflora. Aust. J. Agric. Res. 48:165-180.

Woodford, S.T., and M.R. Murphy. Dietary alteration of particle breakdown and passage from the rumen in lactating dairy cattle. J. Dairy Sci. 71:687-696.

Ying, Y., and M.S. Allen. 1998. Effects of fineness of grinding and conservation method of corn grain on ruminal starch digestion kinetics in Holstein heifers before and after calving. J. Dairy Sci. 81 (Suppl. 1): 318.

Ying, Y., M.S. Allen, M.J. VandeHaar, and N.K. Ames. 1998. Effects of fineness of grinding and conservation method of corn grain on ruminal and whole tract digestibilities and ruminal microbial production of Holstein cows in early lactation. J. Dairy Sci. 81(Suppl. 1): 339.

Ying, Y., and M.S. Allen. 2005. Effects of corn grain endosperm type and conservation method on site of digestion, ruminal digestion kinetics and microbial nitrogen production of lactating dairy cows. J. Dairy Sci. 83 (Suppl. 1): 393.

Zimmerman, C.A., A.H. Rakes, R.D. Jaquette, B.A. Hopkins, and W.J. Croom, Jr. 1991. Effects of protein level and forage source on milk production and composition in early lactation dairy cows. J. Dairy Sci. 74:980-990.

Zinn, R. A., and F. N. Owens. 1986. A rapid procedure for purine measurement and its use for estimating net ruminal protein synthesis. Can. J. Anim. Sci. 66:157-166.