Lowering Ruminally Degradable Protein in Lactating Dairy Cow Diets

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ABSTRACT

Lactating dairy cows convert 25 to 35% of intake N to milk N, and a part of the remaining N ends up in the environment, causing pollution. Dairy cows absorb amino acids available in the small intestine supplied mainly by digestion of microbial protein and ruminally undegraded feed protein (RUP). Ruminally degradable feed protein (RDP) is the major supplier of N for microbial protein synthesis. Most of the excess RDP will be degraded to ammonia and eliminated as urea in urine. Thus, avoiding excess RDP in dairy cattle diets is important in reducing environmental N pollution. The objectives of the work in this dissertation were to test the hypothesis that lactating dairy cows, when fed varying dietary RDP, can maintain feed intake, milk and milk protein yield, ruminal metabolism, passage of nutrients out of the rumen, and N excretion.

The first study investigated the effects of decreasing RDP in lactating dairy cow diets on feed intake, milk production and apparent N efficiency. Forty mid-lactation cows (36 Holstein and 4 Jersey × Holstein cross-breds) were fed a diet containing 11.3% of diet dry matter (DM) as RDP for the first 28 d (covariate period). From d 29 to 47 (treatment period) cows were randomly assigned to 1 of 4 diets containing constant RUP (7.1% of DM) but 11.3, 10.1, 8.8, or 7.6% of DM as RDP. Reducing RDP in diets linearly decreased DM intake and tended to decrease milk yield. Milk protein, fat and lactose contents, milk protein yield, body weight, and plasma essential amino acids were unaffected by reduced dietary RDP. However, milk urea-N concentration and milk fat

yield decreased linearly with reduced dietary RDP. The apparent efficiency of N utilization for milk N production increased linearly as dietary RDP was reduced. As RDP declined in diets, linear reductions in DM intake and milk production suggested that these cannot be maintained below NRC recommendations of RDP for cows in this study.

The aim of the second study was to test the hypothesis that decreasing dietary RDP in lactating dairy cow diets can maintain ruminal metabolism and flow of nutrients out of the rumen and reduce nitrogen excretion. This study was designed as a replicated Latin square with 4 periods of 21 d each. Four treatment diets containing decreasing RDP and constant RUP similar to the first study were used. Three ruminally and duodenally cannulated and 4 ruminally cannulated lactating Holstein cows were randomly assigned to one of the four dietary treatments. A double marker system with Co-EDTA and Yb-labeled forage as markers was used to determine ruminal outflows of nutrients from omasal samples and nutrients reaching the intestine from duodenal samples. Ruminal microbial protein flow was observed using ^{15}N as an external microbial marker. Feed intake, milk yield, milk composition, and urine and feces output were determined in the last week of each period. Ruminal fluid samples were taken 2 and 4 h after feeding to determine ruminal NH_3-N and volatile fatty acid concentrations. Outflows of nutrients from the rumen were determined by analyzing omasal samples collected over a 24 h feeding cycle in the last week of each period. Reducing dietary RDP decreased protein intakes while DM and fiber intakes were unaffected. Ruminal NH_3-N concentrations linearly declined and peptides and amino acids were unaffected with reduced dietary RDP. A trend for a linear decline in ruminal outflows of microbial N and total N was observed with decreasing dietary RDP. Ruminal volatile fatty acids concentrations were

unaltered by feeding treatment diets. Ruminal outflows of DM and acid detergent and neutral detergent fibers were unaffected by treatments. Treatment diets did not have any effect on milk yield and milk composition. However, milk urea-N and milk fat yield decreased linearly with decreasing dietary RDP. Reducing dietary RDP did not affect milk and milk protein yields but did result in greater body protein mobilization. Fecal N output was unaffected however, urine volume and urine N output decreased linearly suggesting reduced environmental N pollution. There was a trend for a linear decrease in total body N balance, but no significant effects on calculated ruminal N balance as dietary RDP decreased. Linear reductions in microbial N leaving the rumen were due to decreased ruminal NH_3-N as peptides plus amino acids and energy supply were unaffected. The linear reduction in milk production and microbial N flow in the first and second studies, respectively, did not support our hypothesis that lactating dairy cows can be fed dietary RDP below current NRC (2001) recommendations without affecting animal performance. The need to raise 15% more cows to alleviate the loss in production may nullify the advantage in reduced N output into the environment by cows fed lower dietary RDP.

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Chapter 1:

Introduction

Ammonia is an important environmental pollutant that impacts the quality of human and animal life (NRC, 2003). Ammonia emissions from dairy operations are an important source of N pollution (Aneja et al., 2008). Ruminants excrete excess dietary N mainly through urine (Wright, 1998). Urea, the major form of urinary N is rapidly converted to ammonia after excretion (Varel et al., 1999). Dairy farming is also controlled by various regulations to control emissions into the environment (Powers, 2002). Thus decreasing N excretion from dairy cows will help reduce ammonia pollution by dairy operations.

Ruminants are only 30% efficient in converting intake N to milk or tissue N (Tamminga et al., 1992) and the remaining N is lost into the surroundings (Olmos Colmenero and Broderick, 2006). Nitrogen efficiency could be as high as 80% in pigs (Chung and Baker, 1992). Assuming similar potential, increasing N efficiency in lactating cows will decrease N excretion from dairy farms. Great improvements in N efficiency could be achieved by reductions in feed N if milk production could be maintained (Ipharraguerre and Clark, 2005).

Feeding excess N may make dairy operations less profitable due to increased feed costs in addition to the fact that it reduces efficiency of nutrient utilization (Tamminga et al., 1992). Soybean meal, the major protein-supplying ingredient in US dairy rations, reached an historic high ($510 /mt) in July 2008 (World Bank Commodity Price Data, 2009). In addition to the economic and environmental impacts, feeding excess CP may affect the fertility of dairy cows (Canfield et al., 1990). Thus, it is beneficial to avoid

excess dietary protein in lactating dairy cow diets without compromising milk production.

Ruminal microbial organisms convert degradable feed protein into peptides, AA and ammonia in the rumen and use them for microbial protein synthesis. Excess ruminal NH_3-N will be absorbed across the rumen wall, converted to urea in the liver and mostly excreted in urine (Broderick et al., 1991). This indicates that feeding RDP to just meet microbial needs will minimize N excretion from the animal. However, feeding inadequate amounts of RDP will compromise dry matter intake (DMI; Firkins et al., 2006) microbial protein production, and energy and protein supply to the cow (Clark et al., 1992).

Microbial growth and protein synthesis are favored by adequate ruminal ammonia N (Allison, 1969), and nitrogen deficient diets will depress microbial growth (Smith et al., 1979). Inadequate ruminal ammonia concentrations due to inadequate RDP may depress fiber digestion (Firkins et al., 1986) that may cause a reduction in DMI (Allen, 2000). Satter and Slyter (1974) suggested that microbial N flow to the small intestine is maximized at ruminal NH_3 concentrations of 5 mg/dl. Later Klusmeyer et al. (1990) noticed that decreasing dietary CP to 11% from 14.5% significantly decreased ruminal ammonia concentrations and increased ruminal pH about 0.3, however, it did not significantly decrease organic matter truly digested in the rumen and microbial N flow to the intestine. In contrast to Satter and Slyter's (1974) observation, in vivo studies conducted in lactating dairy cows indicated that microbial growth was maximized at 9.2 mg/dl ruminal NH_3-N concentration (Reynal and Broderick. 2005).

Klusmeyer et al., (1990) did not observe significant difference in microbial N flow to the duodenum in 11 and 14.5% CP diets fed cows indicating that feeding lower CP maintained microbial protein flow. Gardner and Park (1973) reported a large increase in milk production when CP content of rations increased above 13.2%. The increase in milk production could be due to higher passage of non-NH_3 non-microbial N that supplied essential AA for milk production. However, Christensen et al., (1993, 1994) demonstrated that production could be maintained by feeding lower dietary protein. They did not measure microbial N flow in these studies. Their results suggested that maintaining milk production by reducing dietary RDP and CP might be by maximizing ruminal microbial protein production that supplied essential AA required for milk production.

Only studies where microbial protein flows were measured should be used to define RDP requirements. Studies in which only milk protein responses were recorded after reducing RDP and increasing RUP so that CP remained constant are not appropriate to define RDP requirements because reductions in microbial protein flow caused by RDP deficiency will be compensated by increases in RUP flow to the intestine (Santos et al., 1998).

In addition to dietary N, recycled N could be used for microbial protein synthesis in the rumen. Significant net recycling of blood urea into the rumen has been previously reported (Lapierre and Lobley 2001). Use of recycled N may reduce microbial dependence on RDP. However, NRC did not consider contribution of recycled N for microbial protein synthesis in its model.

The NRC (2001) recommends 9.5 to 10.5% dietary RDP for lactating dairy cows and these recommendations are generally followed. However, it may be concluded that decreasing dietary RDP than recommended will have positive economic and environmental impacts if diets can be constructed to maintain milk and milk protein production in dairy cows. Above discussion suggests that dairy cows may maintain milk production at lower dietary RDP and CP than currently recommended. Milk production may be maintained when essential AA requirements are met by maximizing ruminal microbial protein outflow. Dietary RDP recommendation should be based on microbial N flow measurements in dairy cows fed decreasing dietary RDP. The NRC (2001) do not account for recycled N used for microbial protein synthesis. Thus, a study investigating the effects of lower dietary RDP on intake, ruminal metabolism, recycled N in the rumen, outflow of nutrients from the rumen, and N excretion will help to improve the knowledge base regarding dietary RDP requirements and will help to make better RDP recommendations for lactating dairy cows.

Chapter 2:

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Review of Literature

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Ammonia emission and livestock operations

Ammonia can cause serious environmental problems and health issues in gaseous or particulate phases. For example, gaseous NH_3 can damage foliage (van der Eerden, 1998) or when converted to ammonium (NH_4^+) can pollute surface waters (Russell et al., 1998). At more typical ambient concentrations, volatilized NH_3 reduces air quality by catalyzing the formation of particles with diameters smaller than 2.5 μm (PM$_{2.5}$)(Hering and Friedlander, 1982). Fine particles contribute to global climate change, degrade visibility and can cause increased human mortality (Pope et al., 2002). Other health conditions caused by NH_3 are decreased lung function, aggravation of asthma, and increased respiratory symptoms and disease (EPA, 2001, Pope et al., 2002). Ammonia is toxic at extremely high concentrations. The NRC (2003) considered ammonia emissions from concentrated animal feeding operations (CAFO) as a major air quality concern.

According to Powers (2002), CAFO are required to meet strict regulations regarding emissions into air. The Environmental Protection Agency is required to establish National Ambient Air Quality Standards for pollutants considered harmful to human health by the Clean Air Act amendments of 1990. In addition, release of a hazardous substance in excess of threshold levels (e.g., 45.5 kg of NH_3 over a 24-h period) must be reported according to the Comprehensive Environmental Response, Compensation and Liability Act (CERCLA).

Over $4 billion could be saved annually in particulate-related health costs by a 10% reduction in NH_3 emissions from livestock (McCubbin et al., 2002). There are

approximately 8.4 million dairy cattle in the US (USDA-NASS, 2009). Dairy cattle

contributed 13% of the total NH_3 emissions for the year 2002 while all animal operations

including dairy contributed 55% of the total NH_3 emissions (Aneja et al., 2008). Burkart

and James (1999) reported NO_3 originally produced, as NH_3 by dairy farms in the

Midwest may be a main contributor to the N loading of the Mississippi river and the

hypoxia zone in the Gulf of Mexico. Therefore reducing NH_3 emissions from dairy

operations is imperative to reducing NH_3 emissions into the environment. The dairy

industry must be proactive in reducing NH_3 release into the environment to stay

competitive and socially acceptable businesses.

Nitrogen efficiency

Ruminants are inefficient at converting dietary N to milk or tissue N. For

example, dairy cattle convert intake N to milk N with an efficiency ranging between 28 to

35% (Kalscheur et al., 2006). Tamminga et al. (1992) reported that only 30% of ruminant

intake N is converted to milk or tissue N and most of the remaining N is excreted in urine

and feces. Thus, an increase in N efficiency (conversion of dietary N to productive

output) could reduce N excretion from dairy operations.

Nitrogen efficiency could be improved significantly from 28.2 to 36.5% by

reducing dietary CP from 17.1 to 12.3%, but it also decreased lactation performance

(Kalscheur et al., 2006). When milk production decreases as N efficiency increases the

need for more cows in the herd and their replacements will have negative economic and

environmental impacts (St-Pierre and Thraen 1999). During a 210- d lactation trial in

dairy cows Ipharraguerre and Clark (2005) noticed that as concentration of CP increased

from 14.8 to 19.0%, N efficiency decreased from 32 to 25% and urinary N as a

proportion of intake N increased from 27 to 33%. However, when dietary CP was decreased they also noticed increased loss in BW and BCS and reduced DMI. Thus, an optimum N efficiency needs to be achieved to have best economic and environmental impacts.

Effects of low dietary nitrogen on nitrogen output and milk production

Increasing dietary protein primarily increases urinary N, causing an increase in N excreted through urine without changes in milk or fecal N output (Broderick, 2003, Wright et al., 1998). Decreasing dietary protein decreases N output mainly by a reduction in urinary N output as fecal N losses are relatively constant (NRC, 2001). Jonker et al. (2002) revealed that, feeding 6.6% more N than recommended by NRC (2001) caused a 16% increase in urinary N and 2.7% increase in fecal N loss.

Between 57 and 78% of urinary N is in the form of urea (de Boer et al., 2002) which is rapidly converted to NH_3 during manure collection and storage as compared to fecal N (Varel et al., 1999). Therefore, a sizeable reduction in NH_3 emissions would be achieved by decreasing dietary protein considering the relatively rapid rate of NH_3 volatilization from urine (Meisinger et al., 2001). James et al. (1999) have demonstrated that reducing dietary N intake by Holstein heifers resulted in decreased NH_3 emissions from their manure. In addition, Frank and Swensson (2002) noticed that manure ammonia emissions from cows fed a 19% CP diet were three times higher than those fed a 13% CP diet. Similarly, Burgos et al. (2006) and Jackson et al. (2006) found that manure ammonia emissions decreased linearly when the CP in feed was decreased from 18% to 12% of DM.

It is also important to maintain milk production when dietary CP is lowered to achieve lower N excretion. It appears that dairy cows can remain productive with lower N diets (Christensen et al., 1993, 1994). Other studies show little or no milk yield response to protein supplementation above 12 to 13% of diets fed to cows for a complete lactation (23 kg/d; Chandler et al. 1976), to cows early lactation (22.5 kg/d; Van Horn et al. 1976), and to first lactation heifers (21.8 kg/d; Roffler et al. 1978). Recently Gressley and Armentano (2007) fed lactating dairy cows either a high RDP (10.1%) or a low RDP (7.4%) diet with RUP either 6.0 or 6.1% of DM, respectively and fixed DMI at 90% of *ad libitum* intake. Even though the low-RDP diet was predicted to be 28% below requirements, it did not affect milk yield. However, Klusmeyer (1990) found that milk production was decreased when dietary CP was reduced from 14.5 to 11% of DM. An earlier study by Gardner and Park (1973) reported a large increase in milk production due to protein supplementation of ration containing 13.2% crude protein. Recently, Kalscheur et al. (2006) used decreasing CP (17.1 to 12.3), RDP (11.0 to 6.8) and RUP (6.1 to 5.5) % of DM, respectively, in diets of mid lactation dairy cows and observed that 6.8% RDP diet decreased N excretion but also observed a linear decline in milk and milk protein yield. However, both RDP and RUP contents of the diet were reduced, so it was not clear which component was deficient.

Protein metabolism in the rumen and ruminally degradable protein requirements

A better understanding of ruminal protein metabolism is required to feed cows more efficiently to reduce NH_3 emission into the environment while maintaining milk production. The dietary CP content is the sum of ruminal degradable (non-protein N and true protein) and undegraded protein. Ruminally undegraded protein escapes degradation

by ruminal microbes and is available for metabolism in the intestine. Ruminal bacteria as well as protozoa play important roles in feed degradation in the rumen (Figure 2-1). About 80% of ruminal microbial organisms attach to undigested feed in the rumen (Craig et al., 1987). Outside the bacterial cell, degradable protein will be converted into peptides and AA by cell bound microbial proteases (Brock et al., 1982). These will be taken into the cell where peptidases degrade peptides into AA which could be either used to make microbial protein or could be deaminated to keto acids and ultimately to ammonia and volatile fatty acids (Tamminga, 1979).

Protein requirements of lactating dairy cows are met from the supply of AA reaching the small intestine as ruminal microbial protein, undegraded feed protein, and endogenous secretions. Ruminal N output mainly consists of RUP, microbial protein and ammonia N (Bach et al., 2005). On average 59% of the non-ammonia N that reaches the duodenum is supplied by microbial CP and the remaining is RUP or endogenous protein (Clark et al., 1992).

Feeding RDP below requirements can compromise microbial protein production, ruminal digestion, and energy and protein availability to the cow (Clark et al., 1992; Stokes et al., 1991). Thus, it is critical to provide enough RDP to meet requirements of ruminal microbial organisms. Klusmeyer et al. (1990) observed no differences in microbial N flow to the small intestine when feeding diet containing 5.7 % RDP (11% CP) compared to an 8.7% RDP (14.5% CP) diet. However, reducing dietary protein from 15.5% to 13.2% decreased milk production significantly (Gardner and Park, 1973). Moreover, reductions in RDP may not always lead to reductions in metabolizable protein availability because reductions in microbial N flow can be offset by increases in RUP

flow (Santos et al., 1998). However, feeding RDP is less expensive than feeding RUP. Thus it is important to understand minimum RDP required to maximize microbial protein flow out of rumen and maintain milk production.

Degradable N sources of ruminal microorganisms

Most ruminal bacteria use ammonia as their N source for growth (Allison, 1969). About 80% of the microbial cell N is derived from ammonia N however protozoa cannot use ammonia (Bach et al., 2005). Microbial protein accounts for the majority of the total AA flow into the intestine (Clark et al., 1992). Therefore, it is important to maintain ruminal ammonia concentrations for the maximal microbial protein flow to the intestine. Satter and Slyter, (1974) reported that minimal concentrations of ruminal ammonia required for maximal microbial protein production was 5 mg/dl which corresponds to 13% dietary CP. Kang-Meznarich and Broderick (1980) reported that a minimum ruminal NH_3-N concentration of 8.5 mg/dl was required in nonlactating cows for maximal microbial protein synthesis. Reynal and Broderick, (2005) recommended even higher ruminal NH_3- N (9.2 mg/dl) requirements for maximal microbial protein synthesis in lactating dairy cows.

In addition to ammonia, ruminal microbes can also use other protein degradation products such as peptides and AA for microbial protein production (Atasoglu et al., 2001). Increasing dietary RDP from 7.7 to 12.5% increased free ruminal AA linearly (Reynal and Broderick, 2005). Addition of AA and peptides into the media of cellulolytic and amylolytic bacteria significantly increased bacterial in vitro growth with saturation of growth responses at 10 mg/L (Argyle and Baldwin, 1989). This indicated that peptides and free AA are stimulatory to bacterial growth. When cows were fed once daily with

14.5% CP diets, peptide concentrations were 54 mg/L at 16 h post-feeding (Chen et al.,1987). Their observation suggests the possibility that dairy cows may meet peptides and AA requirements well below 14.5% dietary CP. However, in-vitro conditions may not reflect in-vivo conditions. In general, microbial requirements for peptides, AA and NH_3 can be aggregated and expressed as a RDP requirement as used by NRC (2001).

Another important source of N for ruminal microbes is the large amount of recycled N from blood to the rumen. Recycled urea from blood to the rumen will be converted to ammonia in the rumen. When recycled N is used for microbial protein production, less N will be excreted through urine (Lapierre and Lobley, 2001). Minimizing dietary sources of degradable protein may force microbes to use waste N like recycled urea N for productive purposes. However, it may prioritize more of the RDP to be peptides (Firkins et al., 2006). Both Lapierre and Lobley (2001) and Remond et al. (2002) reported significant net recycling of blood urea into the rumen at ruminal NH_3 concentrations below 9.5 mg/dl and it improved N efficiency and reduced microbial dependence on ruminally degradable protein.

Impacts of varying dietary RDP on ruminal digestion

Varying dietary RDP can influence ruminal fiber digestion. Fiber digestion was reported to increase with the supply of AA (Griswold et al., 1996) and peptides to pure cellulolytic bacteria (Cruz-Soto et al., 1994). However, Jones et al. (1998) observed that fiber digestion decreased linearly with increasing peptide addition in continuous culture fermenters. They reported that diets containing high levels of NSC (46.2 %), excessive peptide concentrations relative to that of ammonia can depress protein digestion and ammonia concentrations, limit the growth of fiber-digesting microorganisms, and reduce

the ruminal fiber digestion and microbial protein production. This indicates that excess RDP may adversely affect fiber degraders in the rumen namely, *Ruminococcus albus*, *R. flavefaciens* and *Fibrobactor succinogenes* populations. However, in-vitro conditions may not be directly applicable in cows as demonstrated by Reynal and Broderick, (2005) who reported increased milk protein yield with increasing dietary RDP.

Firkins et al. (1986) reported higher NDF and apparent OM digestion by feeding more rapidly degradable dry corn gluten feed than slowly degradable dry distiller's grain in steers. They attributed low fiber digestion to low ruminal ammonia concentration for the slow degrading protein source fed steers. In contrast, decreasing concentrations of RDP down to 7.7% in lactating dairy cow diets did not alter ruminal outflow of NDF or its apparent ruminal digestion (Reynal and Broderick, 2005). This result indicated that fibrolytic bacterial growth or function may not be compromised by low RDP content in dairy diets.

Problems associated with NRC (2001) RDP recommendations

The above discussion suggests that microbial requirements for degradable protein are met when dietary CP levels of 14% or higher are fed. Evidence suggests that RDP intake by lactating dairy cows can be reduced substantially with comparable reductions taken in dietary crude protein (Klusmeyer et al. (1990). However, the NRC (2001) predicts a much higher requirement. The NRC (2001) RDP recommendations for dairy cows generally range from 9.5 to 10.5% of dietary DM depending on diet, animal characteristics and production level. More accurate RDP recommendations could be given only by better understanding of the relationship between N inputs and outputs for critical

metabolic processes. Recycling of N from blood to the rumen is poorly represented in NRC (2001).

Feeding recommendations for RDP and RUP in the NRC (2001) were based on a regression approach that was used to evaluate milk and milk protein responses to concentrations of RDP and RUP in the dietary DM. The NRC (2001) used 39 studies to evaluate milk yield responses to dietary RDP concentrations. Experiments in which RDP was decreased generally were designed to balance the CP content to a constant value by increasing the RUP content of the diet when RDP was removed. Results from experiments using such a design where only milk protein production was reported should not be used to derive RDP recommendations because the potential effects of decreasing RDP concentrations on microbial growth and protein supply to the animal will be masked by increasing RUP concentrations (Santos et al., 1998). Reduced microbial protein flow into the intestine due to reduced RDP will not affect milk protein production if the AA supplied by increased RUP substitutes for reduced AA flow in the form of microbial protein. Only experiments where RUP was held constant or where microbial N flow was reported should be used to define RDP recommendations.

The challenge in dairy feed formulation is therefore to balance N and energy to optimize microbial growth, reduce N excretion to the environment and maintain milk production while improving N efficiency. Protein is expensive compared to other feed ingredients and feeding excess protein relative to requirements increases production costs. It appears from previous research that current RDP recommendations may be excessive, resulting in adverse environmental and economic effects. Most of the previous research has focused on the amount and type of RUP to determine the protein

requirement of lactating dairy cows. More studies need to be designed to measure ruminal microbial protein flow to define the RDP requirement of dairy cows in the diet that maintain milk production and decrease N output.

Hypothesis and Objectives

We hypothesized that decreasing RDP in diets could maintain milk and milk contents yield and significantly improve N efficiency in dairy cows without affecting ruminal digestion, flows of nutrients out of the rumen and could reduce N excretion into the environment. Two studies were conducted to test the hypothesis where the first investigated animal production responses and the second studied ruminal outflows of nutrients and N excretion by lactating dairy cows fed decreasing dietary RDP.

The objectives of the first study were to determine intake, milk production and composition, plasma AA concentrations and apparent N efficiency responses of lactating dairy cows fed reduced dietary RDP and constant dietary RUP [based on NRC (2001) predictions]. Another objective of this work was to test the NRC (2001) model for accuracy in predicting RDP requirements in mid-lactation dairy cows. The objectives of the second study were to determine effects on ruminal metabolism, flow of nutrients out of the rumen and excretion of N of lactating dairy cows fed diets used in the first study.
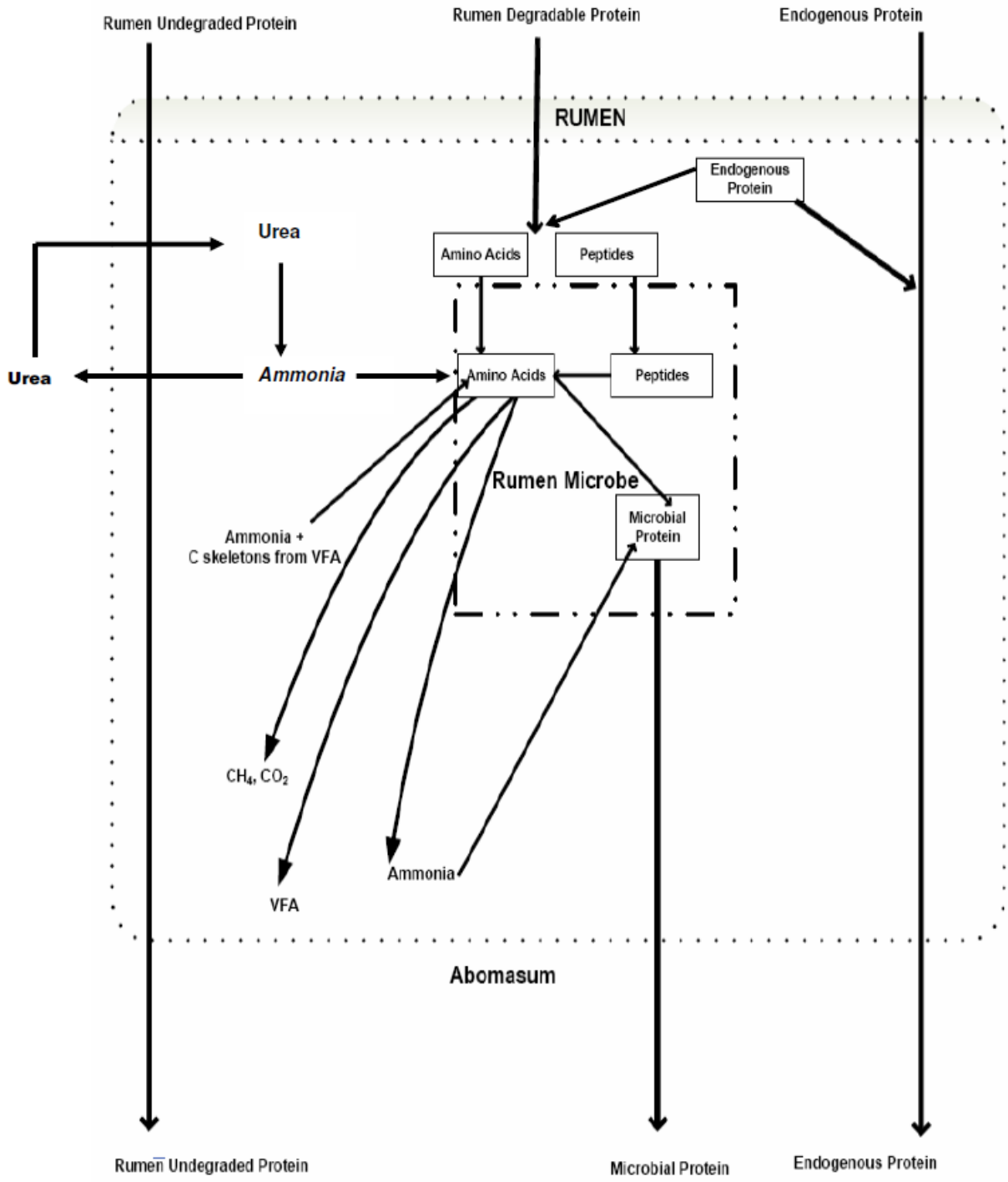
Figure 2-1. A schematic of protein degradation by ruminal bacteria, urea recycling and outflow from the rumen.

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Chapter: 3

Lactation Performance of Mid-Lactation Dairy Cows Fed Ruminally Degradable Protein at Concentrations Lower Than National Research Council Recommendations

ABSTRACT

The aim of this study was to test whether declining dietary ruminally degradable protein (RDP) but with constant ruminally undegraded protein (RUP) alters feed intake, milk production and yield, and the apparent efficiency of N utilization by mid-lactation dairy cows. During the covariate period (d 1 to 28), 40 mid-lactation cows (36 Holstein and 4 Jersey × Holstein cross-breds) were fed a common diet formulated to contain 11.3% of diet dry matter (DM) as RDP. During the treatment period (d 29 to 47), cows were randomly assigned to 1 of 4 diets formulated to contain 11.3, 10.1, 8.8, or 7.6% RDP, whereas ruminally undegraded protein remained constant at 7.1% of DM. All diets contained 47.5% forage and 52.5% concentrate on a DM basis. Dry matter intake was linearly reduced as the dietary RDP declined. The decreasing dietary RDP content was associated with a linear trend for reduced milk yield in cows. Dietary RDP had no effect on body weight or milk fat, protein, and lactose contents in cows. Milk protein yield was not affected by RDP level; however, milk fat yield decreased linearly as dietary RDP was reduced. Concentrations of plasma essential amino acids were unaffected, whereas milk urea-N concentrations decreased linearly as dietary RDP content was reduced. The apparent efficiency of N utilization for milk N production increased linearly from 27.7 to 38.6% as dietary RDP decreased. The dietary RDP requirement of cows in this study was

apparently met between 15.9 and 14.7% dietary crude protein. Milk production responses to lowering dietary RDP suggested a linear declining trend indicating NRC (2001) model recommendations may be accurate for these cows. The linear decrease in milk production will nullify the advantage of increased N use efficiency because more 15% more cows will be required to maintain milk production.

Key words: ruminally degradable protein, protein requirement, milk production, dairy cow

INTRODUCTION

Poor conversion efficiency of dietary N to milk or tissue N gain by ruminants (Bequette et al., 2003) leads to significant losses of N via feces and urine. Urinary N is rapidly converted to ammonia during manure collection and storage as compared with fecal N (Varel et al., 1999). Volatilized ammonia reduces air quality by catalyzing small particle formation (James et al., 1999). The ability to reduce dietary protein levels yet maintain milk production and performance has the potential to reduce N release to the environment by ruminants and may have economic advantages for the producer by reducing feed costs (Tamminga, 1992).

Lactating dairy cows in the United States are generally fed to requirements as set by NRC (2001). These recommendations reflect our current nutritional knowledge. Protein requirements of lactating dairy cows are met from the supply of AA reaching the small intestine in microbial and undegraded feed protein. The degradation of dietary feed

crude protein (CP) in the rumen is important because it supports microbial growth in the rumen. On an average 59% of the non-NH$_3$-N (NAN) that reaches the duodenum is supplied by microbial CP, and the remainder is RUP and endogenous protein secretions (Clark et al., 1992).

Some evidence suggests that ruminants can remain productive at much lower N inputs than are currently recommended and used in practice (Christensen et al.,1993, 1994). Insufficient RDP could lead to a ruminal ammonia deficiency that would depress microbial growth. However, this does not always lead to a reduction in metabolizable protein (MP) availability to the animal because reductions in microbial N flow can be offset by increases in RUP flow (Santos et al., 1998). However, an RDP deficiency can also precipitate depressed fiber digestion, which can lead to reduced dry matter intake (DMI) and energy supply to the animal (Firkins et al., 1986; Allen, 2000; Firkins et al., 2006).

Thus, it is critical to provide enough RDP to meet requirements of ruminal microbes. The NRC (2001) RDP requirements for dairy cows generally range from 9.5 to 10.5% of dietary DM depending on diet, animal characteristics, and production level. Recommendations for RDP and RUP in the NRC (2001) were based on a regression approach using literature data. Few experiments used in the evaluation utilized RDP levels that were well below the current recommendation. Thus, it is possible the current requirements are set too high because of inadequate range in the data used to derive them. Results from research trials where the RDP:RUP ratio was changed while holding CP constant are difficult to interpret if milk protein responses were only reported because decreasing RDP is confounded with the increasing concentration of RUP.

Recommendations of RDP should be based on microbial protein flow and milk production responses. Thus, it would appear that RDP requirements may be higher than necessary under some conditions. Establishment of accurate requirement equations is imperative because overfeeding protein causes decreased animal efficiency and increased N excretion (Kalscheur et al., 2006).

The objectives of this work were to determine effects of reduced dietary RDP with constant RUP [according to NRC (2001) predictions] on intake, milk production and composition, plasma AA concentrations, and apparent N efficiency and to test the NRC (2001) model for accuracy in predicting RDP requirements in mid-lactation dairy cows.

MATER IALS AND METHODS

Animals and Diets

All animal procedures were approved by the Virginia Tech Animal Care and Use Committee. Thirty six Holstein (29 multiparous and 7 primiparous) and 4 primiparous Jersey × Holstein cross-bred cows (147 ± 38 DIM, 594 kg of BW) were used. Cows selected had an average 305-d ME milk yield of 13,209 ± 1,500 kg. Cows were arranged into 4 groups to equalize DIM, parity, BW, milk production, and pregnancy status and then randomly assigned to receive 1 of 4 diets that varied in RDP in the diet DM. Cows were housed in a free-stall unit at the Virginia Tech Dairy Science complex, fed once daily using Calan Broadbent individual animal feeders (American Calan Inc., Northwood, NH), and milked twice daily at 0130 and 1400 h. One cow was removed from the study for health-related issues.

Diets were formulated to meet NRC (2001) recommendations for NE_L, RUP, minerals, and vitamins for a mid-lactation dairy cow weighing 612 kg and producing 36.3 kg of milk per day containing 3.5% fat. Final diets contained 47.5% forage and 52.5% concentrate on DM basis (Table **3-1**). Two concentrate mixes (A and D) were formulated to contain high and low RDP and CP contents and constant RUP content. Concentrate mixes were blended with forages to attain 4 diets containing 11.3 (diet A), 10.1 (diet B), 8.8 (diet C), or 7.6% (diet D) RDP on DM basis and an RUP content of 7.1% of diet DM. Diets were fed as total mixed rations in amounts to maintain 10% daily refusals. Initially, cows were fed diet A during a covariate period (d 1 to 28). During the subsequent treatment period (d 29 to 47), cows were fed their respective experimental diet. Cows were transitioned to the experimental diets over a 4-d period.

Sample Collection and Analysis

Feed intake and refusals were recorded daily. Milk weights were recorded at each milking and BW were recorded twice daily as cows exited the milking parlor. Milk samples were taken on 3 d for each milking during the last week of the covariate and treatment periods. Blood samples were collected from coccygeal vessels into sodium heparin tubes immediately after the afternoon milking on 2 d during the last week of each period, placed on ice, transported to the laboratory, and the blood cells removed by centrifugation. Plasma samples were stored at −20°C until analyses. Two individuals measured the BCS at the end of each period.

Milk samples were submitted to United Federation of DHIA (Blacksburg, VA) for determination of milk true protein, fat and lactose using a Fossomatic 4000 Combi

infrared analyzer (Eden Prairie, MN). Concentrations of MUN were determined using a modification of the Berthelot procedure (ChemSpec 150 Analyzer; Bentley Instruments, Chaska, MN). Urine N, fecal N, and N efficiency were predicted using the following equations (Wattiaux and Karg, 2004):

Urine N output (g/d) = 0.0283 × MUN (mg/dl) × BW (kg);

Fecal N (g/d) = intake N (g/d) – urinary N output (g/d) – milk N (g/d);

N efficiency (%) = milk N (g/d)/intake N (g/d) × 100.

The DM percentages of corn silage and haylage were determined weekly, and diets were adjusted accordingly to maintain a constant forage-to-concentrate ratio (47.5:52.5) on DM basis. The TMR, orts, and major ingredients of the TMR (corn silage, high moisture rolled corn, mixed grass legume silage, cotton seed, and the grain mix) were sampled each day during the last week of each period, composited for the week, and stored at −20°C until analyses. Frozen composited samples were later thawed, oven-dried at 60°C to determine DM, ground through a Wiley mill (1-mm screen; Arthur H. Thomas, Philadelphia, PA), and submitted for nutrient analyses (Dairyland Laboratories, Arcadia, WI). Kjeldahl N, ether extract, ash, and DM contents were determined according to AOAC methods (AOAC, 1997). Acid detergent fiber and lignin were determined according to AOAC (1997; method 973.18) and NDF according to Van Soest et al., (1991). Soluble CP was analyzed as described by Licitra et al. (1996). Starch was measured as dextrose after treating samples with glucoamylase using a YSI 2700 Select Biochemistry Analyzer (application note #319, Yellow Springs, OH) and ether extract by AOAC (1997). Minerals were quantified according to AOAC methods (1997; method 985.01) using an inductively coupled plasma spectrometer (Thermo Jarrell Ash,

Franklin, MA). Packed cell volume (%) was determined by centrifuging heparinized blood in a microhematocrit tube at $15,000 \times g$ for 5 min. Fresh blood was centrifuged ($2,000 \times g$ for 20 min at 4°C) to separate plasma, and AA concentrations were determined by isotope dilution techniques using a gas chromatograph coupled to a mass spectrometer as described previously (El-Kadi et al., 2006).

In Situ Study

To determine ruminal degradability characteristics of CP for each major feed ingredient used in the study, one ruminally cannulated, dry, nonpregnant cow was used. The cow was housed in an individual pen equipped with feeders and clean water. The cow was fed a lactating cow TMR containing 18% CP once daily for ad libitum intake. An in situ analysis of the individual feed ingredients [corn silage, high moisture rolled corn, mixed grass legume silage, concentrate mix A and D, soybean meal (SBM), soybean hulls, and protected SBM (HiVap, Land O' Lakes/Purina Feed, Statesville, NC] was performed on d 8 for 24 h. Samples for the analyses were dried at 60°C and ground to 2 mm. Approximately 4.5 g of the sample were placed in 10×20 cm polyester bags (Ankom Technology, Macedon, NY) with a pore size of 50 µm (±15) and suspended in the rumen in a large (36×42 cm) nylon mesh bag.

Samples were placed in the rumen in reverse order and removed simultaneously at the end of the experiment. The bags resided in the rumen for 2, 4, 8, 12, and 24 h. A 0-h sample was immersed in 39°C water for 20 min. No SBM sample was available in the bag after 24-h incubation for further analysis. After incubation all other bags were rinsed in cold water, washed in a Sears Kenmore washing machine using the knit, cold wash

cycle, and dried at 60°C for 48 h. Residues were ground to 1 mm and analyzed for CP content. Crude protein disappearance was calculated as the difference between the original CP mass and the mass remaining after ruminal fermentation. Digestion rates were calculated by using the Proc NLin procedure of SAS.

Protein degradation rates were estimated according to NRC (2001):

$$Undegraded\ protein\ (\%) = B \times e^{(-kd \times t)},$$

where, B represented the amount insoluble at 0 h, (%), kd represented the degradation rate of B (%/h), and t represented time in the rumen (h).

NRC Model Analysis

The NRC (2001) model was evaluated using treatment means. Observed milk yields, milk composition, and BW were used as inputs. Diet composition was set to values in Table 3-1 and ingredient composition to observed values. Observed DMI were used to set the feeding rate of each ingredient. The observed CP contents of SBM, soyhulls, ground dry corn, and protected SBM were used to calculate the total CP contents of concentrate mixes A and D. These calculated values were compared with the observed CP values of the concentrate mixes and found to deviate slightly. Because the SBM and protected SBM were the major ingredients that contributed to the CP contents of concentrate mixes A and D, the CP values for these 2 ingredients were adjusted to achieve calculated CP contents for the grain mixes that were equal to the observed. These adjusted CP values and the observed NDF and DM values for SBM and protected SBM were used with the observed CP, NDF, and DM for other major forage and grain mix

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ingredients as inputs to the NRC (2001) model to generate predicted RDP, RUP, MP, and energy supplies and animal requirements.

Statistical Analysis

Daily DMI and milk yield in the last week of each period were analyzed statistically. Means of milk composition (n = 6) and blood plasma amino acids (n = 2) were calculated for the last week of each period for each cow. Statistical analyses were performed using PROC MIXED of SAS (2001) with a model that included pretreatment values as a covariate:

$$Y_{ijkl} = \alpha + T_i + P_j + \beta X_{(ij)k} + TP_{ij} + C_{(ij)k} + eijkl,$$

where Y_{ijkl} = l^{th} observation of kth cow of jth parity in i^{th} treatment, α = intercept, T_i = fixed effect of the ith treatment (i = 1 to 4), P_j = fixed effect of the jth parity (j = 1 to 4), β = covariate effect of period 1, TP_{ij} = the fixed interaction between the ith treatment and j^{th} parity, $C_{(ij)k}$ = random effect of the kth cow nested within the ith treatment and j^{th} parity, (k = 1 to 4), and eijkl = residual error. Unless otherwise stated, significance was declared at $P < 0.05$ and a trend was declared at $P \leq 0.10$. All results were reported as least squares means.

RESULTS AND DISCUSSION

The observed chemical composition of experimental diets is presented in (Table 3-2). The dietary DM content varied from 48.0 to 49.6% among diets. Measured CP contents of diets A, B, C, and D were 18.4, 16.8, 15.2, and 13.6% of diet DM, respectively. Chemical composition of individual feed ingredients is reported in Table 3-

3. Concentrate mix D was significantly lower in CP than the formulated value which was due to lower than expected CP in the protected SBM (51.6% vs. the expected value of 53%). Results of the in situ analyses for CP are presented in Table 3-4. The soluble fraction of CP (fraction A) was generally higher than NRC reported values. However, observed degradation rates of major protein sources in the diet were similar to NRC reported values. The degradation rate for SBM was found to be 6.0%/h ± 1%. The NRC reports a value of 7.5%/h, which given the SE of our estimate, would not be significantly different. Soyhulls had an observed rate of 6%/h, whereas the NRC lists a rate of 6.2%/h. We observed a rate of 5.0%/h for rolled, high-moisture corn, whereas the NRC lists a rate of 5.1%/h.

Predicted and observed ruminal CP degradation from concentrate mix A, concentrate mix D, SBM, and protected SBM are presented in Figure 3-1. The rate of disappearance of concentrate mix A and SBM were higher than that of concentrate mix D and protected SBM as expected. The 24-h degradability of the concentrate mixes were 40% units apart as would be expected given the differences in degradation rates of the SBM and protected SBM, the major contributors of CP to the grain mixes.

Least squares means for DMI, milk yield and composition, and BW of cows fed experimental diets are given in Table 3-5. No treatment effects were observed for BW and body condition scores. Dry matter intake of cows fed decreasing dietary RDP in diets were declined linearly ($P < 0.01$). Inadequate RDP can lead to reduced ruminal ammonia concentrations, which causes a depression in fiber degradation (Firkins et al., 1986) and reduces DMI (Allen, 2000). Our results suggested decreasing dietary RDP may not have met the N requirement of ruminal microbes, leading to decreased DMI. Reynal and

Broderick (2005) observed a quadratic effect on DMI decline with decreasing dietary RDP where 7.7% RDP diet fed cows were lower in DMI compared to 9.2 or higher RDP diet fed cows. However, Kalscheur et al. (2006) did not observe changes in DMI when RDP concentrations as low as 6.8% were fed. The linear decline in DMI in our study suggests that ruminal ammonia concentrations are not adequate for maintenance of DMI at dietary RDP less than that recommended by the NRC (2001).

Milk production was not significantly affected by treatment (Table 3-5). However, there was a linear trend ($P < 0.09$) for a loss in production as dietary RDP decreased. The reduced DMI would likely be associated with a reduction in energy supply to the animal and lead to reduction in milk yield. Similarly, when Kalscheur et al. (2006) fed decreasing concentrations of RDP, they observed a linear decline in milk production ($P < 0.01$). The diets in their study were formulated according to the previous NRC (1989) to contain a constant percentage of RUP and increasing percentage of RDP. However, according to the newer NRC (2001), predictions of RUP concentrations in their study decreased from 6.1 to 5.5% as RDP decreased from 11.0 to 6.8%. It was, therefore, not clear whether decreased RUP or RDP caused the trend for a linear decline in milk production in that study. In contrast to our observation, Reynal and Broderick (2005) demonstrated that decreasing dietary RDP from 12.5 to 7.7% did not result in significant loss in milk yield.

It is possible that the period length in our study was too short to allow full dietary responses. This seems unlikely if the low dietary RDP compromised fiber digestion and energy supply because responses to energy restriction are very rapid (Carlson et al., 2006). Responses to a protein deficiency can take longer to manifest due to the buffering

effect of labile protein reserves. However, Krober et al. (2000) observed maximal responses in MUN and milk yield by wk 3 when varying dietary CP content were fed to lactating cows for a period of 5 wk indicating that the 3-wk periods used herein were adequate to test any potential MP deficiencies.

In spite of the linear decline in CP intake (Table 3-5) neither milk CP concentration nor milk protein yield were significantly affected by any of the dietary treatments. Similarly, Armentano et al. (1993) observed no significant changes in milk protein content or yield with changes in dietary RDP. However, Reynal and Broderick (2005) observed increasing linear effects for milk true protein content and a quadratic effect for true protein yield in association with dietary RDP ranging up to 12.5% [predicted from NRC (2001)] with maximum protein production at 10.9% RDP [predicted from NRC (2001)]. Although current NRC (2001) recommendations for RDP range between 9.5 and 10.5% depending on the feeding program and animal characteristics, the NRC (2001) model predicts a quadratic relationship with maximum milk protein yields occurring at 12.2% RDP. However, production responses in the upper range occurred at the expense of a significant increase in estimated urinary N excretion (from 237 to 293 g/d) and a significant decrease in estimated environmental N efficiency (from 94.3 to 87.2 kg of milk per kg of N excreted). Reynal and Broderick (2005) concluded that if optimum N efficiency were the best compromise between the need for profitability and the need for preservation of the environment, the recommended level of RDP from their study would be 9.2% dietary RDP using the NRC (2001) model.

Consistent with the lack of significant dietary effects on milk protein output, no differences in relative proportions of plasma essential AA concentrations were

observed (Table 3-6). The only significant effect was a pair-wise difference for the nonessential amino acid serine for 10.1 and 8.8% dietary RDP. Thus, neither the reduced DMI nor reduced supply of dietary RDP significantly altered plasma AA concentrations or milk protein. In their study, Foldager et al. (1980) reported that when lactating dairy cows were fed an MP-deficient diet (9.3% protein) plasma branched-chain amino acids were decreased and glycine was increased presumably reflecting a shift between dietary sources and tissue mobilization. Because such a shift was not observed herein, it seems unlikely that significant tissue mobilization was occurring.

The apparent efficiency of N use for milk production was 27.7% for cows fed the high RDP diet (Table 3-7). Apparent nitrogen efficiency increased linearly ($P < 0.01$) with decreasing concentrations of RDP in the diet, the lowest RDP diet being most efficient (38.6%) in converting feed protein into milk protein (Table 3-8). Increased N efficiency has important positive environmental implications with respect to air and water quality (Tamminga, 1992). However, 15% more cows fed 7.6% RDP were required to produce same quantity of milk produced by 10.1% RDP fed cows. Reductions in milk yield causing reduced economic return as observed in this study will nullify advantages of improved N efficiency because more cows will be need to fill the gap in production (St-Pierre and Thraen, 1999).

Milk urea nitrogen decreased linearly ($P < 0.01$) from 20.2 to 12.4 mg/dl as dietary RDP declined across treatment diets (Table 3-5). The target range for MUN of Holstein cows is currently 8 to 12 mg/dl (Kohn et al., 2002). However, the results reported herein suggest that an MUN concentration of 12 mg/dl may be inadequate to support maximum production under some conditions because cows on the 8.8% RDP diet

had a mean concentration of 14 mg/dl. The observed values of MUN were higher than expected based on previous work. Results from a split-test (data not presented) ruled out analytical bias at the Virginia DHIA laboratory as a source of the unexpected results. Clearly genetics plays a role in MUN concentrations (Johnson and Young, 2003), which may explain the higher than expected values. Despite this difference, MUN concentrations were clearly responsive to dietary RDP (Table 3-5) and thus are useful for assessing overall N status of the animal.

Milk urea nitrogen can be used as a rapid and noninvasive way to estimate urinary N excretion from dairy cows. According to Wattiaux and Karg (2004), urinary N excretion (g/d) can be predicted as $0.0283 \times BW(kg) \times MUN$ (mg/dl). Predicted daily excretion of urinary N was significantly higher for animals fed high dietary RDP ($P <$ 0.01). Evidence from other studies those measured urinary N excretion, support linear decrease in urinary N excretion with decreasing dietary CP (Davidson et al., 2003; Reynal and Broderick, 2005; Kalscheur et al., 2006). Calculating N excretion in feces assuming no changes in body N mobilization results in a linear reduction in fecal N output ($P < 0.01$) from 178 to 75 g/d as dietary RDP decreased from 11.3 to 7.6% of DM. Such large changes in predicted fecal N output (g/d) are not consistent with the observations of Hristov et al. (2004), who observed no relationship between fecal N output (g/d) and dietary RDP. However, when our results are expressed as a percentage of N intake, the 3 diets with the highest RDP had relatively constant proportions of intake N not accounted for in milk N and predicted urinary output with values of 25, 21, and 24%, respectively. The lowest CP diet had a predicted fecal output plus retained N of 17%. The latter value could be an indication that mobilization did occur, although

increased digestion efficiency cannot be ruled out given the significant reduction in DMI. Hristov et al. (2004) reported that as proportion of intake, fecal N losses were significantly higher for low dietary RDP compared with high dietary RDP. A possible explanation for our results is the apparent slight reduction in RUP as dietary RDP was decreased (Table 3-8). This resulted from slightly lower CP concentrations in concentrate D as compared with the formulated diet (Table 3-3).

Milk fat percentage was not affected by diet, but milk fat yield decreased linearly ($P < 0.02$) from 1.43 to 1.15 kg/d as RDP in the diet decreased from 11.3 to 7.6% of DM (Table 3-5). Armentano et al. (1993) reported no changes in milk fat content or yield with changes in dietary RDP. Methionine and lysine may play a role in milk fat synthesis through increased de novo synthesis of short- and medium- chain fatty acids or through increased synthesis of chylomicra and very low density lipoproteins (NRC, 2001). However, no significant changes in plasma methionine or lysine concentrations were observed (Table 3-6), suggesting that another mechanism was responsible.

Table 3-8 reports NRC (2001) predicted protein supplies and requirements using observed treatment means for DMI, ingredient composition, milk production, and milk composition. As noted previously, these predictions indicated that RUP did not remain constant as originally formulated but declined from 7.1 to 6.4% of diet DM as dietary RDP decreased from 11.3 to 7.6%. But this predicted decline should not have resulted in an MP deficiency for the 3 diets with the greatest CP content because the loss in RUP was roughly 100 g/d for each step down in CP with the base diet being over 200 g/d in excess of requirements. Cows on high dietary RDP (11.3 and 10.1%) produced less milk

than MP allowable milk production according to NRC (2001) predictions indicating that those diets were adequate in RDP. Conversely, milk yields were higher than predicted for the 8.8 and 7.6% dietary RDP, indicating the model overestimated requirements for these cows. For the 8.8% RDP diet the predicted RDP supply was only 87% of that required with a RDP balance of -308 g/d. As dietary RDP declined, the NRC (2001) model predicted higher RUP requirements to meet predicted MP requirements given the predicted decline in microbial yields. As additional dietary RUP was not provided, negative RUP and MP balances were predicted. Depression in DMI with reductions in RDP limited RDP supply along with decreased RUP caused the trend for reduced milk yield. However, cows on the 7.6% RDP diet produced 25% more milk than NRC (2001) predicted from MP allowable milk.

CONCLUSIONS

Mid-lactation dairy cows fed decreasing dietary RDP linearly decreased DMI, milk yields, milk fat yields and maintained milk protein yields during this study. This observation suggests that ruminal microbial RDP requirements may be met `with levels of RDP recommended in the current NRC (2001). Feeding less RDP and CP improved apparent N efficiencies from 27.7 to 38.6% with a trend for lost milk production. Such improvements in N efficiency will have positive environmental impacts. However, 15% more cows required to maintain production may nullify the environmental benefits.

ACKNOWLEDGMENTS

Figure 3-1. In situ crude protein degradability of predicted and observed concentrate mix A (—, ■) concentrate mix D (– · – , ●) soybean meal (···· , ♦) and protected soybean meal (– –, ▲) in the rumen
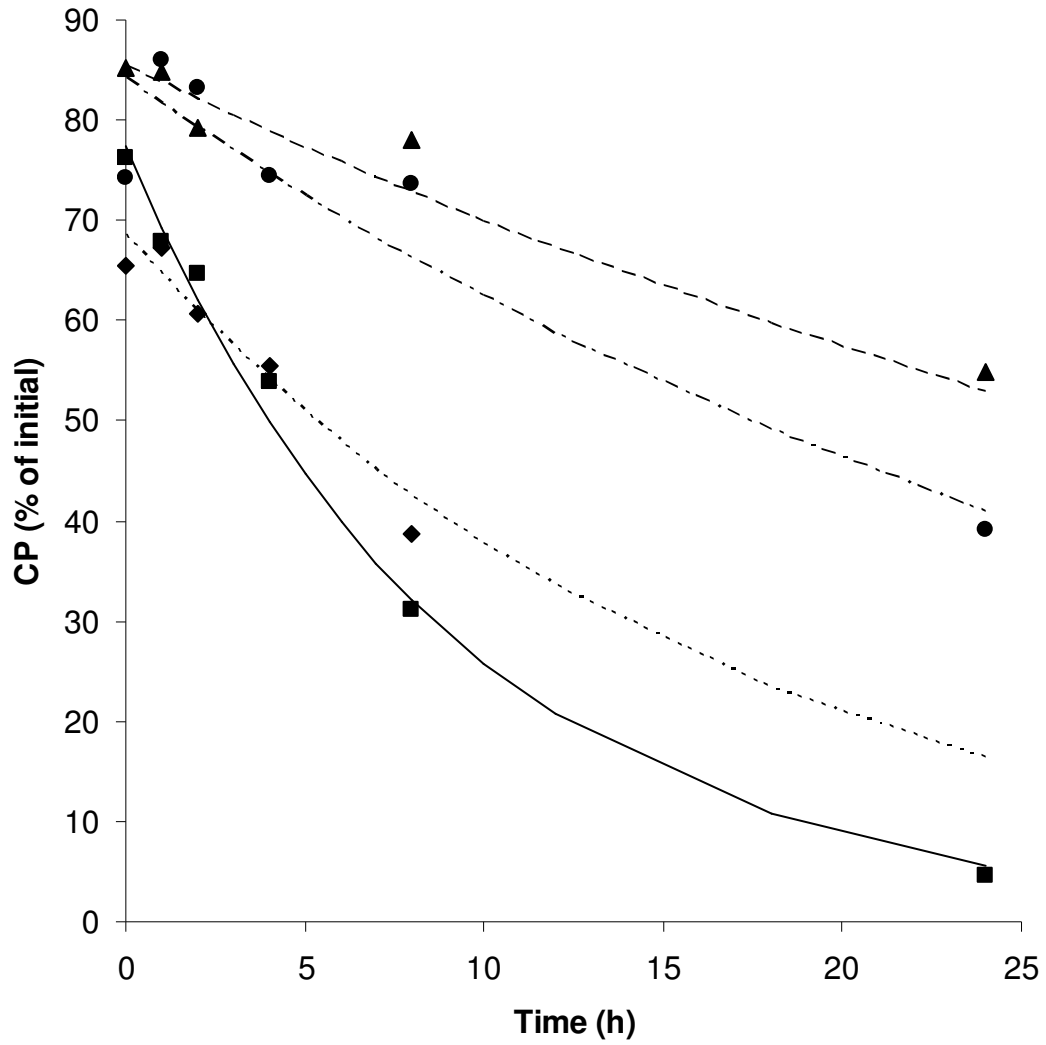
Table 3-**1.** Formulated composition of experimental diets.

Ingredients	RDP, % of diet DM[1]			
	11.3	10.1	8.8	7.6
	_____ (% of DM) _____			
Corn silage	39.7	39.7	39.7	39.7
Mix grass + Legume silage	7.8	7.8	7.8	7.8
Whole linted cotton seed	2.9	2.9	2.9	2.9
Rolled high moisture corn grain	15.5	15.5	15.5	15.5
Soybean hulls	9.7	11.4	13.0	14.7
Soybean meal, solvent –ext (48% CP[2])	20.4	13.6	6.8	0.0
Protected soybean meal[3]	0.0	4.1	8.3	12.4
Ground dry corn grain	0.6	1.3	2.0	2.7
Tallow	0.9	1.2	1.5	1.8
Limestone	1.8	1.7	1.7	1.6
Dicalcium phosphate[4]	0.0	0.1	0.2	0.3
Sodium bicarbonate	0.2	0.2	0.2	0.2
Salt	0.5	0.5	0.5	0.5
Trace mineral and vitamin mix[5]	0.1	0.1	0.1	0.1
NRC Estimates[6]				
RDP, % of DM	11.3	10.1	8.8	7.6
RUP[7], % of DM	7.1	7.1	7.1	7.1
NFC[8], % of DM	41.9	41.9	41.8	41.8
NDF[9], % of DM	30.0	30.8	31.7	32.5
ADF[10], % of DM	20.3	20.9	21.5	22.1
Crude fat, % of DM	4.1	4.6	5.0	5.5
NE_L[11]Mcal/kg	1.6	1.6	1.6	1.6
RDP supplied, g/d	2,611	2,328	2,045	1,762
RDP required, g/d	2,311	2,319	2,328	2,336
RDP balance, g/d	301	9	-282	-574
RUP supplied, g/d	1,646	1,648	1,649	1,651
RUP required, g/d	1,239	1,374	1,510	1,645
RUP balance, g/d	407	273	140	6
MP supplied[12], g/d	2,793	2,690	2,586	2,483
MP allowable milk, kg/d	44.2	41.6	39	36.4

[1]Ruminally degradable protein (RDP), % of diet dry matter (DM) according to national research
council (NRC 2001).
[2] CP= Crude protein
[3] Hivap®, Land O' Lakes/Purina Feed, Statesville, NC
[4] Contained 22% Ca and 19.3% P.
[5]Land O' Lakes/Purina Feed, Statesville, NC; formulated to provide (per kg of DM) 25×10^5 IU
of vitamin A, 400,000 IU of vitamin D, and 10×10^5 IU of vitamin E, 0.1 mg of Co, 12 mg of

Cu, 0.7 mg of I, 60 mg of Fe, 48 mg of Mn, 48 mg of Zn, 0.3 mg of Se.

[6]calculated using the NRC model (2001) and observed input values.

[7]RUP = Ruminally undegraded protein.

[8]NFC = Non-fiber carbohydrate.

[9]NDF = Neutral detergent fiber.

[10]ADF = Acid detergent fiber.

[11]NE_L = Net energy lactation.

[12]Metabolizable protein (MP) supplied: assumes microbial yields are compromised by an RDP deficiency.

Table 3-2. Observed chemical composition of experimental diets.

Item	RDP, % of diet DM[1]			
	11.3	10.1	8.8	7.6
DM, % of diet	49.6	49.1	48.0	48.1
CP[2], % of DM	18.4	16.8	15.2	13.6
Soluble protein, % of CP	41.9	41.4	40.9	40.4
ND-ICP[3], % of CP	11.3	12.6	14.0	15.8
AD-ICP[4], % of CP	4.5	4.8	5.1	5.6
OM[5], % of DM	92.7	92.8	92.9	93.0
NDF[6], % of DM	31.4	32.8	34.1	35.4
ADF[7], % of DM	18.6	20.0	21.4	22.8
Lignin, % of DM	3.2	3.3	3.4	3.5
Ether extract, % of DM	4.53	4.77	4.83	5.16

[1]Ruminally degradable protein (RDP) % of diet dry matter (DM) according to national research council (NRC 2001).
[2]CP = Crude protein.
[3]ND-ICP = Neutral detergent insoluble crude protein.
[4]AD-ICP = Acid detergent insoluble crude protein.
[5]OM = Organic matter.
[6]NDF = Neutral detergent fiber.
[7]ADF = Acid detergent fiber.

Table 3-3. Observed chemical composition of individual ingredients in the experimental diet.

Item	Conc. Mix A	Conc. Mix D	Corn silage	Haylage	Cotton Seed	HMC[1]	SBM[2]	SH[3]	Protected SBM[4]
DM[5], % of feed	88.2	89.2	36.6	48.9	85.6	73.7	86.2	82.9	88.8
OM[6], % of DM	87.2	88.1	96.2	86.5	95.7	98.7	-	-	-
NDF[7], % of DM	27.5	38.0	40.3	42.9	48.4	8.4	5.2	64.0	3.6
ADF[8], % of DM	17.2	29.5	20.5	36.9	40.3	3.6	-	-	-
Lignin, % of DM	2.5	3.4	2.7	7.4	12.4	1.9	-	-	-
CP[9], % of DM	35.0	22.3	7.8	19.3	22.7	7.7	54.6	11.8	51.6
Soluble protein, % of CP	25.2	21.0	53.2	67.0	22.6	40.4	-	-	-
ND-ICP[10], % of CP	6.3	11.5	21.8	24.9	17.8	13.6	-	-	-
AD-ICP[11], % of CP	3.7	5.4	6.4	6.3	7.3	3.1	-	-	-

[1] HMC = High moisture corn grain.
[2] SBM = soybean meal.
[3] SH = soyhulls.
[4] Protected SBM = HiVap®, Land O' Lakes/Purina Feed, Statesville, NC.
[5] DM = Dry matter.
[6] OM = Organic matter.
[7] NDF = Neutral detergent fiber.
[8] ADF = Acid detergent fiber.
[9] CP = Crude protein.
[10] ND-ICP = Neutral detergent insoluble CP.
[11] AD-ICP = Acid detergent insoluble CP.

Table 3-4. Crude protein degradation results from in-situ analyses.

Item	A[1], %	B, %	k, h[-1]
Corn silage	77 ± 0.6	23	0.018± 0.003
Mix grass + Legume silage	76 ± 1.2	24	0.048± 0.01
Rolled High moisture corn	41 ± 0.9	59	0.047± 0.003
Soybean hulls	32 ± 3.0	68	0.062± 0.01
Soybean meal, solvent–ext (48% CP[2])	31 ± 2.2	69	0.065± 0.01
Protected soybean meal[3]	15 ± 1.8	85	0.018± 0.002
Concentrate Mix A	23 ± 1.8	77	0.106± 0.01
Concentrate Mix D	16 ± 3.9	84	0.028± 0.01

[1]A = soluble, B = insoluble (100 − A), k = degradation rate.
[2]CP = Crude Protein.
[3]Protected SBM = HiVap®, Land O' Lakes/Purina Feed, Statesville, NC.

Table 3-5. Least squares means for intake, milk yield, milk composition, and body weight of dairy cows fed the experimental diets.

| Item | RDP, % of diet DM[1] | | | | SEM | Contrasts[2] | | |
	11.3	10.1	8.8	7.6		L	Q	C
Intake, kg/d							── (*P* <) ──	
DM	24.1	23.9	23.2	20.4	0.57	0.01	0.33	0.57
CP[3]	4.44	4.02	3.52	2.79	0.09	0.01	0.10	0.70
NDF[4]	7.58	7.83	7.89	7.22	0.19	0.24	0.02	0.52
ADF[5]	4.49	4.79	4.97	4.66	0.12	0.21	0.02	0.49
Milk Production								
Milk yield, kg/d	41.2	42.1	40.3	36.6	1.95	0.09	0.26	0.92
Milk lactose, %	4.87	4.88	4.86	4.88	0.03	0.92	0.96	0.61
Milk true protein, %	2.98	3.00	3.01	2.92	0.05	0.45	0.25	0.66
Milk fat, %	3.43	3.13	3.22	3.33	0.20	0.82	0.32	0.71
Milk lactose, kg/d	2.03	2.08	1.95	1.73	0.11	0.06	0.26	0.86
Milk true protein, kg/d	1.23	1.26	1.21	1.07	0.06	0.11	0.20	0.60
Milk fat, kg/d	1.43	1.33	1.28	1.15	0.08	0.02	0.91	0.71
MUN[6], mg/dl	20.2	17.6	14.2	12.4	0.62	0.01	0.58	0.40
BW[7], kg	612	611	626	611	4.9	4.86	0.19	0.04
BCS[8]	3.1	3.1	3.1	3.1	0.05	0.31	0.81	0.85

[1]Ruminally degradable protein (RDP), % of diet dry matter (DM).
[2]Contrasts: L = linear, Q = quadratic, C = cubic.
[3]CP = Crude protein.
[4]NDF = Neutral detergent fiber.
[5]ADF = Acid detergent fiber.
[6]MUN = Milk urea nitrogen.
[7]BW = Body weight.
[8]BCS = Body condition score.

Table 3-6. Relative proportions of free AA[1] (as % of total AA) in plasma of cows fed the experimental diets.

	RDP, % of diet DM[2]					
Item	11.3	10.1	8.8	7.6	SEM	*P*
	% of total AA					
Essential AA						
His	2.5	2.2	2.2	2.4	0.001	NS[3]
Ile	6.6	6.5	6.3	6.5	0.003	NS
Leu	7.3	7.3	7.0	7.5	0.004	NS
Lys	4.6	4.3	4.3	4.2	0.002	NS
Met	0.8	0.7	0.8	0.7	0.005	NS
Phe	1.6	1.6	1.6	1.9	0.001	NS
Thr	5.2	5.2	4.0	4.5	0.003	NS
Trp	1.7	1.6	1.6	1.6	0.001	NS
Val	14.9	14.8	13.5	14.0	0.007	NS
Nonessential AA						
Ala	12.5	12.7	12.7	14.2	0.7	NS
Asp	0.4	0.3	0.4	0.3	0.03	NS
Gln	11.1	10.8	10.4	9.9	0.006	NS
Glu	2.6	2.6	2.6	2.4	0.002	NS
Gly	17.5	19.7	21.5	19.5	0.02	NS
Pro	4.3	4.4	4.3	4.4	0.002	NS
Ser	4.1[ab]	3.5[a]	4.4[b]	4.5[b]	0.002	0.05
Tyr	1.9	1.8	1.8	1.9	0.001	NS

[1]AA= Amino acids.
[2]Ruminally degradable protein (RDP), % of diet dry matter (DM).
[3]NS = non-significant.
 Means in a row without common superscript differ at *P* < 0.

Table 3-7. Nitrogen efficiency of cows fed the experimental diets.

Item	RDP , % of diet DM[1]				SEM	Contrasts[2]		
	11.3	10.1	8.8	7.6		L	Q	C
							(P <)	
Intake N, g/d	719	613	544	453	14.6	0.01	0.62	0.36
Milk N, g/d	197	191	193	169	6.6	0.01	0.20	0.27
Predicted Urine N[3], g/d	350	304	248	210	11.3	0.01	0.74	0.62
Predicted Fecal N[4], g/d	178	128	133	75	13.4	0.01	0.78	0.07
N efficiency[5], %	27.7	30.9	35.5	38.6	1.2	0.01	0.99	0.60

[1]Ruminally degradable protein (RDP), % of diet dry matter (DM).
[2]Contrasts: L = linear, Q = quadratic, C = cubic.
[3]Predicted urine N output = $0.0283 \times$ MUN (mg/dl) $\times$ body weight (kg).(Wattiaux and Karg, 2004).
[4]Predicted fecal N = N intake – predicted urinary N – milk N.
[5]Apparent N efficiency (%) = $100 \times$ Milk N (g/d) / Intake N (g/d).

Table 3-8. Predicted protein requirements and allowable milk production using the NRC[1] (2001) model and observed diet composition and dry matter intake.

NRC prediction[3]	RDP, % of diet DM[2]			
	11.3	10.1	8.8	7.6
CP[4], % of DM	18.3	16.9	15.4	14.0
RDP, % of DM	11.2	10.0	8.7	7.6
RUP[5], % of DM	7.1	6.9	6.7	6.4
NE_L[6] Mcal/kg	1.61	1.61	1.61	1.65
RDP required, g/d	2,388	2,379	2,331	2,097
RDP supplied, g/d	2,707	2,380	2,022	1,545
RDP balance, g/d	319	1	-308	-552
RUP required, g/d	1,463	1,531	1,682	1,676
RUP supplied, g/d	1,708	1,649	1,551	1,299
RUP balance, g/d	245	118	-131	-376
MP[7] balance, g/d	211	101	-112	-320
NE_L allowable milk, kg/d	42	43.2	41.3	35.4
MP allowable milk, kg/d	46	44.4	37.8	29.3

[1]NRC = National research council.

[2]Ruminally degradable protein (RDP), % of diet dry matter (DM).

[3]Values predicted using actual dry matter intake, ingredient composition, milk yield, and milk composition for each treatment (NRC, 2001).

[4]CP = Crude protein.

[5]RUP = Ruminally undegraded protein.

[6]NE_L = Net energy lactation.

[7]MP = Metabolizable protein.

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Chapter 4:

Nitrogen digestion and metabolism of lactating dairy cows fed varying dietary ruminally degradable protein.

ABSTRACT

This study investigated the effects of varying dietary ruminally degradable protein (RDP) on ruminal metabolism, nutrient passage from the rumen, and nitrogen excretion in lactating dairy cows. Three ruminally and duodenally cannulated and 4 ruminally cannulated lactating Holstein cows were used in a replicated Latin square design with 4 treatments and 4 periods of 21 d each. Cows were randomly assigned to one of four dietary sequences. Diets were formulated to contain 11.3, 10.1, 8.8, or 7.6% RDP with 7.1% ruminally undegraded protein as a percentage of dietary dry matter (DM). All diets contained 48% forage and 52% concentrate on a DM basis. Ruminal outflows of nutrients were determined from omasal and duodenal samples using a double marker system with Co and Yb as markers. Ruminal outflows of nutrients were also determined from omasal samples using a single marker system and either indigestible neutral detergent fiber, Cr, Yb or Co as markers and using a triple marker system and Co, Yb and indigestible neutral detergent fiber markers. The double marker system and the Yb single marker system provided nutrient flows comparable to previously published results. Decreasing dietary RDP did not significantly alter DM intake. Ruminal NH_3-N concentrations decreased linearly from 14.5 to 5.5 mg/dl, and there was a trend for reduced ruminal outflows of microbial N and total N with decreasing dietary RDP. Ruminal digestibility of organic matter, energy, and fiber were not significantly affected indicating ruminal ammonia concentrations were adequate to support fiber digesting bacteria. Calculated ruminal N balance was not significantly affected suggesting that

urea transport from blood was not enhanced by the low ruminal ammonia concentrations. Milk yield and milk composition were unaltered excepting milk urea-N, which decreased linearly with decreasing dietary RDP. Milk and fecal N outputs were unaffected, but urine N output decreased and nitrogen balance tended to decline linearly with decreasing dietary RDP. Neither ruminal amino acid plus peptide concentrations nor fermentable energy were not altered by diet, so the trend for reduced flow of microbial and total N from the rumen suggests that microbial N synthesis in the rumen was limited by lack of ammonia in the low RDP diets.

Key words: ruminally degradable protein, microbial growth, dairy cow, protein requirements

INTRODUCTION

The ability of dairy cows to convert intake N to milk N (N efficiency) ranges from 14 to 45% with an average of 25% (Huhtanen and Hristov, 2009). Tamminga et al. (1992), observed that in ruminants only 30% of intake N is converted to saleable products and most of the remaining N is excreted in urine and feces. Urea, the major urinary N compound, is rapidly converted to ammonia while fecal N is converted at a much lower rate (Varel et al., 1999). Ammonia is toxic at extremely high concentrations and has numerous environmental and human health effects even at low concentrations (Erisman and Monteny, 1998, Sutton et al., 1993). Reducing dietary N intake reduces ammonia emissions from manure (Frank and Swensson, 2002) and increases apparent N efficiency and does not always result in significant reductions in milk yield (Christensen et al., 1993). As dairy cattle account for 13% of total ammonia emissions (Aneja et al., 2008), accurate estimation of dietary N requirement is critical.

Amino acids (AA) required by lactating dairy cows are supplied via microbial and undegraded feed protein flowing from the rumen. An average of 59% of non-ammonia nitrogen (NAN) reaching the duodenum is supplied by microbial crude protein (CP) produced in the rumen (Clark et al. 1992) using degradable protein. The meta-analysis conducted by Ipharraguerre and Clark, (2005) demonstrated that feeding supplemental sources of ruminally undegraded protein (RUP) compared with soybean meal (SBM) decreased intestinal supply of microbial N but increased the supply of non-ammonia non-microbial N (NANMN). Strategies focused on improving microbial protein flow to the intestine by meeting ruminal RDP requirements will reduce undegraded protein needs of lactating dairy cows.

Ruminal bacteria mainly use dietary protein degradation products such as ammonia (Allison, 1969), peptides and AA (Argyle and Baldwin, 1989) in support of growth and protein synthesis. However, significant net recycling of blood urea into the rumen supplements dietary degradable N sources (Lapierre and Lobley, 2001, Remond et al., 2002) and may act to buffer ammonia concentrations in the rumen when low RDP diets are fed. Ruminal microbes are thought to have an ammonia requirement of 5 mg/dl, which corresponds to 13% dietary CP (Roffler and Satter, 1975a, b and Satter and Slyter, 1974). When ruminally available N is deficient, degradation of OM can be reduced (Smith, 1979) due largely to inhibition of fiber digesting bacteria (Firkins et al., 1986). Reductions in fiber digestion can lead to reductions in DMI, energy supply, and milk production (Allen, 2000, Kalscheur et al., 2006).

Some studies suggest adequate ruminal microbial metabolism at lower ammonia-N concentrations. Klusmeyer et al. (1990) observed no changes in microbial growth or microbial N flow from the rumen when dairy cows were fed 11% CP diets (5.7 % RDP) resulting in ruminal ammonia concentrations of 2.5 mg/dl. Using in-vitro techniques Argyle and Baldwin (1989),

demonstrated that growth of mixed microbial populations was maximized at AA and peptides concentrations of 10 mg/L. Chen et al. (1987) reported that feeding 14.5% CP diets led to a ruminal peptide concentration of 54 mg/L at 16 h post-feeding.

Taken together, these studies suggest that diets below 14.5% CP (7-8% dietary RDP) should still provide adequate ammonia, AA, and peptides to maximize microbial growth. However, the NRC (2001) predicts a much higher requirement (9.5% of DM as RDP) using a regression approach that was used to evaluate milk and milk protein responses to concentrations of RDP and RUP in the dietary DM. Ruminally degradable protein requirements must be based on microbial N flow out of the rumen in dairy cows. To redefine NRC (2001) recommendations, in-vivo measurements of ammonia, AA and peptides and ruminal outflow of microbial N with decreasing dietary RDP are necessary.

Based on these observations, we hypothesized that microbial growth and fiber digestion could be maintained at dietary concentrations below current NRC requirements. The objectives of this work were to determine the effects of varying dietary RDP on ruminal metabolism and microbial growth, post-ruminal nutrient flow, and N excretion in mid-lactation dairy cows with the goal of improving our knowledge of RDP requirements.

MATERIALS AND METHODS

Animals and Diets

The Virginia Tech Animal Care and Use Committee approved all animal procedures. This study was conducted as a replicated Latin square with 4 periods of 21 d each. There were 3 ruminally and duodenally cannulated lactating Holstein cows (2 multiparous and 1 primiparous; 72±12 DIM, 580±48 kg BW) in the first replication and 4 ruminally cannulated lactating

Holstein cows (3 multiparous and 1 primiparous; 80 ± 18 DIM, 617 ± 57 kg BW) in the second replication. One cow was inadvertently removed from the study by the farm crew after the 3rd period of the first replication and one cow missed the first 2 periods of sampling in the second replication due to health related issues. During the first 16 d of each period, cows were housed in a free-stall unit at the Virginia Tech Dairy Science complex with free access to water and feed. Animals were fed once daily at 1100 h. using Calan Broadbent individual animal feeders (American Calan, Inc., Northwood, NH), and milked twice daily at 0130 and1400. On d 16 of each period, cows were moved to individual stalls in the metabolism unit for feeding, milking and sampling. Cows were fed once daily at 1100 and milked twice daily at 0700 and 1900 from d 17- 21.

Cows were randomly assigned to one of four dietary sequences that varied in the proportion of dietary RDP. Two concentrate mixes were formulated to contain high and low RDP content with constant RUP content using the NRC model (2001). A third concentrate mix was formulated from corn and soybean hulls with and without Cr_2O_3 and included at a constant rate across diets for purposes of testing different markers. All diets contained 48% forage (a blend of 83% corn silage and 17% haylage). Concentrate mixes were blended with forages in varying proportions to produce four diets containing 11.3, 10.1, 8.8, or 7.6% RDP on a DM basis and a constant RUP content of 7.1% of dietary DM (Table 4-1). Diets were formulated to meet the NRC (2001) recommendations for energy, RUP, minerals, and vitamins for a lactating Holstein cow (70 DIM) weighing 612 kg and producing 36.3 kg milk per day containing 3.5% fat. Final diets contained 48% forage and 52% concentrate on a DM basis. Diets were fed as total mixed rations in amounts to achieve between 5 and 10% daily refusals on an AF basis. Dry matter

content of corn silage and haylage were determined weekly, and diets were adjusted accordingly to maintain a constant forage-to-concentrate ratio on a DM basis.

Sample Collection and Analysis

Feed and milk

Feed offered and refused was recorded daily for individual animals. Milk yield was recorded twice daily throughout the study. Milk samples were collected at 8 consecutive milkings during the last week of each period. Major ingredients of the TMR (corn silage, mixed grass legume silage, cottonseed and grain mixes), TMR, and orts were sampled daily during the last week of each period (sampling week), composited by week and stored at -20° C until analyses. Frozen samples were later thawed, oven dried at 60° C to constant weight and ground through a Wiley mill (1-mm screen; Arthur H. Thomas, Philadelphia, PA) and submitted for nutrient analyses (Dairyland Laboratories, Arcadia, WI). Total N concentration was determined by combustion using a Perkin-Elmer 2410 Series II N analyzer (Perkin-Elmer, Norwalk, CT). AOAC methods (AOAC, 1996) were used to ether extract (method 920.39), and ash (method 942.05). Acid detergent fiber and lignin were determined according to AOAC (1997; method 973.18) and NDF according to Van Soest et al. (1991). Soluble CP, ND-ICP and AD-ICP were analyzed as described by Licitra et al. (1996). Starch was measured as dextrose after treating samples with glucoamylase using a YSI 2700 Select Biochemistry Analyzer (Application Note #319, Yellow Springs, OH). Minerals were quantified according to AOAC (1997; method 985.01) using an inductively coupled plasma spectrometer (Thermo Jarrell Ash, Franklin, MA). Gross energy of ground TMR and orts samples was determined by bomb calorimetry (model 1271, Parr Instruments, Moline, IL).

Milk samples were submitted to the United Federation of DHIA laboratory (Blacksburg, VA) for determination of milk true protein, fat, and lactose using a Fossomatic 4000 Combi infrared analyzer (Eden Prairie, MN). Concentrations of MUN were determined using a modification of the Berthelot procedure (ChemSpec 150 Analyzer; Bentley Instruments, Chaska, MN). Total N concentration in milk was determined on freeze dried samples by combustion using a Perkin-Elmer 2410 Series II N analyzer (Perkin-Elmer, Norwalk, CT).

Urine and Feces

On day 16 of each period, cows were fitted with a urinary catheter (22 French, 75 cc; C.R. Bard, Inc., Covington, GA). All excreted urine and feces were collected, mixed thoroughly and weighed at 11.00 daily from d 18 to 21. The urinary catheter was connected to tygon tubing that drained into new 20 L plastic containers. Containers were capped and tubing passed into the container via a hole drilled into the cap to minimize loss of ammonia. During the first period of the first replication, collection containers were held at room temperature. In subsequent periods, urine collection containers were chilled to prevent ammonia loss (Knowlton et al., 2010). At the end of each 24 h collection, all excreted urine was pooled and a 50 ml representative sample was acidified with 50% H_2SO_4 and stored at -20° C. Feces from each cow were mixed thoroughly and samples were taken and stored at -20° C until analyses. Frozen samples of feces were later thawed, composited by cow and period, oven dried at 60 ^{o} C to constant weight through a Wiley mill (1-mm screen; Arthur H. Thomas, Philadelphia, PA) and analyzed for ash, OM, NDF, ADF and acid hydrolysis of fat (Dairyland Laboratories, Arcadia, WI) as described above. Total N concentrations in urine and dried ground feces samples were determined by combustion using a Perkin-Elmer 2410 Series II N analyzer (Perkin-Elmer, Norwalk, CT). Gross energy of fecal

samples was determined by bomb calorimetry (model 1271, Parr Instruments, Moline, IL).

Markers of Digesta and Microbial Flows

Ruminal outflow of digesta was assessed via the omasal digesta sampling technique (Huhtanen et al., 1997) and duodenal sampling. Chromium sesquioxide (Cr_2O_3) (Ouellet et al., 2002), $YbCl_3$ (Harvatine et al., 2002) LiCo-EDTA (Uden et al., 1980) and indigestible NDF (INDF; Dado and Allen, 1995) were used as digesta flow markers. The INDF of ruminal digesta was not analyzed and it was used only as an internal marker in this study. The Cr_2O_3 marker was incorporated into a corn/SH mix that contained 1% Cr_2O_3, 20% corn grain and 79% soybean hulls. During the first 10 d of each period, cows were fed a diet that contained a premix of 20% corn grain and 80% soybean hulls at a rate of 12.8% of dietary DM. From d 11 to 14, the corn soyhulls mix containing Cr_2O_3 as a marker was substituted for the 20% corn, 80% soyhulls premix to achieve dosing rates of 10 or 3.4g Cr/d/cow during the first and second replications of the study, respectively. From d 15 to 20, the marker mix was directly deposited into the rumen and mixed by hand at 8 h intervals to ensure that the entire marker was consumed.

Corn silage was labeled with Yb using $YbCl_3$ in an aqueous solution at a concentration of 35% (w/w) as described by Harvatine et al., (2002). Yb-labeled corn silage was directly deposited into the rumen and mixed by hand at 8 h intervals from d 15 to 20 at a rate of 0.27 or 0.11g Yb/cow/d during the first and second replications, respectively. Lithium Co-EDTA was prepared as described by Uden et al., (1980), dissolved in distilled water, deposited into the rumen from day 15 to 20 at a rate of 0.37 (first replication) and 0.33g Co/cow/d (second replication), and hand mixed at 8 h intervals. Concentrations of INDF in omasal and duodenal digesta, TMR, orts, and feces was determined by 120-h in vitro fermentation in buffered rumen media without addition of pepsin (Dado and Allen, 1995) at the Ohio State University,

Columbus, OH. Microbial N flow from the rumen was measured by dosing ^{15}N at a rate of 104.5 mg/cow/d (Reynal and Broderick, 2005). (^{15}NH$_4$)$_2$SO$_4$ (10% atom excess) was dissolved in distilled water and deposited in the rumen from d 16 to 20 at the same time as the Co marker.

Ruminal, omasal and duodenal sampling and analysis

On d 10 of each period (before initiation of dosing), ruminal samples were collected and stored at -20°C for for later analysis of background ^{15}N content. Nutrient flows were calculated using single (Cr, Yb or INDF), double (Co and Yb) and triple marker (Co, Yb and INDF) methods as described by France and Siddons (1986). On d 17 of each period, ruminal samples were collected from cows at 0800, 1000, 1300 and 1500 h. Samples were strained through 2 layers of cheesecloth and ruminal pH was measured immediately. Sub-samples of strained ruminal fluid collected at 1300 and 1500 h were preserved by addition of 0.2 mL of 50% (vol/vol) H$_2$SO$_4$, and stored at -20°C.

Ruminal contents were evacuated at 2 h post-feeding on d 20 and 2 h before feeding on d 21 of each period as described by Dado and Allen, (1995). Total contents were thoroughly mixed and 500 mL sub-samples were taken and stored at -20°C. Remaining ruminal contents were returned to the rumen within 45 min of initiating evacuations.

Ruminal fluid samples were thawed and centrifuged at 30,000 × g for 20 min at 4°C. Ruminal ammonia concentrations were determined following the procedure of Broderick and Kang (1980). Trichloroacetic acid (TCA) soluble ruminal N content (assumed to contain peptides, AA, and NH$_3$-N) was determined as described by Griswold et al. (2003). Ruminal peptide and AA-N fractions were determined by subtracting NH$_3$-N from TCA soluble N. Concentrations of VFA in ruminal fluid were measured by NMR spectroscopy as described by Beckonert et al. (2007).

Beginning on d 18, omasal and duodenal digesta samples (400 mL) were collected every 4 h over a 48 h period with sampling time advanced by 2 h on the second day to yield samples representing each 2 h interval of a 24 h period. Samples were stored at -20 $^{\circ}$C. Omasal and duodenal samples were subsequently thawed and 200 mL sub-samples were obtained from each of the 12 sampling times and pooled by cow and period to yield a 2.4-L composite. The composites were separated into 3 phases [large particle (LP), small particle (SP), and fluid (FP)] as described by Reynal and Broderick (2005). Separated phases were frozen, freeze dried, and then ground through a Wiley mill (1-mm screen; Arthur H. Thomas, Philadelphia, PA). These samples were analyzed for Cr, Co and Yb concentrations (Reynal and Broderick, 2005) using direct current plasma emission spectroscopy (Combs and Satter, 1992; Spectro CirOS VISION ICP Model FVS12, Spectro Analytical Instruments, Mahwah, NJ).

Additional digesta samples were collected from the omasum and duodenum (250 mL) at 8 h intervals during the 48 h collection described above and stored at 4°C. Samples from these six sampling times were pooled by cow and period and bacteria were isolated by differential centrifugation (Reynal and Broderick (2005). Resulting composites of particle-associated bacteria (PAB) and fluid-associated bacteria (FAB) were stored at -20°C and subsequently freeze-dried and ground with a mortar and pestle prior to analysis.

Freeze dried and ground ruminal samples from d 10 (background),, digesta phase samples, PAB and FAB were analyzed for ^{15}N enrichment of NAN (Hristov et al., 2001). Total N concentration in omasal and duodenal digesta samples (LP, SP and FP) was determined by combustion using a Perkin-Elmer 2410 Series II N analyzer (Perkin-Elmer, Norwalk, CT). Gross energy of omasal and duodenal digesta phase samples was determined by bomb calorimetry (model 1271, Parr Instruments, Moline, IL).

Calculations

Nutrient flows to the omasum and duodenum were calculated from the Co and Yb markers as described by France and Siddons (1986). Microbial N in the fluid phase (MNFP, g/g as is basis) was calculated as:

$$MNFP = NFP \times (^{15}N\ enrichment\ FP\ /\ ^{15}N\ enrichment\ FAB),$$

where NFP and FAB represented fluid phase N content, (g/g as is basis) and fluid associated bacteria respectively. Concentrations of MN in the particulate phase (MNPP, g/g as is basis) were calculated in a similar manner from the small and large particle phases. Microbial N (MN, g/g as is basis) in the digesta was calculated as the sum of MNFP and MNPP. Peptide-N plus AA-N (mg/dl) was calculated as TCA soluble N minus ammonia N. Ruminal N balance (g/d) represents the net balance of N flux between blood and the rumen and was calculated as:

$$Ruminal\ N\ Balance\ (RNB) = Intake\ N - Total\ ruminal\ N\ outflow.$$

Digestibility coefficients of nutrients were calculated in the following manner using DM digestibility as an example.

$$TTDC = (DM\ intake - Fecal\ output\ of\ DM)\ /\ DM\ intake,$$

$$RDCa = (DM\ intake - Ruminal\ outflow\ of\ DM)\ /\ DM\ intake,$$

$$RDCt = (DM\ intake - (Ruminal\ outflow\ of\ DM - Ruminal\ outflow\ of\ microbial\ DM))\ /$$
$$DM\ intake,$$

$$PRDC = (Ruminal\ out\ flow\ of\ DM - Fecal\ output\ of\ DM)\ /\ Ruminal\ outflow\ of\ DM$$

where TTDC (kg digested / kg total) represented the apparent total tract digestibility coefficient, RDCa (kg digested / kg total) represented the apparent fractional ruminal digestibility coefficient, RDCt (kg digested / kg total) represented the true ruminal digestibility coefficient,

and PRDC (kg digested / kg total) represented the apparent post-ruminal digestibility

coefficient. Overall N efficiency (%) was calculated as:

$N\ efficiency = Milk\ N\ /\ Intake\ N \times 100,$

N balance (g/d) was calculated as:

$N\ balance = N\ intake\ -\ Milk\ N\ -\ Urinary\ N\ -\ Fecal\ N$

Statistical Analysis

The effects of varying dietary RDP on DMI, milk yield, milk components, and measures

of ruminal, omasal, and duodenal digesta, urinary and fecal excretion were analyzed with the

following statistical model using the MIXED procedure of SAS (V. 9.1; SAS Inst. Inc., Cary,

NC).

$Y_{ijkl} = \mu + Si + P_j(S_i)\ + C_k(S_i) + T_l\ + e_{ijkl,}$

where, μ = the overall mean, Si = the fixed effect of square (i = 1 or 2), $P_j(S_i)$ = the fixed effect

of period (j = 1….4) nested within square, $C_k(S_l)$ = the random effect of cow (k = 1……4) nested

within square, T_l = the fixed effect of treatment (l = 1….4), e_{ijkl} = residual error assumed to be

normally distributed. Repeated measures analyses were conducted on pH measurements with an

autoregressive order-one covariate structure. Preplanned contrasts were designed to test for

linear, quadratic and cubic effects of decreasing dietary RDP. Statistical significance was

declared at $P < 0.05$ and a trend was declared at $P \leq 0.10$, unless otherwise noted.

RESULTS

Nutrient Composition of Diets

Dietary treatments were similar to those reported in a prior study (chapter 3) except for

the use of dry ground corn in place of rolled high moisture corn grain. Observed chemical

composition of experimental diets and of the concentrate mixes and individual feed ingredients are presented in Table 4-2 and Table 4-3. Measured CP contents were 17.8, 16.9, 15.9, and 15.0 % of DM corresponding to formulated values of 18.4, 17.2, 15.9, and 14.7 % of dietary DM. Soluble protein content decreased in association with reduced dietary RDP content. Diets were similar in lignin, OM, and ND-ICP content.

Intakes and Production

Intake of DM, OM, ADF, NDF, starch, and energy were not affected by treatments (Table **4-4**). Nitrogen intake decreased linearly ($P < 0.01$) from 553 g/d to 448 g/d with decreasing dietary RDP. By design fat intake increased linearly with decreasing dietary RDP.

Milk production and milk composition were not affected by diet (Table 4-4). Milk protein yields were similar across treatments, but milk N yields decreased quadratically ($P < 0.04$) with decreasing dietary RDP. In association with decreasing dietary RDP from 11.3 to 7.6% of DM, MUN concentrations decreased linearly ($P < 0.03$) from 18.5 to 14.2 mg/dl. Nitrogen efficiency increased quadratically with decreasing dietary RDP. The highest N efficiency (40.8%) was observed in cows fed 8.8% dietary RDP. Milk fat yields decreased linearly ($P < 0.01$) with a trend for quadratic reduction ($P < 0.06$) with decreasing dietary RDP.

Urine and Feces

Fecal DM output was not affected by treatment, but urinary output decreased linearly from 20.4 to 13.9 kg/d as dietary RDP decreased (Table 4-5). Urinary N excretion decreased linearly ($P < 0.02$) from 214 to 155 g/d and N balance declined quadratically ($P < 0.05$) with decreasing dietary RDP. Fecal excretions of OM, N, ADF, NDF, fat and energy were not altered by treatments. Fecal starch excretion increased linearly ($P < 0.05$) from 148 to 177 g/d in association with feeding decreasing dietary RDP. Fecal DM, ADF, NDF, fat, starch, and energy

content were unaffected by treatments (Appendix Table 6-1). Fecal N content decreased linearly ($P < 0.05$) from 2.81 to 2.66% with decreasing dietary RDP.

Ruminal Fermentation and Nutrient Digestion

Feeding varying dietary RDP did not significantly alter concentrations of acetate, propionate, butyrate, or total VFA (Table 4-6). The ratio of acetate to propionate in ruminal fluid did not change significantly in association with varying dietary RDP. Ruminal pH decreased from 6.0, 1 h before feeding to 5.4, 4 h after feeding (Appendix Figure 6-1). However no significant ruminal pH changes were observed for cows fed different treatment diets (Table 4-6).

Ruminal DM, ADF, fat, starch, and energy content were not significantly different across treatments (Table 4-6). As dietary RDP decreased, the NDF content of ruminal contents increased linearly ($P < 0.01$) from 54.8 to 57.9% of DM and N content and pool size varied quadratically ($P < 0.02$ and $P < 0.05$, respectively) with the lowest values observed for the 10.1 and 8.8% RDP diets. Least squares means of ruminal NH_3-N concentrations decreased from 14.9 to 5.5 mg/dl ($P < 0.01$) from cows fed high to low dietary RDP (Table 4-6). Peptide plus AA -N concentrations in ruminal fluid were unaffected by decreasing dietary RDP although there was a linear numerical increase.

The proportion of omasal digesta segregating into different phases remained similar across treatments (Appendix Table 6-2). As dietary RDP decreased nutrient composition of the different phases for omasal samples were similar except for starch in the FP ($P < 0.01$) and NDF in the SP ($P < 0.02$). The proportion and nutrient composition of different phases of duodenal digesta except duodenal fat percentage ($P < 0.04$) were not significantly different with decreasing dietary RDP (Appendix Table 6-3).

Marker and Site Comparison

Ruminal outflows of nutrients were calculated using single, double and triple marker methods. Single marker calculations using INDF, Cr and Yb are presented in (Appendix: Table 6-4, Table 6-5 and Table 6-6). Dry matter outflow calculated using Cr was higher and that calculated using INDF was lower than that found using the double marker calculation but was not significantly different. The double marker method (Co and Yb; Table 4-9) and Yb single marker method gave results comparable to previously published reports. The triple marker method, presented in appendix (Table 6-7), gave biologically improbable nutrient flow results. Results of comparison of omasal and duodenal flows of nutrients using double marker method (Co and Yb) and single marker method (Yb or Cr or Co or INDF) are presented in Table 4-7. Passage of nutrients to the duodenum was numerically higher than nutrients flowing out of the omasum and DMI.

Ruminal Outflows of Nutrients

Nutrient flow calculations using the double marker method (Co and Yb) indicated that total N flow out of the rumen, which includes NAN and NH_3-N, tended ($P < 0.09$) to decline from 587 g/d to 469 g/d as dietary RDP was lowered from 11.3 to 7.6% of DM (Table 4-8). Similarly decreasing dietary RDP tended ($P < 0.08$) to decrease NAN flow (microbial N plus non-ammonia, non-microbial N (NANMN)). Non-ammonia N flows out of the rumen ranged from 562 to 461 g/d for high to low dietary RDP. Total N or NAN flows expressed as a percentage of N intakes did not indicate any significant differences among cows fed varying dietary RDP. Decreasing dietary RDP decreased microbial N flow from the rumen from 289 to 193 g/d ($P < 0.09$) indicating a trend towards significance. Varying dietary RDP did not significantly influence NANMN (dietary plus endogenous NAN) flows out of the rumen of these

cows. Microbial N or NANMN expressed as a percentage of NAN was not affected by treatments. Microbial N production per kg of OM apparently digested in the rumen was not significantly affected by changes in dietary RDP. Ruminal N balance was unaffected by changes in dietary RDP. The double marker method (Co and Yb) demonstrated that ruminal outflows of DM, OM, ADF, NDF, fat, starch and energy were not significantly different by feeding dairy cows with decreased dietary RDP (Table 4-9).

Digestibility of Nutrients

Treatment diets did not affect RDCt or RDCa of N (Table 4-8) or other nutrients (Table 4-9). Similarly PRDC of N and other nutrients were not significantly affected by changes in dietary treatments (Table 4-8 and Table 4-9). A linear reduction in TTDC of N ($P < 0.01$), a quadratic tendency in TTDC of NDF ($P < 0.06$) and a linear tendency of starch ($P < 0.06$) were noticed when dietary RDP decreased from 11.3 to 7.6% (Table 4-9).

DISCUSSION

It was hypothesized that ruminal metabolism and nutrient flow from the rumen would be sustained, ruminal microbes would utilize more urea N from blood, and urinary N excretion would be reduced leading to improved animal efficiency at RDP concentrations below requirements. The hypothesis was tested by feeding lactating cows with reduced RDP content in diets and measuring passage of N and other nutrients out of the rumen and ruminal concentrations of ammonia-N, peptides and AA-N, VFA, and pH. Cows were also monitored for feed intake and milk production. In addition, N output in feces and urine were determined by total collection of feces and urine.

Marker Methods

Nutrient flows out of the rumen were calculated using single, double and triple marker methods. Comparison of results from these methods indicated that the double marker method using Co and Yb as markers of the liquid and particulate phases provided results comparable to previously published data and thus was assumed to be the most representative of the true flows and so that method of calculation was used for determining the effects of reduced dietary RDP on nutrient outflow from the rumen.

Ipharraguerre et al. (2007) reported that measurement of nutrient flows from the rumen to the lower digestive tract of dairy cows could vary significantly due to several methodological factors (e.g., microbial markers, site of sampling). According to France and Siddons (1986), when digesta is sampled from the digestive tract, it is difficult to get a representative sample that compromises calculations of digesta flow when a single flow marker is used. Use of multiple flow markers allows reconstitution of a true digesta sample. In the current study, results from the use of Cr and INDF as single markers gave results that appeared to be biologically improbable and thus sampling was likely unrepresentative. However, flows calculated using Co, Yb, and INDF in a triple marker system also generated flows that were clearly biologically improbable for a number of individual samplings. This might be due to difficulty in sampling corn kernels and large fiber particles by aspiration through the omasal sampler orifice (Ipharraguerre et al., 2007) that lead to errors in calculation of INDF concentration in the omasal true digesta. Flows of nutrients including DM, OM, ADF, NDF and N at the duodenum were numerically greater than the corresponding nutrient intakes and thus were also biologically improbable. This might be due to malfunction of closed T shape duodenal cannula (Faichney, 1980) used in the current study.

Microbial protein outflow measurement may be affected by sampling methods. Ipharraguerre et al. (2007) reported that omasal sampling might overestimate microbial NAN flow at the omasal canal due to the loss of large particles during sampling. Multiple markers used in the current study might not be able to correct for this bias.

Ruminal N metabolism and microbial growth

Microbial protein synthesis in the rumen is affected by dietary protein and energy levels (Clark, 1975 and Clark and Davis, 1980). Nitrogen availability for ruminal microbes is mainly in the forms of NH_3-N, peptide and AA-N. Linear reductions ($P < 0.09$) in ruminal microbial protein flow with decreasing dietary RDP might have been due to inadequate ruminally available N or ruminal dietary energy supply. Declining microbial N flow caused a similar trend in total N ($P < 0.09$) flows out of rumen because ruminal outflows of NANMN flows were unaltered. Linear reductions in microbial flow indicated that microbial growth was responsive to dietary RDP throughout the range tested, high RDP diets tested were insufficient and NRC recommendations for RDP were inadequate to support maximum microbial growth rates. Observed DM and fermentable OM intakes were not high in cows fed treatment diets which provided less ruminally available N. However, it would seem the ratio of the 2, which is related to the concentration of RDP, would be more important in determining a ruminal N deficiency than overall RDP intake.

The decline in microbial flow could have been caused by the linear decline in ruminal NH_3-N concentrations with decreasing RDP (Smith, 1979). The lowest concentration is equivalent to the 5 mg/dl reported by Satter and Slyter (1974) to be sufficient to maintain maximal microbial protein production. However, other researchers have found higher ruminal NH_3-N requirements for maximal microbial protein production. Kang-Meznarich and Broderick

(1980) and Reynal and Broderick (2005) reported that microbial protein synthesis was not maximized until ruminal NH_3-N concentrations exceeded 8.5 and 11.8 mg/dl, respectively, consistent with the observation in the current study.

Ammonia requirements of ruminal microbes are influenced by dietary characteristics (Chikunya et al., 1996). Erdman et al. (1986) observed that ruminal NH_3-N concentrations for maximum microbial protein synthesis and digestion increased with increasing fermentability of feed from less than 5 mg/dl for alfalfa hay- based diets to 25 mg/dl for ground corn and solvent SBM diets. Ruminal digestion of OM and energy were unaltered in cows as dietary RDP decreased. In addition, ruminal VFA concentrations and acetate: propionate ratios were unaffected. These observations demonstrated that there were no differences in feed fermentability among diets that could have caused the observed change in microbial growth rates. Therefore, ruminal NH_3-N requirements were not influenced by fermentability of feed among diets in the current study. Numerical reductions in OM intake and microbial yield per kg of fermented OM were observed as RDP was reduced. The latter suggests a reduction in efficiency as ruminal N supply is reduced. This might have led to changes in microbial growth without changes in fermented OM.

Ruminal pH 1 h before feeding averaged 6.0, and it averaged 5.7 before and after feeding across all treatments and neither were significantly different due to changes in diets. It is known that the efficiency of microbial protein synthesis remains constant within a wide range of ruminal pH (Bach et al., 2005) as optimal pH of ruminal proteolytic enzymes ranges from 5.5 to 7.0 (Kopecny and Wallace 1982). However, as pH decreases ruminal cellulolytic bacterial counts will be decreased (Endres and Stern, 1993) affecting fiber degradation, reducing access of proteolytic bacteria to protein and indirectly diminishing protein degradation (Bach et al., 2005).

Ruminal pH declined after feeding in the current study averaging 6.0 and 5.7 before and after feeding without significant changes in ruminal pH and fiber digestion with changes in diets. Thus pH did not cause the reduced rates of microbial growth.

Argyle and Baldwin (1989) observed that peptide and AA-N supply can affect microbial growth, and thus, a deficiency can precipitate a reduction in microbial protein synthesis. However, results indicated that peptide plus AA concentrations in ruminal fluid were unaltered with decreasing dietary RDP, suggesting that it did not cause the observed reduction in microbial growth. It is possible that specific peptides or AA required for maximal growth rates may have declined in concentrations without causing an overall decline in peptide plus AA concentrations.

Therefore, it can be concluded that ruminal AA and peptides, pH and energy were not contributing to the trend in decreased microbial protein outflow from the rumen. The likely cause of the decline in microbial protein flow from the rumen was the linear decrease in ruminal NH_3-N concentration associated with decreased dietary RDP.

Recycled N from blood to the rumen in the form of urea is a significant source of N for microbial protein production (Lapierre and Lobley, 2001). The calculated negative ruminal N balance in the current study indicates that the balance of NH_3-N absorption and urea N transfer from blood favors a net influx of N into the rumen. Urea-N transfer into the rumen has been observed to be negatively correlated with ruminal NH_3-N concentrations (Kennedy and Milligan, 1980). However, decreasing ruminal NH_3-N concentrations did not result in a decrease in ruminal N balance indicating that the net balance of urea-N and NH_3-N transfer were unaffected. However, given the linear declines in both ruminal NH_3-N and blood urea N (indicated by milk urea; Oltner and Wiktorsson, 1983) concentrations, it is possible that the stimulatory affect of reduced ruminal NH_3-N concentrations on urea transfer were offset by reduced blood urea

concentrations resulting in no change. Results from the current study contradicted Remond et al. (1993) reports of positive relationship between ruminal N balance and ruminal ammonia concentrations in sheep. This may be due to inherent differences between sheep and cattle or other contributing factors such as diet.

Ruminal Digestion of Other Nutrients and Rumen Pool Sizes

Early evidence suggests that ruminal NH_3-N concentrations of 3.3 mg/dl were adequate to support maximum ruminal digestion of OM in nonlactating cows (Kang-Meznarich and Broderick, 1981). These results were consistent with recent reports of Reynal and Broderick (2005) and Boucher et al. (2007) and the current study. Feeding decreasing dietary RDP did not alter ruminal digestion flow of OM, fiber and energy from the rumen and ruminal VFA concentrations suggesting that treatment diets were adequate to support maximal rates of ruminal digestion of OM including fiber.

Ruminal outflow of starch was low resulting in a very high RDCA (0.91) of starch. Excess fermentation of starch in the rumen leads to excess VFA production, affects buffering and nutrient absorption, reduces pH and causes depressed DMI (Robinson and Kennelly, 1989; McCarthy et al., 1989). In the current study DMI was not depressed due to the effects of excess starch fermentation suggesting overestimation of starch digestibility. In addition, excess starch fermentation can cause low digestibility of NDF (Grant and Mertens, 1992). However, no reduction in ruminal NDF digestibility was noticed in the current study. Thus the high starch digestibility might be due to other reasons. Data compiled by Reynolds et al. (1997) demonstrated that on average 48% of starch intake was apparently digested in the rumen of lactating dairy cows. However, segregation of corn kernels during omasal sampling can result in underestimation of starch flows and overestimation of starch digestibility (86%) in the rumen

(Ipharraguerre et al., 2007). In the current study, corn kernels occasionally lodged in the inlet port on the omasal sampler despite the use of a larger bore sampling hole (16 mm diameter) than originally specified. These corn kernels likely derived from the corn silage as all other corn was finely ground. Use of a kernel processor on the forage harvestor may prevent such problems. If there was sampling bias, the effects should have been constant across treatments and thus would not bias interpretation of treatment effects.

Ruminal fiber digestibility in the current study appeared to be normal with fiber digestibilities ranging between 42 and 55% for NDF. Fiber digestion by ruminal bacteria can also be adversely affected when ruminal NH_3-N concentrations are low (Firkins et al., 1986) in association with feeding protein with a low degradability (Stock et al.1981). Major fiber digesting bacterial populations in the rumen (*Ruminococcus albus*, *R. flavefaciens* and *Fibrobactor succinogenes;* Forsberg et al., 1997) are susceptible to an NH_3-N deficiency. In the present study, ruminal outflows and RDCa of NDF and ADF were not altered by dietary treatments, suggesting that the reduced ruminal NH_3-N concentrations were not low enough to compromise fiber digesting bacteria growth and activity. This may be explained by the fact that the lowest ruminal NH_3-N concentrations were higher than the minimum ruminal NH_3-N for maximum fiber digestion suggested by Kang-Meznarich and Broderick (1981).

Ruminal digesta contents were not significantly different across treatments further supporting the conclusion that ruminal digestion was not altered. If digestion of fiber is reduced, an increase in the size of the ruminal fiber pool would be expected which can lead to depressed DMI (Allen, 2000). However, no significant reductions in DMI were noticed in the current study. It can be concluded that neither ruminal digestion of OM, fiber and energy nor ruminal pool sizes were significantly altered due to decreased ruminal N availability.

Post-ruminal and Total Tract Digestion and Absorption

Post-ruminal digestibility coefficients of nutrients were not significantly altered with changes in dietary treatment suggesting that PRDC were not significantly influenced by dietary RDP. Christensen et al. (1993) reported that post-ruminal and total tract apparent digestibilities of OM, starch, and NDF were not affected by the amount of CP in the diets and apparent post-ruminal digestibility of N increased with increasing concentrations of dietary CP. In the present study, with decreasing dietary RDP linear reductions in TTDC of N was observed without significant changes in RDCa and PRDC. When dietary CP fed to dairy cows was increased, apparent N digestibility was increased as a result of dilution of metabolic fecal N and greater intake SBM a highly digestible protein source (Broderick, 2003). As dietary N intake decreased in the current study with decreasing dietary RDP protected SBM content in diets was increased. The greater intake of highly digestible SBM in higher RDP diets might have caused significantly greater apparent TTDC of N in higher RDP diet fed cows. There was no clear indication whether this reduction occurred in the rumen or postruminally.

When dietary RDP decreased there was a decreasing linear trend in TTDC of starch ($P < 0.06$) along with linear increase in fecal starch excretion. However, RDCa and PRDC of starch were unaffected. Wheeler et al. (1975) reported that when diets containing corn were fed to cattle significant amounts of starch was excreted in feces. In the current study increased fecal starch excretion might be due to increased concentration of corn in the diet with decreasing dietary RDP.

Broderick and Reynal, (2009) reported greater total TTDC of NDF with increasing dietary lignosulfonate-treated SBM. Protected SBM that was high in ND-ICP would escape rumen digestion but still be digestible in intestine (Fox et al., 2004) perplexing ruminal and total

tract digestibilities of NDF. A similar dietary strategy was used herein, however, the protected SBM used for this work was not generated by a browning reaction as is the lignosulfonate treated product. The NDF, ADF, AD-ICP, and ND-ICP contents were all similar between the protected and unprotected SBM. It is unclear why there was a quadratic trend ($P < 0.06$) for a change in the TTDC of NDF.

Post ruminal digestion of ADF and NDF was not significantly different from 0 which is less than expected (Christensen et al., 1993). Omasal sampling of larger fiber particles may have also been unrepresentative as noted for starch. However, the sampling port was much larger than the threshold particle size for passage from the rumen (Poppi et al., 1980), and thus this would not seem to be a valid reason for the observations.

A declining linear trend in total ruminal N outflow ($P < 0.09$), suggested that essential AA available for absorption in the small intestine was affected. However, milk protein output was unaffected which was consistent with observations in Chapter 3. Amino acids absorbed from the small intestine are required for milk protein synthesis, and microbial protein is a major source of AA for absorption in the small intestine (Clark et al., 1992). Previous reports indicated that effects on milk protein synthesis due to RDP deficiency may be compensated by increases in RUP flow to the intestine (Santos et al., 1998). However, in the current study, as dietary RDP declined dietary RUP remained constant across treatments. Thus, decreasing total N reaching the small intestine due to decreased microbial N flow might decrease AA available for absorption in the intestine.

Milk Production, N Balance, N Excretion and N Efficiency

Milk production, milk protein content, and milk protein yield were not affected by dietary RDP despite the apparent reduction in N flow to the small intestine and the reduction in TTDC

for N. This suggests the cows were fed metabolizable protein in excess of their requirements when consuming the 10.1% RDP diet, they drew upon body reserves to buffer the deficiency, or they improved post-absorptive efficiency of AA use. However, feeding the same diet to a larger herd of cows in a previous study (Chapter 3) a trend of loss in milk yield was observed. Similarly, Kalscheur et al. (2006) reported linear reductions in milk and milk protein yields with decreasing dietary RDP from 11 to 6.8 % of DM suggesting that production was compromised by lack of protein supply. However, in the above study dietary RUP was declined along with dietary RDP and thus it was not clear whether the loss in production was due to reduced RDP or RUP.

Energy supply, if restricted, will cause a rapid decline in milk yield (Carlson et al., 2006). In the current study, dry ground corn grain and tallow contents were increased 1.4 and 1.8%, respectively, from high to low RDP diets to make the diets isoenergetic. Dry matter intake, apparent digestible energy as well as milk yield in the current study were not affected by dietary treatments, indicating energy supplies to these cows were not compromised.

Calculated N balance tended to decline linearly ($P < 0.1$) from positive to negative values in response to decreased N supply suggesting the loss of microbial N flow was at least partially buffered by mobilization of body protein (Botts et al., 1979). This suggested that animals were trying to meet essential AA requirements for milk protein synthesis by using protein from tissues (Swick and Benevenga, 1977). As total N flow out of rumen trended to decline linearly with decreasing dietary RDP, MUN and urinary N output decreased linearly indicating that reductions in N supply led to reduced protein catabolism. Although we hypothesized the rumen would have improved N efficiency when RDP was reduced, the lack of a change in ruminal N balance indicates N losses from the rumen were constant across diets.

Therefore the gains in animal N efficiency and reductions in urinary N output must have derived from improved post-absorptive efficiency.

When dietary RDP decreased, urinary output was reduced in the current study. This result was in agreement with previous studies where a reduction in urine volume was observed with reductions in dietary CP (Broderick, 2003; Wattiaux and Karg, 2004). Daily urine production depends on intakes of digestible Na, K, and N and their excretion in milk and urine (Bannink et al., 1999). In addition to urinary volume, urinary N output were decreased, as fewer AA were catabolised when decreasing dietary RDP supplied decreased amounts of N at the intestine for absorption. Reductions in urinary N would decrease urea excretion. Urea, the major urinary N component, is rapidly converted to ammonia, which contributes to environmental N loading (Varel et al., 1999). Evidence from other studies supports linear decreases in urinary N excretion with decreasing dietary CP levels (Davidson et al., 2003; Castillo et al., 2001). Decreasing dietary RDP and N intake did not alter fecal N excretion, demonstrating that excess N was mainly channeled to urine for excretion. Fecal N output (g/d) results are consistent with the observations of Hristov et al. (2004) who observed no relationship between fecal N output (g/d) and dietary RDP. Thus, decreasing dietary RDP decreases ammonia emissions from dairy operations by reducing urinary N excretion.

As dietary RDP was reduced, N efficiency was increased linearly ($P < 0.02$) indicating increased efficiency of N use for milk N secretion. Apparent N efficiency was reported to increase by feeding decreasing dietary RDP in previous studies (Chapter 3; Kalscheur et al., 2006). When dietary RDP declined, there was a linear decline in N intake and consistent milk protein output resulting in greater N efficiency. Results demonstrated that the highest N efficiency (40.8%) was for 8.8% RDP diet fed cows while 7.6% dietary RDP fed cows

responded differently to cope up with the N deficiency and yielded less milk N. Increasing N efficiency by decreasing dietary RDP reduces N output into surroundings. Thus, increased N efficiency in dairy cows reduces atmospheric N load and ammonia pollution (Tamminga, 1992). If the numerical reductions in milk yield from the 10.1% RDP diet to the 7.6% RDP diet are real, 16% more cows would be required on the lower protein diet to achieve the same milk output. This would negate a significant proportion of the gain in animal efficiency due to the need to milk more animals to supply national demand resulting in only a 4.5% reduction in urinary N output per unit of milk produced (St-Pierre and Thraen, 1999). However, if the 8.8% RDP could be fed without loss of production, a 12% reduction in urinary N output would be realized with no additional animals required.

CONCLUSIONS

Decreasing dietary RDP from 11.3 to 7.6% of DM in lactating dairy cows tended to decrease ruminal outflows of microbial N and total N and decrease N balance. It did not alter ruminal N balance, OM digestion, fiber digestion or energy supply to the animal. Of the major determinants of microbial flow, ruminal NH_3-N levels were the most likely to have contributed to the observed trend for a decline in microbial N flow out of the rumen as dietary RDP decreased. As dietary RDP was decreased, N efficiency was improved and significant reductions in urinary N excretion were observed.

ACKNOWLEDGMENTS

Table 4-1. Formulated composition of experimental diets.

Ingredients	RDP, % of diet DM[1]			
	11.3	10.1	8.8	7.6
	(% of DM)			
Corn silage	39.9	40.0	40.0	40.1
Mix grass + Legume silage	7.9	7.9	7.9	7.9
Whole linted cottonseed	2.9	2.9	2.9	2.9
Soybean hulls	9.6	11.5	13.5	15.4
Soybean meal, solvent-extracted(48% CP[2])	20.3	13.5	6.8	0.0
Protected soybean meal[3]	0.0	4.0	7.9	11.9
Ground dry corn grain	16.9	17.4	17.8	18.3
Tallow	0.9	1.2	1.6	1.9
Calcium carbonate	0.5	0.4	0.4	0.3
Dicalcium phosphate[4]	0.0	0.1	0.2	0.3
Sodium bicarbonate	0.2	0.2	0.2	0.2
Salt	0.5	0.5	0.5	0.5
Trace mineral and vitamin mix[5]	0.4	0.4	0.4	0.4
NRC Estimates[6]				
RDP, % of DM	11.3	10.1	8.8	7.6
RUP[7], % of DM	7.1	7.1	7.1	7.1
NFC[8], % of DM	43.1	43.0	42.9	42.8
NDF[9], % of DM	30.1	31.1	32.0	33.0
ADF[10], % of DM	20.4	21.1	21.8	22.5
Crude fat, % of DM	4.1	4.6	5.1	5.6
NE_L[11] Mcal/kg	1.6	1.6	1.6	1.6
RDP supplied, g/d	2576	2295	2013	1732
RDP required, g/d	2297	2305	2314	2322
RDP balance, g/d	279	-11	-300	-590
RUP supplied, g/d	1637	1631	1626	1620
RUP required, g/d	1239	1380	1520	1661
RUP balance, g/d	399	252	105	-42
MP supplied[12], g/d	2777	2663	2548	2434
MP allowable milk, kg/d	44.0	41.2	38.3	35.5

[1]Ruminally degradable protein (RDP), % of diet dry matter (DM) according to national research council (NRC 2001).
[2] CP= Crude protein
[3] Hivap®, Land O' Lakes/Purina Feed, Statesville, NC
[4] Contained 22% Ca and 19.3% P.
[5]Land O' Lakes/Purina Feed, Statesville, NC; formulated to provide (per kg of DM) 25×10^5 IU of vitamin A, 400,000 IU of vitamin D, and 10×10^5 IU of vitamin E, 0.1 mg of Co, 12 mg of Cu, 0.7 mg of I, 60 mg of Fe, 48 mg of Mn, 48 mg of Zn, 0.3 mg of Se.
[6]calculated using the NRC model (2001) and observed input values.
[7]RUP = Ruminally undegraded protein.
[8]NFC = Non-fiber carbohydrate.

[9]NDF = Neutral detergent fiber.
[10]ADF = Acid detergent fiber.
[11]NE_L = Net energy lactation.
[12] Metabolizable protein (MP) supplied: assumes microbial yields are compromised by an RDP deficiency.

Table 4-2. Observed chemical composition of experimental diets.

Item	RDP, % of diet DM[1]			
	11.3	10.1	8.8	7.6
DM, % of diet	49.7	48.2	47.6	48.7
CP[2], % of DM	17.8	16.9	15.9	15.0
Soluble protein, % of CP	35.6	34.6	33.6	32.6
ND-ICP[3], % of CP	1.1	1.1	1.2	1.1
AD-ICP[4], % of CP	2.3	2.4	2.5	2.5
OM[5], % of DM	93.4	93.5	93.6	93.7
NDF[6], % of DM	34.6	34.9	35.1	35.4
ADF[7], % of DM	19.9	20.3	20.7	21.1
Lignin, % of DM	2.2	2.1	2.1	2.1
Ether extract, % of DM	4.6	5.2	5.8	6.4

[1]Ruminally degradable protein (RDP) % of diet dry matter (DM).
[2]CP = Crude protein.
[3]ND-ICP = Neutral detergent insoluble crude protein.
[4]AD-ICP = Acid detergent insoluble crude protein.
[5]OM = Organic matter.
[6]NDF = Neutral detergent fiber.
[7]ADF = Acid detergent fiber.

Table 4-3. Observed chemical composition of individual ingredients in the experimental diet.

Item	High RDP Mix. [1]	Low RDP Mix.	Corn/SH Mix.	Corn silage	Haylage	Cotton Seed	SBM[2]	SH[3]	Protected SBM[4]
DM[5], % of feed	86.8	88.1	87.6	32.3	47.9	87.7	87.3	89.0	91.1
OM[6], % of DM	90.8	92.1	95.5	96.2	88.4	96.0	92.8	94.5	93.1
NDF[7], % of DM	19.6	21.9	52.9	39.0	47.5	46.1	7.0	62.7	6.4
ADF[8], % of DM	12.9	16.2	38.5	23.9	38.2	34.2	3.9	44.7	4.7
Lignin, % of DM	1.4	0.9	1.9	1.7	6.3	9.6	0.9	2.1	0.7
CP[9], % of DM	29.6	21.9	12.9	8.5	18.3	19.1	54.8	14.4	50.9
Soluble protein, % of CP	18.9	10.7	21.9	52.5	55.8	17.8	15.8	26.5	5.7
ND-ICP[10], % of CP	2.0	2.6	3.6	1.8	4.1	2.7	0.9	4.4	0.8
AD-ICP[11], % of CP	0.7	0.9	1.2	1.2	1.4	1.9	0.7	0.7	0.8

[1] RDP = Ruminal degradable protein.
[2] SBM = soybean meal.
[3] SH = soyhulls.
[4] Protected SBM = HiVap®, Land O' Lakes/Purina Feed, Statesville, NC.
[5] DM = Dry matter.
[6] OM = Organic matter.
[7] NDF = Neutral detergent fiber.
[8] ADF = Acid detergent fiber.
[9] CP = Crude protein.
[10] ND-ICP = Neutral detergent insoluble CP.
[11] AD-ICP = Acid detergent insoluble CP.

Table 4-4. Least squares means for intakes and milk production and N efficiency of dairy cows fed experimental diets.

Item	RDP, % of diet DM[1]				SEM	Contrasts[2]	
	11.3	10.1	8.8	7.6		L	Q
Intakes						——— (P <) ———	
DM, kg/d	19.4	18.5	18.1	18.6	0.70	0.31	0.17
OM[3], kg/d	18.1	17.2	16.9	17.5	0.65	0.36	0.17
N, g/d	553	498	460	448	18.4	0.01	0.13
ADF[4], kg/d	3.85	3.75	3.74	3.93	0.14	0.65	0.19
NDF[5], kg/d	6.69	6.43	6.34	6.60	0.24	0.68	0.18
Fat, g/d	888	959	1044	1183	38.5	0.01	0.27
Starch, kg/d	4.41	4.19	4.08	4.19	0.16	0.17	0.17
GE[6], Mcal/d	82	78	77	79	3.0	0.34	0.19
Milk Production							
Milk yield, kg/d	32.7	34.5	34.1	29.8	2.10	0.33	0.14
Milk true protein, %	2.83	2.78	2.80	2.79	0.09	0.67	0.70
Milk fat, %	4.15	3.57	4.06	3.53	0.38	0.45	0.94
Milk lactose, %	4.79	4.68	4.79	4.71	0.13	0.75	0.88
MUN[7], mg/dl	18.5	17.7	14.2	14.2	1.55	0.03	0.80
Milk true protein, kg/d	0.92	0.95	0.95	0.83	0.06	0.28	0.15
Milk fat, kg/d	1.25	1.24	1.20	0.97	0.06	0.01	0.06
Milk N, g/d	161	180	178	150	10.1	0.47	0.04
N efficiency[8], %	28.3	37.2	40.8	33.2	1.63	0.02	0.01

[1]Ruminally degradable protein (RDP), % of diet dry matter (DM).
[2]Contrasts: L = linear, Q = quadratic.
[3]CP = Crude protein.
[4]NDF = Neutral detergent fiber.

[5]ADF = Acid detergent fiber.
[6]GE = Gross energy.
[7]MUN = Milk urea nitrogen.
[8]N efficiency = Milk N ×100 / Intake N.

Table 4-5. Least squares means of feces and urine excretions, digestible energy and N balance of dairy cows fed experimental diets.

Item	RDP, % of diet DM[1]					Contrasts[2]	
	11.3	10.1	8.8	7.6	SEM	L	Q
Fecal excretion							
DM, kg/d	5.54	5.72	5.85	5.80	0.31	0.50	0.69
OM[3], kg/d	4.97	5.15	5.22	5.22	0.29	0.49	0.73
N, g/d	157	159	156	155	9.32	0.86	0.82
ADF[4], kg/d	2.18	2.34	2.40	2.28	0.14	0.61	0.36
NDF[5], kg/d	2.82	3.12	3.24	3.16	0.20	0.19	0.30
Fat, g/d	239	242	244	273	29.4	0.22	0.48
Starch, g/d	148	147	167	177	21.9	0.05	0.62
GE[6], Mcal/d	23.1	23.8	24.3	24.1	1.40	0.53	0.71
Urine							
Output, kg/d	20.4	16.6	14.3	13.9	1.06	0.01	0.14
Urine N, g/d	214	188	165	155	15.3	0.02	0.62
DE[7], Mcal/d	58.8	54.4	51.8	55.1	3.20	0.21	0.12
N balance[8], g/d	23.1	-25.7	-45.3	-15.3	20.2	0.10	0.05

[1]Ruminally degradable protein (RDP) % of diet dry matter (DM).
[2]Contrasts: L = linear, Q = quadratic.
[3]OM = Organic matter.
[4]ADF = Acid detergent fiber.
[5]NDF = Neutral detergent fiber.
[6]GE = Gross energy.
[7]DE (Apparent digestible energy) = Gross energy intake – Fecal energy output.
[8]N balance= Intake N - Milk N - Urinary N - Fecal N.

Table 4-6. Least squares means for ruminal metabolism, composition and pool size of dairy cows fed experimental diets.

	RDP, % of diet DM[1]					Contrasts[2]	
Item	11.3	10.1	8.8	7.6	SEM	L	Q
Ruminal metabolism						$(P<)$	
NH_3-N, mg/dl	14.9	11.5	8.03	5.52	0.83	0.01	0.55
Peptide+AA–N[3], mg/dl	7.19	8.69	10.9	11.4	2.03	0.15	0.81
pH	5.71	5.69	5.62	5.71	0.07	0.82	0.34
Total VFA[4], mM	101	110	95.3	100	7.83	0.65	0.76
Acetate (A), mM	61.9	65.4	54.2	64.6	6.44	0.93	0.60
Propionate (P), mM	26.5	29.8	26.6	27.0	2.73	0.90	0.61
Butyrate, Mm	11.5	14.6	13.4	13.5	1.17	0.39	0.23
A:P ratio	2.32	2.23	2.06	2.41	0.16	0.88	0.14
Composition of Ruminal Contents							
DM, % of digesta	17.8	19.0	18.3	18.9	0.45	0.28	0.57
N, % of DM	3.10	2.77	2.71	2.96	0.16	0.35	0.02
Ash, % of DM	6.84	6.31	6.78	6.33	0.16	0.17	0.79
ADF[5], % of DM	36.1	36.5	38.8	38.1	1.46	0.28	0.69
NDF[6], % of DM	54.8	57.0	57.4	57.9	0.56	0.01	0.13
Fat, % of DM	6.35	6.15	6.68	6.43	0.22	0.46	0.90
Starch, % of DM	2.90	2.86	2.64	3.44	0.63	0.63	0.48
Energy, kcal/g DM	4.48	4.47	4.44	4.48	0.03	0.66	0.34
Ruminal Pool Sizes							
Digesta, kg	11.9	11.5	11.3	13.5	1.09	0.18	0.11
OM[7], kg	11.1	10.7	10.6	12.6	1.05	0.17	0.11
N, g	390	318	310	400	39.2	0.87	0.05
ADF, kg	4.45	4.17	4.42	5.12	0.41	0.17	0.19
NDF, kg	6.51	6.49	6.47	7.80	0.64	0.06	0.12
Fat, g	758	712	752	860	78.1	0.19	0.21
Starch, g	334	333	341	515	123	0.35	0.48
GE[8], Mcal/g DM	53	51	50	60	4.82	0.16	0.09

[1]Ruminally degradable protein (RDP) % of diet dry matter (DM).
[2]Contrasts: L = linear, Q = quadratic.
[3]AA-N = Amino acid N.

[4]VFA   = Volatile fatty acid.
[5]ADF   = Acid detergent fiber.
[6]NDF   = Neutral detergent fiber.
[7]OM   = Organic matter.
[8]GE   = Gross energy.

Table 4-7. Comparison of least squares means of nutrients passage to the omasum and duodenum using a double marker (Co and Yb) method and single marker (Yb or Cr or Co or INDF) methods.

Item	Omasum	Duodenum	SEM	($P <$)
DMM[1], (Co & Yb)				
DM[2], kg/d	16.8	22.0	2.06	0.37
OM[3], kg/d	13.7	18.5	1.53	0.35
ADF[4], kg/d	4.68	5.69	0.72	0.75
NDF[5], kg/d	6.75	7.66	1.00	0.67
N, kg/d	0.73	0.76	0.04	0.94
SMM[6], Yb				
DM, kg/d	17.7	23.5	1.90	0.48
OM, kg/d	15.2	19.4	1.73	0.45
ADF, kg/d	4.46	5.45	0.55	0.68
NDF, kg/d	6.42	7.41	0.71	0.59
N, kg/d	0.78	0.81	0.06	0.87
SMM, Cr				
DM, kg/d	22.5	23.7	2.49	0.74
OM, kg/d	19.3	20.2	2.14	0.84
ADF, kg/d	5.36	5.86	0.69	0.97
NDF, kg/d	7.74	7.79	0.84	0.83
N, kg/d	1.00	0.87	0.14	0.86
SMM, Co				
DM, kg/d	15.0	17.2	1.06	0.55
OM, kg/d	12.8	15.3	0.85	0.82
ADF, kg/d	3.77	4.40	0.41	0.92
NDF, kg/d	5.40	5.94	0.56	0.99
N, kg/d	0.65	0.65	0.03	0.39
SMM, INDF[7]				
DM, kg/d	8.46	10.5	1.06	0.67
OM, kg/d	7.19	8.57	0.97	0.69
ADF, kg/d	2.12	2.40	0.32	0.62
NDF, kg/d	3.03	3.23	0.44	0.71
N, kg/d	0.37	0.35	0.04	0.32

[1]DMM = Double marker method.
[2]DM = Dry matter.
[3]OM = Organic matter.
[4]ADF = Acid detergent fiber.
[5]NDF = Neutral detergent fiber.
[6]SMM = Single marker method.
[7]INDF = indigestible NDF.

Table 4-8. Least squares means for N flow out of the rumen and N digestibility of dairy cows fed experimental diets. Flows were calculated using double marker (Co and Yb) method.

Item	RDP, % of diet DM[1]				SEM	Contrast[2]	
	11.3	10.1	8.8	7.6		L	Q
						── (P <) ──	
Total N, g/d	587	546	576	469	37.5	0.09	0.38
RNB[3], g/d	-30.3	-48.5	-133	-22.0	36.4	0.74	0.11
NAN[4], g/d	562	517	524	461	51.6	0.08	0.89
MN[5], g/d	289	245	223	193	36.1	0.09	0.85
NANMN[6], g/d	257	267	300	262	33.8	0.76	0.51
Ammonia N, g/d	27.0	30.0	43.2	5.5	29.5	0.72	0.50
Total N, % of N Intake	106	112	128	105	9.35	0.75	0.15
NAN, % of N Intake	104	107	125	108	6.0	0.35	0.16
NANMN, % of NAN	42.6	44.1	54.0	53.9	5.21	0.14	0.89
MN, % of NAN	57.4	55.9	46.0	46.1	5.21	0.14	0.88
MN g/kg OM	25.6	24.5	21.8	19.7	3.70	0.30	0.90
RDCa[7], kg digested/kg intake	-0.06	-0.14	-0.33	-0.05	0.14	0.74	0.15
RDCt[8], kg digested/kg intake	0.51	0.45	0.23	0.45	0.07	0.28	0.16
PRDC[9], kg digested/kg PRF[10]	0.68	0.66	0.73	0.55	0.05	0.27	0.21
TTDC[11], kg digested/kg intake	0.71	0.68	0.66	0.65	0.02	0.01	0.28

[1]Ruminally degradable protein (RDP) % of diet dry matter (DM).
[2]Contrasts: L = linear, Q = quadratic.
[3]RNB (Ruminal N balance) = Intake N – Total ruminal N outflow.
[4]NAN = Non-Ammonia N.
[5]MN = Microbial N.
[6]NANMN = Non-ammonia non-microbial N.
[7]RDCa (fractional ruminal digestibility coefficient) = (Intake N – Total ruminal N outflow) / Intake N.
[8]RDCt (true ruminal digestibility coefficient) = (Intake N – (Total ruminal N outflow - Ruminal microbial N outflow)) / Intake N.
[9]PRDC (post-ruminal digestibility coefficient) = (Post-ruminal flow – Fecal out put) / Post-ruminal flow.
[10]PRF = Post-ruminal flow.
[11]TTDC (total digestive tract digestibility coefficient) = (Intake – Fecal output) / Intake.

Table 4-9. Least squares means for ruminal outflow and digestibility of nutrients in dairy cows fed experimental diets. Flows were calculated using double marker (Co and Yb) method.

Item	RDP, % of diet DM[1]					Contrast[2]	
	11.3	10.1	8.8	7.6	SEM	L	Q
DM						— ($P <$) —	
PRF[3], kg/d	12.4	12.4	11.7	11.9	0.59	0.36	0.86
RDCa[4], kg digested/kg intake	0.36	0.34	0.35	0.36	0.03	0.94	0.59
RDCt[5], kg digested/kg intake	0.54	0.49	0.52	0.54	0.04	0.95	0.43
PRDC[56], kg digested/kg PRF	0.47	0.48	0.50	0.46	0.02	0.70	0.30
TTDC[7], kg digested/kg intake	0.71	0.69	0.67	0.69	0.02	0.11	0.12
OM[8]							
PRF, kg/d	10.8	10.3	11.2	10.9	0.58	0.69	0.65
RDCa, kg digested/kg intake	0.40	0.41	0.32	0.37	0.03	0.28	0.52
PRDC, kg digested/kg PRF	0.52	0.48	0.54	0.50	0.02	0.93	0.99
TTDC, kg digested/kg intake	0.72	0.70	0.69	0.70	0.02	0.12	0.14
ADF[9]							
PRF, kg/d	2.43	2.72	2.22	2.01	0.39	0.42	0.57
RDCa, kg digested/kg intake	0.48	0.29	0.39	0.47	0.08	0.88	0.19
PRDC, kg digested/kg PRF	12.6	-1.03	-20.0	-2.05	13.7	0.36	0.26
TTDC, kg digested/kg intake	0.43	0.38	0.35	0.43	0.04	0.91	0.11
NDF[10]							
PRF, kg/d	3.63	3.84	3.78	3.45	0.45	0.80	0.58
RDCa, kg digested/kg intake	0.55	0.45	0.42	0.45	0.05	0.26	0.26
PRDC, kg digested/kg PRF	0.81	-4.95	-3.21	-2.01	3.15	0.66	0.27
TTDC, kg digested/kg intake	0.56	0.49	0.48	0.52	0.03	0.24	0.06
Fat							
PRF, kg/d	1.10	0.92	1.03	1.25	0.13	0.44	0.20
RDCa, kg digested/kg intake	-0.22	0.07	0.01	-0.08	0.15	0.61	0.25
PRDC, kg digested/kg PRF	0.76	0.77	0.76	0.79	0.04	0.70	0.75
TTDC, kg digested/kg intake	0.73	0.74	0.75	0.76	0.03	0.14	0.94
Starch							
PRF, g/d	415	437	401	402	42	0.70	0.80

RDCa, kg digested/kg intake	0.91	0.89	0.91	0.91	0.02	0.96	0.43
PRDC, kg digested/kg PRF	0.63	0.69	0.63	0.65	0.09	0.99	0.80
TTDC, kg digested/kg intake	0.97	0.96	0.96	0.96	0.01	0.06	0.97
GE[11]							
PRF, Mcal/d	53	55	48	47	4.0	0.17	0.62
RDCa, kg digested/kg intake	0.37	0.32	0.34	0.38	0.04	0.79	0.16
PRDC, kg digested/kg PRF	0.42	0.48	0.52	0.46	0.04	0.45	0.17
TTDC, kg digested/kg intake	0.72	0.70	0.68	0.69	0.02	0.16	0.19

[1]Ruminally degradable protein (RDP) % of diet dry matter (DM).

[2]Contrasts: L = linear, Q = quadratic.

[3]PRF = Post-ruminal flow.

[4]RDCa (fractional ruminal digestibility coefficient) = (Intake - Ruminal outflow) / Intake.

[5]RDCt (true ruminal digestibility coefficient) = (Intake – (Ruminal outflow - Ruminal microbial outflow) / Intake.

[6]PRDC (post-ruminal digestibility coefficient) = (Post-ruminal flow – Fecal output) / Post-ruminal flow.

[7]TTDC (total digestive tract digestibility coefficient) = (Intake – Fecal output) / Intake.

[8]OM = Organic matter.

[9]ADF = Acid detergent fiber.

[10]NDF = Neutral detergent fiber.

[11]GE = Gross energy.

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Chapter 5

Overall Conclusions

The hypothesis of the first study was that mid-lactation dairy cows would be able to maintain milk production and improve apparent N efficiency by feeding RDP below NRC (2001) recommendations. The first objective of this study was to assess effects of varying dietary RDP on feed intake, milk yield and milk composition, plasma essential AA and apparent N efficiency when dietary RDP was reduced and RUP held constant. The second objective was to test the NRC (2001) model for accuracy in predicting RDP requirements in mid-lactation dairy cows.

Results from this production study demonstrated that DMI was linearly decreased with a linear trend for reduced milk yield for the cows fed the lowest dietary RDP. This suggested that 7.6% dietary RDP might not have met ruminal microbial RDP requirements in this study. Milk protein, fat and lactose contents and milk protein yield were unaffected. Milk urea N concentration was linearly reduced by lowering dietary RDP indicating a lower AA catabolism. Plasma AA concentrations were unaffected by varying levels of RDP in diets. Decreasing concentrations of MUN would be predicted to result in reduced urinary N excretion. The apparent N utilization efficiency of milk production was linearly increased by feeding lower dietary RDP. The trend for lost milk production was consistent with NRC (2001) model recommendations for dietary RDP for these cows. The linear decrease in milk production will nullify the advantage of increased N use efficiency because 15% more cows will be required to produce the same quantity of milk.

The aim of the second experiment was to study digestion and excretion of nutrients in lactating dairy cows fed decreasing dietary RDP. Both the first and second studies used the same dietary RDP and RUP treatments. It was hypothesized that, when lactating dairy cows were fed

decreasing dietary RDP and constant RUP, ruminal outflows of nutrients and ruminal metabolism would be unaffected, less AA would be catabolized and N excretion as urinary N could be reduced without affecting milk production. The objectives of this study were to test the effects of varying dietary RDP on ruminal metabolism, ruminal digestion, microbial and total N flows out of the rumen and N efficiency and milk production and N excretion in urine and feces in lactating dairy cows.

Results from this study demonstrated that reducing dietary RDP significantly decreased ruminal NH_3-N concentrations, did not influence ruminal peptides plus amino acid levels and tended to decrease ruminal outflow of microbial and total N. Decreasing dietary RDP did not influence calculated ruminal N balance. Organic matter digestion and ruminal energy supply were unaltered by reducing dietary RDP suggesting that microbial energy requirements were met. Dietary RDP level did not alter ruminal fiber digestion indicating fiber digesting bacterial population was unaffected by changes in dietary RDP. Milk production and protein yield were unaffected. Increased body protein mobilization indicated by declining negative N balance and decreased AA catabolism demonstrated by decreasing MUN might have compensated the deficit in essential AA availability. Milk and fecal N outputs did not change but urine volume and urine N output were decreased significantly with lowering dietary RDP. This suggested that ammonia emission from urinary N would be decreased significantly by lowering RDP in dairy cow diets. Thus, feeding dairy cows with decreasing dietary RDP could significantly affect animal's N supply and could reduce urinary N output.

In conclusion, dairy cows could maintain digestion and reduce N excretion with lower dietary RDP than NRC (2001) recommendations. However, DMI might be affected by feeding RDP lower than NRC recommendations. Decreasing dietary RDP did not affect urea recycled into the rumen. There was a trend for a reduction in microbial N flow by lowering dietary RDP levels suggesting that ruminal NH_3-N requirements were not met. Decreasing dietary RDP could limit milk production. Reduction in N flow to the intestine was apparently buffered by release of N from body tissue. This was evidenced by a reduction in N balance as dietary RDP was reduced. The need to raise 15% more cows to alleviate the loss in production might nullify the advantage in reduced N output into the environment by cows fed lower dietary RDP. These observations caution against feeding dietary RDP below NRC (2001) recommendations to mid-lactation dairy cows.

Appendix: Additional figure and tables for Chapter 4

Figure 6-1. Change in ruminal fluid pH before and after feeding experimental diets in dairy cows.
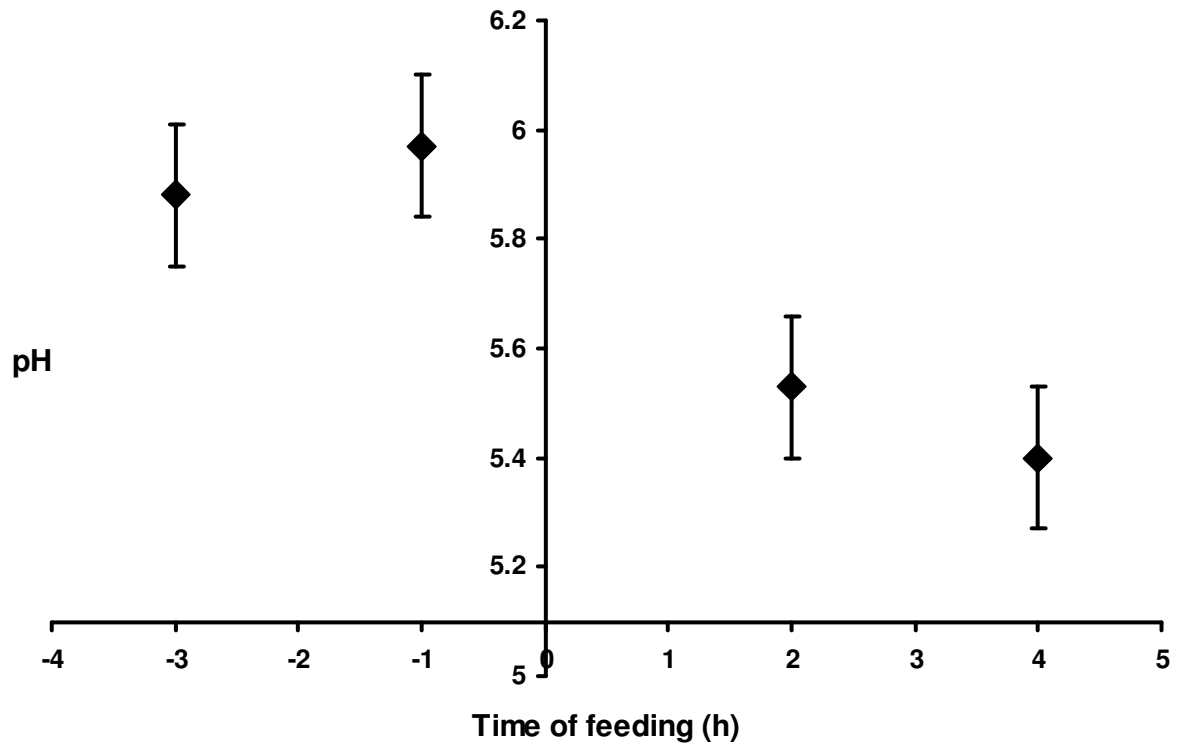
Table 6-**1.** Least squares means of feces composition of cows fed experimental diets.

Item	RDP, % of diet DM[1]				SEM	Contrasts[2]	
	11.3	10.1	8.8	7.6	SEM	L	Q
Feces composition						— (P <) —	
DM, %	15.5	16.1	16.1	16.2	0.49	0.22	0.58
Ash, % of DM	10.5	9.93	10.9	9.85	0.31	0.26	0.34
N, % of DM	2.81	2.79	2.66	2.66	0.05	0.01	0.69
ADF[4], % of DM	39.6	40.6	41.5	39.5	1.59	0.91	0.33
NDF[5], % of DM	50.6	54.4	55.3	54.1	1.45	0.10	0.10
Fat, % of DM	4.25	4.39	4.41	4.91	0.39	0.18	0.56
Starch, % of DM	2.58	2.56	2.88	3.08	0.35	0.09	0.61
GE[6], kcal/g DM	4.16	4.17	4.15	4.16	0.04	0.96	0.92

[1]Ruminally degradable protein (RDP), % of diet dry matter (DM).
[2]Contrasts: L = linear, Q = quadratic.
[3]CP = Crude protein.
[4]ADF = Neutral detergent fiber.
[5]NDF = Acid detergent fiber.
[6]GE = Gross energy.

Table 6-2. Least squares means for omasal composition of dairy cows fed experimental diets.

| Item | RDP, % of diet DM[1] | | | | SEM | Contrasts[2] | |
	11.3	10.1	8.8	7.6		L	Q
Fluid Phase						⎯ (P <) ⎯	
Proportion	0.78	0.77	0.75	0.77	0.02	0.39	0.36
DM	2.86	2.56	2.63	2.55	0.19	0.27	0.47
N, % of DM	5.46	4.70	4.96	4.09	0.36	0.03	0.87
Ash, % of DM	32.3	35.6	33.8	36.0	1.84	0.19	0.71
ADF[3], % of DM	1.08	2.32	1.04	2.32	1.38	0.70	0.99
NDF[4], % of DM	6.62	6.70	7.93	7.05	2.78	0.80	0.82
Fat, % of DM	8.88	8.93	9.31	11.2	1.10	0.09	0.30
Starch, % of DM	1.03	0.91	0.87	0.82	0.06	0.01	0.37
GE[5], kcal/g DM	3.61	3.36	3.58	3.53	0.18	0.99	0.33
Small Particle Phase							
Proportion	0.12	0.11	0.13	0.12	0.01	0.48	0.72
DM	12.5	12.4	11.6	11.9	0.73	0.48	0.75
N, % of DM	5.58	5.67	6.12	5.77	0.36	0.56	0.56
Ash, % of DM	11.8	11.5	11.9	11.8	0.24	0.72	0.71
ADF, % of DM	14.6	10.0	13.7	16.7	2.47	0.25	0.06
NDF, % of DM	25.9	20.4	27.0	31.1	2.79	0.03	0.04
Fat, % of DM	15.0	13.4	16.7	17.5	1.48	0.15	0.41
Starch, % of DM	5.47	5.79	6.13	5.36	0.67	0.99	0.34
GE, kcal/g DM	4.74	4.87	4.76	4.85	0.08	0.60	0.83
Large Particle Phase							
Proportion	0.10	0.12	0.12	0.12	0.01	0.55	0.43
DM	24.3	23.9	24.8	23.8	1.67	0.91	0.85
N, % of DM	2.73	3.06	2.87	2.81	0.19	0.96	0.32
Ash, % of DM	4.52	4.92	4.70	5.07	0.46	0.25	0.94
ADF, % of DM	46.2	43.6	49.0	38.4	2.79	0.20	0.17

NDF, % of DM	62.7	60.3	62.0	57.0	1.78	0.09	0.48
Fat, % of DM	4.78	5.15	5.60	5.31	0.50	0.40	0.52
Starch, % of DM	5.37	4.88	4.06	5.00	0.54	0.46	0.21
GE, kcal/g DM	4.30	4.16	4.29	4.21	0.05	0.52	0.54

[1]Ruminally degradable protein (RDP), % of diet dry matter (DM).
[2]Contrasts: L = linear, Q = quadratic.
[3]ADF = Neutral detergent fiber.
[4]NDF = Acid detergent fiber.
[5]GE = Gross energy.

Table 6-**3.** Least squares means for duodenal composition of dairy cows fed experimental diets.

Item	RDP, % of diet DM[1]				SEM	Contrasts[2]	
	11.3	10.1	8.8	7.6		L	Q
Fluid Phase						—— (P <) ——	
Proportion	0.83	0.83	0.78	0.81	0.03	0.55	0.63
DM	1.68	1.83	1.48	1.83	0.16	0.87	0.46
N, % of DM	5.98	5.23	5.85	6.62	0.50	0.44	0.35
Ash, % of DM	39.5	39.9	35.0	40.6	1.73	0.57	0.09
ADF[3], % of DM	0.34	0.72	0.97	-0.13	0.30	0.54	0.26
NDF[4], % of DM	1.59	1.65	0.97	0.72	0.65	0.42	0.83
Fat, % of DM	4.48	4.16	7.84	6.86	1.05	0.22	0.76
Starch, % of DM	1.52	1.22	1.62	1.47	0.09	0.48	0.32
GE[5], kcal/g DM	2.51	2.63	2.70	2.79	0.28	0.57	0.97
Small Particle Phase							
Proportion	0.06	0.05	0.07	0.07	0.01	0.33	0.86
DM	15.3	16.2	15.6	17.2	0.99	0.36	0.76
N, % of DM	5.56	5.70	5.11	5.29	0.43	0.55	0.96
Ash, % of DM	5.99	6.05	5.71	5.27	0.66	0.51	0.71
ADF, % of DM	11.1	6.97	10.3	7.29	0.79	0.16	0.45
NDF, % of DM	18.9	13.8	16.9	12.2	1.31	0.11	0.88
Fat, % of DM	17.4	18.1	19.3	23.1	2.01	0.19	0.48
Starch, % of DM	7.88	9.56	11.0	11.0	1.05	0.16	0.48
Large Particle Phase							
Proportion	0.11	0.12	0.16	0.12	0.03	0.66	0.53
DM	29.2	32.6	27.5	31.2	2.04	0.92	0.95
N, % of DM	2.63	3.61	1.85	2.31	0.53	0.37	0.66
Ash, % of DM	9.04	8.14	5.80	4.96	2.82	0.37	0.99
ADF, % of DM	30.8	34.5	39.3	34.9	4.08	0.46	0.42
NDF, % of DM	43.5	44.9	55.8	43.4	4.02	0.63	0.22

Fat, % of DM	7.46	8.77	6.05	6.80	0.45	0.04	0.32
Starch, % of DM	16.4	16.2	8.84	20.1	3.76	0.85	0.26
GE, kcal/g DM	4.23	4.17	4.07	4.36	0.11	0.61	0.21

[1]Ruminally degradable protein (RDP), % of diet dry matter (DM).

[2]Contrasts: L = linear, Q = quadratic.

[3]ADF = Neutral detergent fiber.

[4]NDF = Acid detergent fiber.

[5]GE = Gross energy.

Table 6-4. Least squares means for ruminal outflow and digestibility of nutrients of dairy cows fed experimental diets. Flows were calculated using single marker indigestible neutral detergent fiber (INDF) method.

Item	RDP, % of diet DM[1]					Contrasts[2]	
	11.3	10.1	8.8	7.6	SEM	L	Q
DM							── (P <) ──
Post ruminal flow, kg/d	11.4	7.75	8.89	10.2	1.17	0.58	0.05
RDCa[3], kg digested/kg intake	0.43	0.55	0.53	0.55	0.04	0.15	0.33
OM[4]							
Post ruminal flow, kg/d	9.44	6.43	7.34	8.30	0.87	0.48	0.05
RDCa, kg digested/kg intake	0.50	0.60	0.59	0.61	0.03	0.10	0.35
N							
Post ruminal flow, g/d	508	333	376	397	0.06	0.28	0.14
RDCa, kg digested/kg intake	0.12	0.28	0.21	0.27	0.09	0.40	0.63
ADF[5]							
Post ruminal flow, kg/d	2.27	1.58	1.91	1.11	0.17	0.13	0.12
RDCa, kg digested/kg intake	0.42	0.56	0.50	0.57	0.04	0.05	0.28
NDF[6]							
Post ruminal flow, kg/d	3.62	2.52	2.75	3.25	0.33	0.51	0.04
RDCa, kg digested/kg intake	0.48	0.62	0.60	0.59	0.35	0.11	0.11
Fat							
Post ruminal flow, g/d	1000	554	835	1076	152	0.45	0.06
RDCa, kg digested/kg intake	-0.08	0.38	0.24	0.27	0.12	0.16	0.17
GE[7]							
Post ruminal flow, Mcal/d	46.5	34.8	34.9	42.7	4.6	0.54	0.06

[1]Ruminally degradable protein (RDP), % of diet dry matter (DM).
[2]Contrasts: L = linear, Q = quadratic
[3]RDCa (fractional ruminal digestibility coefficient) = (Intake - Ruminal outflow) / Intake.
[4]OM = Organic matter.
[5]ADF = Acid detergent fiber.

[6]NDF = Neutral detergent fiber.
[7]GE = Gross energy.

Table 6-5. Least squares means for ruminal outflow and digestibility of nutrients of dairy cows fed experimental diets. Flows were calculated using single marker (Cr) method.

	RDP, % of diet DM[1]					Contrast[2]	
Item	11.3	10.1	8.8	7.6	SEM	L	Q
DM						—— (P <) ——	
Post ruminal flow, kg/d	12.7	19.8	16.6	16.8	3.06	0.51	0.25
RDCa[3], kg digested/kg intake	0.32	-0.05	0.08	0.08	0.14	0.39	0.23
OM[4]							
Post ruminal flow, kg/d	10.9	16.6	14.0	13.8	2.24	0.57	0.22
RDCa, kg digested/kg intake	0.38	0.06	0.17	0.19	0.12	0.43	0.19
N							
Post ruminal flow, g/d	541	835	727	692	0.14	0.61	0.27
RDCa, kg digested/kg intake	-0.01	-0.67	-0.58	-0.59	0.29	0.26	0.29
ADF[5]							
Post ruminal flow, kg/d	3.14	4.15	3.56	3.19	0.47	0.80	0.12
RDCa, kg digested/kg intake	0.13	-0.10	0.02	0.17	0.09	0.50	0.04
NDF[6]							
Post ruminal flow, kg/d	4.87	6.39	5.33	5.38	0.67	0.85	0.27
RDCa, kg digested/kg intake	0.22	0.02	0.15	0.16		0.89	0.20
Fat							
Post ruminal flow, g/d	1.06	1.70	1.57	1.77	0.37	0.28	0.56
RDCa, kg digested/kg intake	-0.41	-0.56	-0.36	-0.53	0.32	0.88	0.97
GE[7]							
Post ruminal flow, Mcal/d	55	85	60	69	1.2	0.72	0.38

[1]Ruminally degradable protein (RDP), % of diet dry matter (DM).
[2]Contrasts: L = linear, Q = quadratic.
[3]RDCa (fractional ruminal digestibility coefficient) = (Intake - Ruminal outflow) / Intake.
[4]OM = Organic matter.
[5]ADF = Acid detergent fiber.

[6]NDF = Neutral detergent fiber.
[7]GE = Gross energy.

Table 6-6. Least squares means for ruminal outflow and digestibility of nutrients of dairy cows fed experimental diets. Flows were calculated using single marker (Yb) method.

Item	RDP, % of diet DM[1]					Contrast[2]	
	11.3	10.1	8.8	7.6	SEM	L	Q
DM						—— $(P <)$ ——	
Post ruminal flow, kg/d	12.5	12.0	11.6	12.3	0.40	0.62	0.20
RDCa[3], kg digested/kg intake	0.36	0.34	0.33	0.35	0.14	0.73	0.65
OM[4]							
Post ruminal flow, kg/d	10.4	10.1	9.74	10.2	0.32	0.52	0.17
RDCa, kg digested/kg intake	0.43	0.41	0.39	0.42	0.03	0.53	0.80
N							
Post ruminal flow, g/d	584	525	511	496	0.02	0.40	0.66
RDCa, kg digested/kg intake	-0.06	-0.08	-0.14	-0.07	0.09	0.78	0.60
ADF[5]							
Post ruminal flow, kg/d	2.62	2.77	2.84	2.63	0.18	0.91	0.34
RDCa, kg digested/kg intake	0.33	0.26	0.19	0.33	0.06	0.82	0.14
NDF[6]							
Post ruminal flow, kg/d	3.88	3.97	4.06	4.25	0.21	0.23	0.81
RDCa, kg digested/kg intake	0.43	0.37	0.32	0.36	0.06	0.47	0.75
Fat							
Post ruminal flow, g/d	1.21	1.04	1.11	1.24	0.37	0.60	0.02
RDCa, kg digested/kg intake	-0.34	-0.09	-0.18	-0.12	0.12	0.22	0.28
GE[7]							
Post ruminal flow, Mcal/d	50.3	48.7	46.9	50.2	2.06	0.74	0.16

[1]Ruminally degradable protein (RDP), % of diet dry matter (DM).
[2]Contrasts: L = linear, Q = quadratic.
[3]RDCa (fractional ruminal digestibility coefficient) = (Intake - Ruminal outflow) / Intake.
[4]OM = Organic matter.
[5]ADF = Acid detergent fiber.
[6]NDF = Neutral detergent fiber.

[7]GE = Gross energy.

Table 6-7. Least squares means for ruminal outflow of nutrients of dairy cows fed experimental diets. Flows were calculated using triple marker (Co, Yb and INDF) method.

| Item | RDP, % of diet DM[1] | | | | | Contrast[2] | |
	11.3	10.1	8.8	7.6	SEM	L	Q
DM						⸺ (P <) ⸺	
Post ruminal flow, kg/d	-115	-17.7	-285	-86.6	106	0.70	0.63
OM[3]							
Post ruminal flow, kg/d	-97.8	-15.4	-231	-68.3	88.6	0.75	0.65
N							
Post ruminal flow, g/d	-6.84	-0.95	-14.2	-3.95	5.73	0.86	0.70
ADF[4]							
Post ruminal flow, kg/d	-11.6	-4.47	-45.7	-9.77	17.1	0.64	0.41
NDF[5]							
Post ruminal flow, kg/d	-30.5	-4.72	-71.3	-21.8	27.3	0.74	0.66
Fat							
Post ruminal flow, g/d	-10.7	-1.72	-26.0	-8.17	9.70	0.70	0.65
GE[6]							
Post ruminal flow, Mcal/d	-0.68	-0.01	-1.42	-0.40	0.57	0.86	0.70

[1]Ruminally degradable protein (RDP), % of diet dry matter (DM).
[2]Contrasts: L = linear, Q = quadratic.
[3]OM = Organic matter.
[4]ADF = Acid detergent fiber.
[5]NDF = Neutral detergent fiber.
[6]GE = Gross energy.