The Pennsylvania State University

The Graduate School

College of Agricultural Sciences

INVESTIGATIONS INTO THE NUTRITIONAL EFFICIENCY OF DAIRY HEIFERS LIMIT FED DIETS CONTAINING DIFFERENT LEVELS OF FORAGE AND CONCENTRATE

A Dissertation in

Animal Science

by

Geoffrey I. Zanton

Submitted in Partial Fulfillment
of the Requirements
for the Degree of

Doctor of Philosophy

May 2009

The dissertation of Geoffrey I. Zanton was reviewed and approved* by the following:

Arlyn J. Heinrichs
Professor of Dairy and Animal Science
Dissertation Advisor
Chair of Committee

Naomi S. Altman
Associate Professor of Statistics

Michael L. O'Connor
Professor of Dairy Science

Gabriella A. Varga
Distinguished Professor of Animal Science

Terry D. Etherton
Distinguished Professor of Animal Nutrition
Head of the Department of Dairy and Animal Science

*Signatures are on file in the Graduate School

ABSTRACT

Limit feeding high concentrate or high digestibility diets for growing dairy heifers may offer an economical alternative to *ad libitum* consumption of high forage, low digestibility diets that are traditionally fed to dairy heifers. Literature pertaining to economic, physiological, and nutritional responses to alterations in feeding level and dietary concentrate level are reviewed. It is concluded that limit feeding higher concentrate diets do not affect growth or first lactation milk production compared to dairy heifers fed high forage diets when equivalent levels of gain are obtained. It is also concluded that limit feeding high concentrate diets does not need to be excluded as a management option and may offer an opportunity for heifer growers to reduce feed costs and environmental output under certain circumstances. The level of dietary crude protein resulting in maximum efficiency of nutrient utilization under a limit feeding, high concentrate management system and differences from limit-fed high forage feeding are not available from the literature. Therefore, the objective of the reported experiment was to evaluate efficiency of nutrient and N utilization of dairy heifers limit-fed a low forage/high concentrate and high forage diets at equal ME intakes and 4 levels of N intake.

The hypothesis of this experiment is that a low forage (LF) ration will be utilized with a greater efficiency than a high forage ration (HF) by dairy heifers and that the response will be affected by level of N intake. To test this hypothesis, 8 Holstein heifers (beginning at 362 ± 7 kg and 12.3 ± 0.4 mo) were fed eight rations according to a split-plot, 4 x 4 Latin square design. Treatments were formulated to contain 25% or 75%

forage (corn silage and chopped wheat straw) and fed at 4 levels of N intake [0.94 (Low), 1.62 (MLow), 2.30 (MHigh), 2.96 (High) g N/kg $BW^{0.75}$ per d]. Diets were limit-fed to maintain equal ME intake. Organic matter (OM) intake was greater for heifers fed HF, but, due to increased OM digestibility of LF (74.0 vs 67.6% ± 0.9; P <0.01), digestible OMI was unaffected by forage level (P > 0.50). OM digestibility was affected by an interaction between forage level and N intake (P < 0.01); increasing to a plateau of 78.01% at 18.43 %CP for LF and 68.78% at 13.90 %CP for HF fed heifers. Apparent N digestibility was greater for heifers fed LF and increased from 47.7% to 80.8% between Low and High N intake. Less N appeared in the feces of heifers fed LF than HF (45.56 vs 52.60 g per d). Urea-N excretion was not different between forage levels, but increased linearly with N intake. Concentration of plasma urea N (PUN) was significantly higher for LF and with increasing N intake. Like urea-N excretion, daily urinary N excretion was affected only by N intake. Retained N responded linearly to increased levels of NI. As a result of a significant interaction between forage level and NI on fecal N excretion and numerical differences in urine N, retained N at maximum N intake was greater for LF than HF. In spite of this observation, the percent of N intake that was retained only tended to be affected by an interaction and was not significantly affected by forage level. It is concluded that increasing N intake increases the digestibility of OM, the magnitude of which depends on the level of dietary forage provided. Furthermore, differences in N utilization between LF and HF in this trial were small and were not evident until N intake increased to impractical levels.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ACKNOWLEDGEMENTS

It is truly impossible for me to adequately thank all of the people that have helped me in one form or another in preparation for and throughout the time spent working on my doctorate. Throughout the course of my time at PSU, Dr. Heinrichs has played an important part of my evolution as a dairy scientist. He has given me excellent mentorship, guidance, example, and encouragement since the time that I interviewed for graduate school. I am also humbled by the confidence that he had in me to explore new and unusual areas of research and hope that my diversions from the beaten path have been as beneficial to others as they have been to my education. I would also like to express appreciation to my other committee members for their support and encouragement. Drs. Naomi Altman, Michael O'Connor, and Gabriella Varga have been helpful to me in more ways than I can express. I would also like to thank Dr. Ron Kensinger for his time and support and Dr. Lester Griel for his irreplaceable veterinary assistance.

I would also like to thank Maria Long who has helped immeasurably with laboratory work and in experimental preparation and execution. I would also like to thank all of the multitudes of undergraduate students that have helped me in both the lab and the barn, of which there are far too many to name. There are many graduate students that have given me help and support. I would especially like to thank Dr. Neil Brown, Dr. Jorge Elizondo, Dr. Sylvia Kehoe, Meghan Moody, and Gustavo Lascano for their support and friendship.

I am proud to have grown up on a dairy farm and can not begin to express my deep appreciation to my parents Jim and Mary Jo Zanton for the countless sacrifices that

they have made on my brothers' and sisters' and my behalf. Their patience, support, and love has provided me strength and opportunity throughout my life. I would also like to thank Jenna, Tricia, Marie, Lisa, Tom, and Steve for all they did for me to make this possible.

Finally, it is impossible to imagine life without Sara. There is no way that I could have entered much less completed graduate school without your support and commitment. Together we have received many blessings since our move from Wisconsin to Pennsylvania, but above all of these are Marianna and Sam. There is no way that I can thank you all for all you have given me.

Chapter 1

INTRODUCTION

Raising dairy heifers from birth to first parturition is an expensive proposition owing to the long duration of this timeframe, the relatively low efficiency of converting consumed nutrients to tissue for growth, and, most importantly, due to the absence of income until the onset of lactation. Dairy heifers have been traditionally fed diets where the great majority of the consumed nutrients derive from forages fed for ad libitum consumption where the fibrousness of the diet limits voluntary dry matter intake. The continuance of this practice, however, is a lost opportunity for the dairy producer to devote a greater proportion of the forages on the farm to the lactating dairy cow for which the marginal efficiency of nutrient utilization is higher, as well as through lost physiological efficiency associated with the digestion of forages and metabolism of the end-products of forage digestion. Throughout this period, the dairy heifer is also not producing any income and is negatively contributing to the environmental sustainability of the dairy farm. Feeding practices which would enhance the economic stability, environmental sustainability, and physiological efficiency of dairy heifers are required—while maintaining or improving future lactation performance.

Recent research activities have focused on nutritional methods to enhance the efficiency of raising dairy heifers through the application of limit-fed, energy-dense rations. The motivations for moving toward this feeding system are several-fold. First, it has been shown through meta-analytic statistical procedures that milk production in the

first lactation does not decrease in response to increasing average daily gain over the entire conceivable range of daily gains, as was generally believed. Instead, milk production is maximized at an intermediate rate of gain (Zanton and Heinrichs, 2005). Therefore, nutrient consumption should be provided at a level commensurate with the animals' precise requirement to sustain these levels of gain. An outcome of this result is that feeding amounts and feed costs should be compared on the ability of a feed to provide the nutrients required to obtain this optimal level of growth, which differs conceptually from ad libitum feed consumption of high forage diets where the animal selects the nutrients consumed. When compared on strictly economic terms, even with the current increase in grain costs, concentrate feedstuffs are considerably more cost effective when supplied to provide energy and protein to the animal than forages. Therefore, meeting the energy and protein requirements of the animal with concentrates could provide an opportunity to enhance the efficiency of dairy heifer production.

For any management or nutritional concept to be widely applicable at the farm level, the concept must not only improve the outcome variable of interest, but the alteration must not have detrimental effects on other important variables. To evaluate the concept of high concentrate, limit-fed rations for dairy heifers growth and lactation experiments have been conducted to assess outcomes important to dairy producers (Hoffman et al., 2007; Zanton and Heinrichs, 2007). In these experiments, animals were fed diets of differing energy densities at a level of intake controlled to obtain an equal level of average daily gain between dietary groups. The results of these experiments demonstrated that feed efficiency was improved when a higher energy density diets were limit-fed, with skeletal growth and first lactation milk production unaffected by dietary

treatment, milk production was numerically improved in both trials and fat-corrected milk and milk fat production were significantly increased in heifers limit-fed the high concentrate diets (Zanton and Heinrichs, 2007). Therefore, it can be concluded that growth and production outcomes were not impeded by the utilization of high concentrate diets, provided that the intake of the diets were restricted, and in some cases productive outcomes were improved.

Additionally, a potential reduction in environmental wastes may be allowed by the use of higher concentrate/digestibility, limit-fed diets. It is logical, from a qualitative perspective, that when a more highly digestible feed is provided at a lower level than a less digestible feed, less manure will be produced. The quantitative advantage of this dietary alteration, if present, was not available from the literature. From previous results in the literature it could be inferred that reducing intake enhances the efficiency of nutrient utilization on a proportional level and, depending on the diet and the level of dry matter restriction, the absolute level of efficiency can remain unaltered since the enhanced proportional efficiency can compensate for the reduced feed intake (Tyrrell and Moe, 1975; Murphy and Loerch, 1994). Responses to restricting the intake of high forage diets showed that for each one kilogram decrease in dry matter intake, manure output was decreased by 2.62 kilograms (Zanton and Heinrichs, 2008); a substantial reduction with practical implications on farms limited by land availability for manure disposal. Improvements that can be realized when both diet intake and composition are altered simultaneously can be considerably greater than that which is observed when intake is altered alone, however (10.2 kg manure/kg DMI, for instance, from the results of Moody et al., 2007). Nitrogen retention and excretion was reduced linearly, but the efficiency of

nitrogen utilization was maximized at an intermediate level of dry matter intake (Zanton and Heinrichs, 2008).

Considering the potential for increased efficiency, reduced feed costs, and reduced environmental excretion, limit-feeding high concentrate diets to dairy heifers has been studied. The overall aim of this research has been to indentify nutritional opportunities upon which dairy producers may capitalize without sacrificing future lactation performance, maintaining structural growth characteristics, while reducing environmental outputs and feed expenditures through the application of limit feeding high concentrate diets to growing dairy heifers. Literature on the effects of limit feeding high concentrate diets is reviewed and results from an experiment evaluating efficiency of nutrient and N utilization of dairy heifers limit-fed a low forage/high concentrate and high forage diets at equal ME intakes and 4 levels of N intake is presented.

REFERENCES

Hoffman, P. C., C. R. Simson, and M. Wattiaux. 2007. Limit feeding of gravid Holstein heifers: Effect on growth, manure nutrient excretion, and subsequent early lactation performance. J. Dairy Sci. 90:946–954.

Moody, M. L., G. I. Zanton, J. M. Daubert, and A. J. Heinrichs. 2007. Nutrient utilization of differing forage-to-concentrate ratios by growing Holstein heifers. J. Dairy Sci. 90:5580–5586.

Murphy, T. A., and S. C. Loerch. 1994. Effects of restricted feeding of growing steers on performance, carcass characteristics, and composition. J. Anim Sci. 72:2497–2507.

Tyrrell, H. F., and P. W. Moe. 1975. Effect of intake on digestive efficiency. J. Dairy Sci. 58:1151–1163.

Zanton, G. I., and A. J. Heinrichs. 2005. Meta-analysis to assess effect of prepubertal average daily gain of Holstein heifers on first-lactation production. J. Dairy Sci. 88:3860–3867.

Zanton, G. I., and A. J. Heinrichs. 2007. The Effects of Controlled Feeding of a High-Forage or High-Concentrate Ration on Heifer Growth and First-Lactation Milk Production. J. Dairy Sci. 90:3388–3396.

Zanton, G. I., and A. J. Heinrichs. 2008. Rumen digestion and nutritional efficiency of dairy heifers limit-fed a high forage ration to four levels of dry matter intake. J. Dairy Sci. 91:3579–3588.

Chapter 2

LITERATURE REVIEW

2.1 DAIRY HEIFER FEEDING MANAGEMENT AND ECONOMICS

The profitability and sustainability of dairy farming depends vitally on efficient management practices that result in maximizing milk production at a minimum monetary and environmental cost. While practices of managing lactating dairy cattle occupy the greatest share of time, effort, and costs associated with dairy farming, the total costs of raising dairy heifers are the second largest contributor to the annual operating expenses of a dairy farm (Tozer and Heinrichs, 2001). This large contribution toward operating expenses would indicate that an opportunity exists to reduce whole farm expenses by reducing the expenditures on raising dairy heifers. Since management decisions that are made during the rearing period affect both future productivity (Sejrsen et al., 1982; Sejrsen et al., 2000; Zanton and Heinrichs, 2005) and the duration until lactation commences (Gardner et al., 1977; Gardner et al., 1988; Hoffman et al., 1996; Ettema and Santos, 2004), decisions concerning heifer management must be balance between reducing current expenditures, maximizing future revenue, while minimizing the time that a heifer is in an unproductive state (Hoffman and Funk, 1992). The impact of heifer management on whole farm management can be readily appreciated by simply evaluating the time partitioning of the life of a cow on Pennsylvania dairy farms (Figure 1). While age at first calving (**AFC**) declines as milk production increases, the level of

improvement does still not fall within the recommended range of AFC of 22-24 months (Heinrichs, 1993; Hoffman, 1997; Ettema and Santos, 2004). Contrasting these

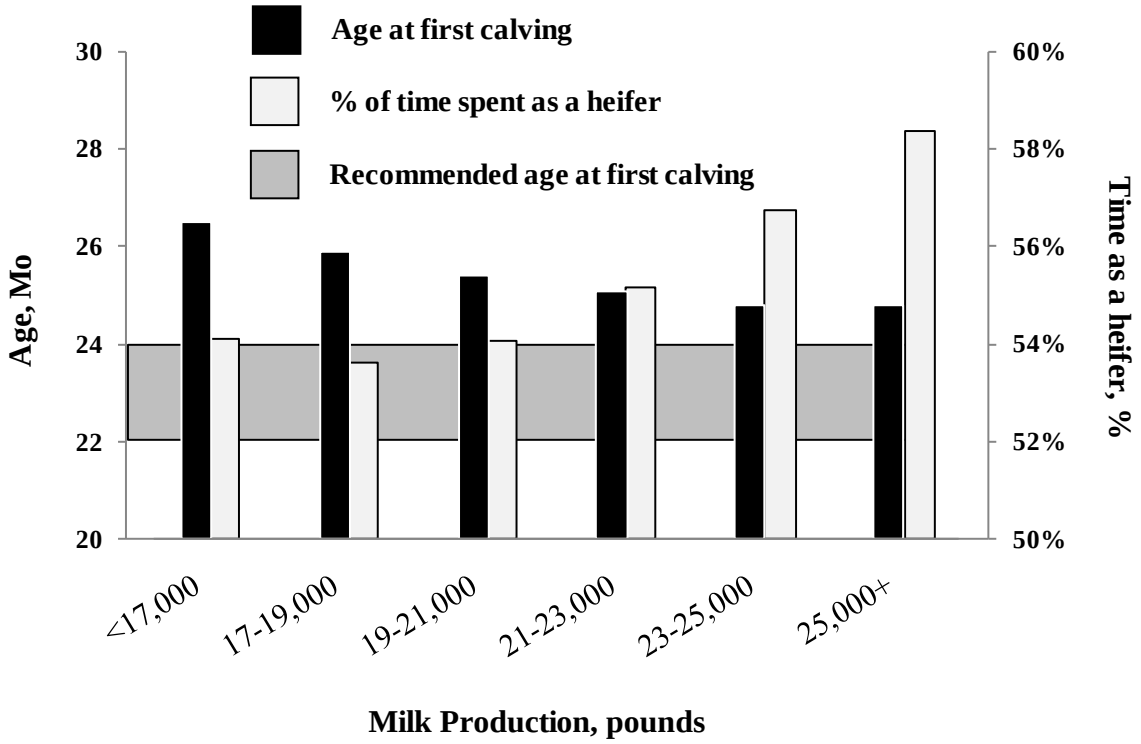

Figure **2-1**: Age at first calving and percent of time spent as heifers by herd milk production level for dairy cows in Lancaster DHIA in 2007.

improvements in AFC is proportion of the time that an average cow spends in the unproductive, heifer stage of life. Due to reductions in average animal age on higher producing dairy farms, the proportion of life as a heifer is 58%; over half of the life of an average cow is spent costing money without making money.

Feed costs represent up to 60%, the greatest proportion, of total expenses associated with dairy heifer rearing (Gabler et al., 2000) and feed management practices impact total manure output and characteristics (Nennich et al., 2005). Therefore, feed and nutritional management of growing dairy heifers is an important control point for total

farm profitability and sustainability. Dairy heifers are traditionally offered forages as a primary source of nutrition from the time of weaning until parturition, with the proportional allotment of forage increasing with age (Heinrichs, 1996). While this practice has been used for many years, feeding forage may not always be the most cost effective method for providing for the nutrition of dairy heifers. Since dairy heifers require energy and nutrients, evaluation of the cost effectiveness of feeding programs must be evaluated relative to a diet's ability to provide these components. Considered on this basis, in the United States, feedstuffs which provide concentrated sources of nutrients have historically been the most cost effective. Figure 2A shows the price received for corn, soybeans, or hay from from April 1998 through September 2008 by Pennsylvania farmers (National Agriculture Statistics Service, United States Department of Agriculture) and soybean meal prices (*Feedstuffs* ingredient market data representing current trading value of soybean meal as of the month reported). As can be seen, on a dry matter basis hays have historically yielded a price between corn and soybean meal; however when expressed relative to the level of metabolizable energy (B) or crude protein (C), corn and soybean meal were the cheaper source of energy or protein, respectively. This difference relates to the higher concentration of energy and protein in corn and soybean meal, and reflects the reduced level of dry matter that would need to be provided to meet the nutrient requirement of the animal for growth. It is also important to note that the price of these feedstuffs, with some exceptions, tend to vary in parallel. While price conditions are different at various locations around the country and across time, this historical price differential is not a guarantee of future cost effectiveness of feed ingredients and ultimately must be evaluated on an individual farm basis. Should

market conditions favor the feeding of concentrates at a greater proportion than have traditionally occurred, however, animal performance and future productivity must

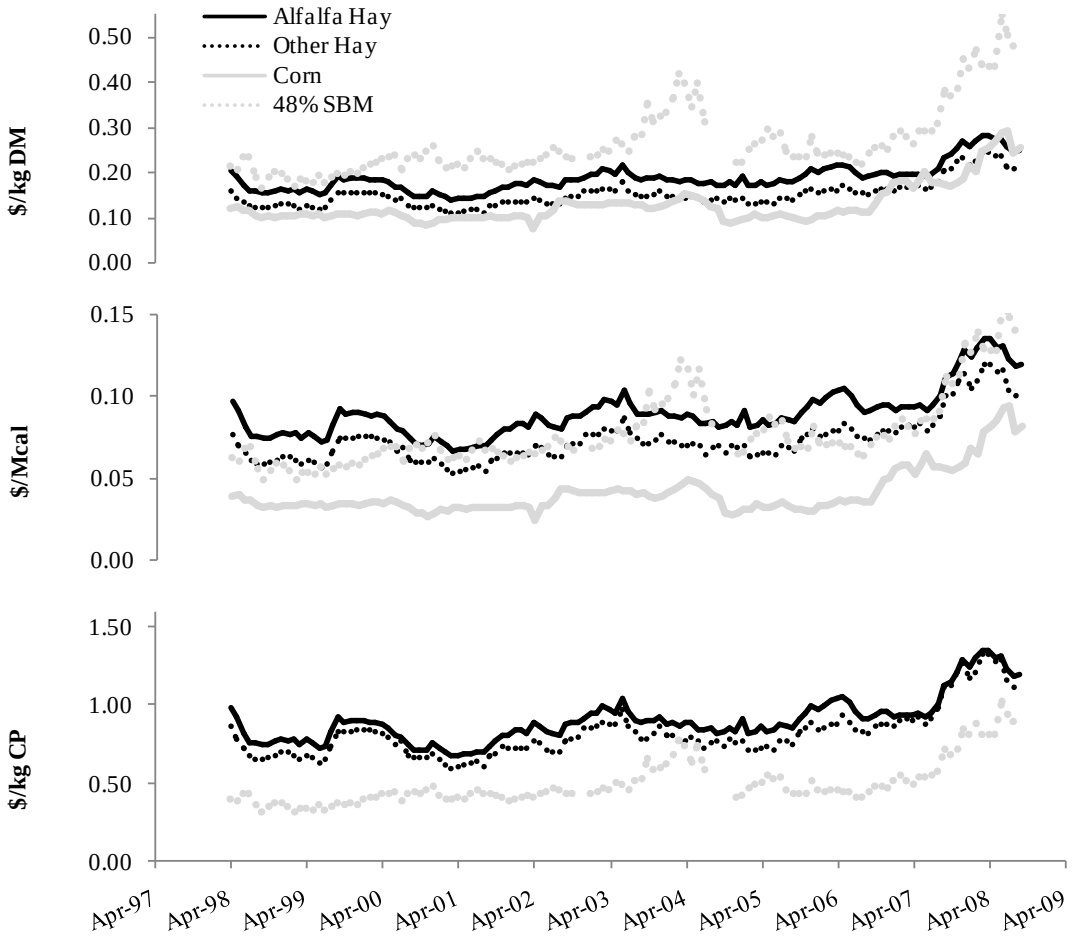

Figure 2-2: Cost of feed ingredients to supply dry matter, metabolizable energy, and crude protein over time.

be known to ensure that potential savings in feed costs are not offset by poorer animal performance and health outcomes.

2.2 DAIRY HEIFER GROWTH AND FUTURE PRODUCTIVITY

2.2.1 Mammary Development and Milk Production

It is well documented that increasing intake of concentrates results in greater levels of average daily gain (**ADG**). While increasing the concentration of concentrated feed ingredients may represent an alternative means of providing the nutrients required for growth and development of the dairy heifer, impact on future productivity must be known. Swanson (1960) observed that when identical twin heifers were grown at normal, control rate of gain or an accelerated rate of gain induced by the inclusion of high levels of concentrates in the diet, the accelerated gain heifers produced less milk in the first and second lactation and had lower levels of mammary secretory tissue. These conclusions with respect to ADG have been replicated by many experiments (Gardner et al., 1977; Little and Kay, 1979; Sejrsen et al., 1982; Capuco et al., 1995; Van Amburgh et al., 1998; Lammers et al., 1999; Radcliff et al., 2000; Meyer et al., 2006b; Rincker et al., 2008); provided that the differences in feeding rates were confined to the allometric phase of mammary development since ADG increases during the pre-weaning (Brown et al., 2005) or post-pubertal (Sejrsen et al., 1982; Hoffman et al., 1996) has positive or no effect on mammary development, respectively.

The mechanisms responsible for the reduction in milk production has been attributed to several metabolic hormones that are altered by level of feeding (Sejrsen and Purup, 1997). Lower concentrations of growth hormone has been correlated with reduced mammary development for heifers fed for high ADG as a result of correlation analysis (Sejrsen et al., 1983) or experimentally induced alterations in growth hormone

concentrations (Sejrsen et al., 1986; Radcliff et al., 1997). First lactation milk production responses to administered growth hormone during the pre-pubertal period does not always correspond to the enhanced mammary gland development observed, however (Radcliff et al., 2000; Capuco et al., 2004). However, since bovine mammary tissue does not bind growth hormone (Akers, 1985), other factors have been investigated as contributing to reduced mammary gland development with increased ADG. The effect has been attributed to the downstream modulators of growth hormone action such as insulin-like growth factor (**IGF-I**; Silva et al., 2005) and alterations in the IGF binding proteins (Berry et al., 2001), increases in leptin circulation (Silva et al., 2002a; Silva et al., 2008) as a result of greater body fat mass (Silva et al., 2002b), or simply due to reduced time for adequate mammary development as a result of the termination of the pre-pubertal allometric growth phase earlier for heifers grown at a faster rate (Meyer et al., 2006a; Meyer et al., 2006b). The extent to which each of these and other factors contribute individually or collectively to the potential for reduced mammary development is still the subject of a considerable research effort.

Several experiments have not observed any differences in mammary development or lactation responses when ADG has been increased prior to puberty in contrast to accepted theory (Peri et al., 1993; Radcliff et al., 1997; Waldo et al., 1998; Abeni et al., 2000). Recently, it has been suggested from an analysis of literature data, that the discrepancy in lactation responses to ADG observed in the previously cited experiments, was due to the response profile not a being linear response (Zanton and Heinrichs, 2005). Quadratic responses in first-lactation milk production to alterations in ADG has been reported previously (Foldager and Sejrsen, 1987; Sejrsen et al., 2000) and consistency

between the responses reported in these reviews and the analysis reported by Zanton and Heinrichs (2005) is shown in Figure 3. As seen, the responses in milk

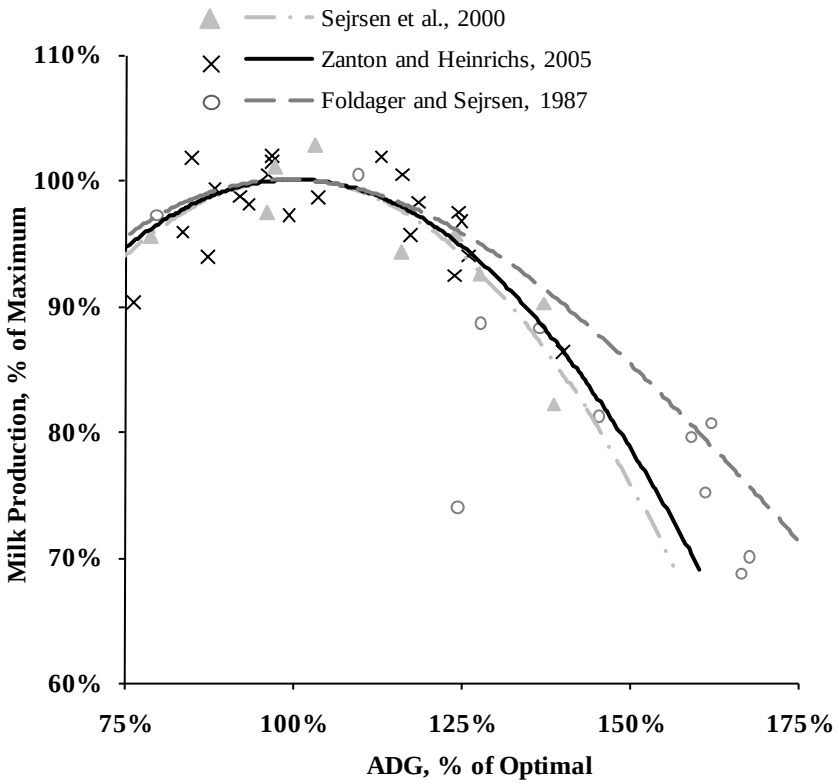

Figure 2-3: Curvilinear response in milk production as a percent of predicted maximum milk production related to average daily gain as a percent of optimal average daily gain.

production do not respond as a linearly reducing function to increases in ADG, but increase over a range of lower ADG levels and then decrease after reaching a maximum. For the Holstein heifer experiments analyzed by Zanton and Heinrichs (2005) this maximum occurred at ADG of 799 g/d and led the authors to conclude that, for the current population of Holstein heifers, this ADG would optimize first lactation milk production.

Considering that rapid ADG would result in a sooner return-on-investment due to the capacity for the heifer to reach breeding age earlier, many hormonal and dietary alterations have been investigated in combination with high ADG in an attempt to negate or reduce the negative effects on mammary development produced by high ADG. Additional dietary crude protein (Whitlock et al., 2002), rumen undegradable protein (Capuco et al., 2004), and fat (Thibault et al., 2003) have been investigated for this purpose without effect. Capuco et al. (1995) conducted an experiment in which pre-pubertal dairy heifers were fed diets consisting of predominantly corn silage or alfalfa silage for 2 levels of ADG. Though subsequent milk production for heifers fed these diets was not different between groups (Waldo et al., 1998), mammary growth was said to be inhibited for heifers receiving the corn silage diet but not for the alfalfa silage diet. While this result was interpreted with respect to the greater protein intake for heifers consuming the alfalfa diet reducing the negative effects of rapid gain (Whitlock et al., 2002), an alternative interpretation for these results was that the rapid fermentability of the corn silage diet in combination with rapid ADG was resulting in decreased mammary development. Since most of the experiments that evaluate mammary gland development or first lactation milk production in response to greater ADG alter the diets provided by enhancing the fermentability as well as the availability, this result would offer an explanation for the negative impacts on mammary development that is less dependent on ADG, but on ADG in combination with a highly fermentable diet. This may also, however, minimize the opportunity for concentrate inclusion in the diets of growing dairy heifers.

In the literature, there are several examples where the effect of diet fermentability and heifer ADG have been attempted to be separated (Hof and Lenaers, 1984; Carson et al., 2000; Hoffman et al., 2007; Zanton and Heinrichs, 2007). First lactation results are shown in Table 1, accompanied by the ADG obtained in the experiments and the growth phase in which the dietary alteration was made. None of these experiments reported

Table **2-1**: The effect of forage level at constant ADG on first lactation milk production.

Reference Feeding period	n	Forage, % DM	ADG, g/d	Milk, kg/d	% greater than HF diet
Zanton and Heinrichs, 2007	17	75	837	31.7	9.46
Pre-pubertal	17	25	837	34.7	
Hoffman et al., 2007	15	94	754	30.6	0.33
Post-pubertal	16	80	871	30.7	4.89
	15	63	835	32.2	
Carson et al., 2000	10	54	950	21.6	2.78
Pre-pubertal	9	29	930	22.2	
Sejrsen and Foldager, 1992	8	50	498	16.1	2.48
Rearing period	8	30	475	16.5	
Hof and Lenaers, 1984	19	82	669	14.1	2.13
Rearing period	17	19	643	14.4	

significant differences in ADG, so the inferences are restricted to differences in diet type. As can be seen from these experiments, reducing the forage level in the diet, though a potential confounding factor in some of the experiments reported previously, does not appear to negatively impact milk production. In fact, though none of the numerical differences were found to be significant, the low forage diets consistently resulted in improved first lactation milk production. In an attempt to determine whether this numerical increase in first lactation milk production is significant, the data were combined and analyzed statistically; the results are shown in Figure 4. The results of these experiments and analysis indicate that, if ADG is equal between dietary groups,

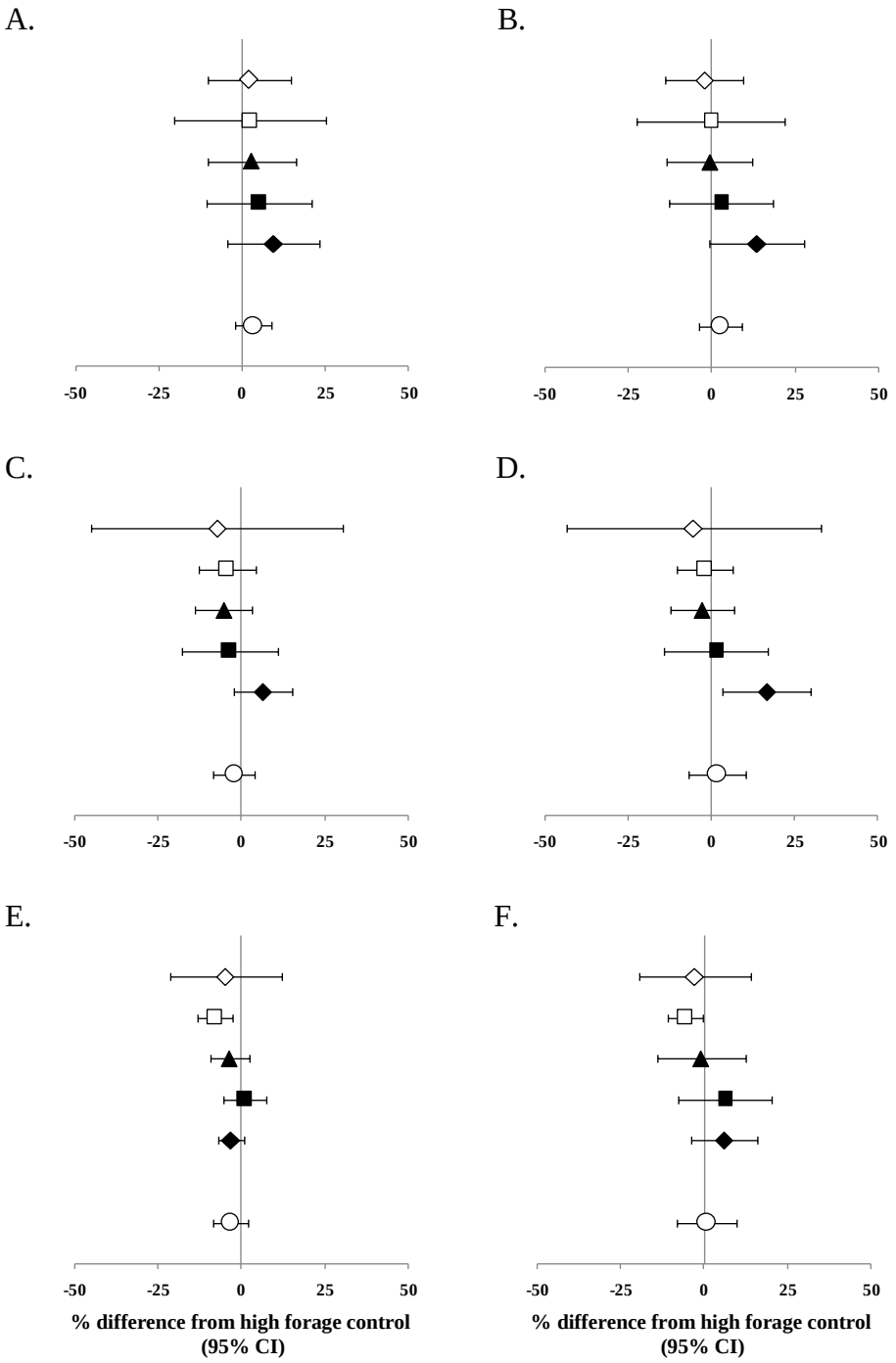

Figure 2-4: Milk production (A), fat-corrected milk production (B), milk fat percentage (C), milk fat yield (D), milk protein percent (E), and milk protein yield (F) differences between dairy heifers limit-fed low or high forage diets; % difference of low forage from high forage.
Where ◊ is Hof and Lenaers, 1984; □ is Sejrsen and Foldager, 1992; ▲ is Carson et al., 2000; ■ is Hoffman et al., 2007; ♦ is Zanton and Heinrichs, 2007; and ○ is the overall average. Error bars are the 95 % confidence intervals of the difference.

first lactation milk production will not be inhibited by altering the concentrate level in the diet. The animals from the experiment conducted in this laboratory (Zanton and Heinrichs, 2007) were subsequently followed through the first two lactations and, as shown in Table 2, productive outcomes are not significantly different between forage feeding groups. Thus, limit feeding high concentrate diets to growing dairy heifers need not be specifically excluded as a management option based on lactation performance as the results presented show no differences between animals fed a forage diet as a control.

Table 2-2: Overall first and second lactation performance of Holstein cows limit-fed high forage or high concentrate diets prior to puberty as heifers.

	High Forage	High Concentrate	SE	*P* <
First Lactation				
Heifers Freshening	17	17		
Cows Finishing	12	15		
Cows finishing lactation open	1	0		
Transition Disorders				
Displaced Abomasum	3	3		
Uterine Infection	0	1		
Production , 305 d ME				
Milk, kg	13,624	14,303	820	0.481
4% FCM, kg	13,148	14,240	798	0.248
Fat, kg	513	568	34	0.181
Protein, kg	425	430	22.82	0.860
Fat, %	3.78	3.97	0.16	0.320
Protein, %	3.12	3.01	0.06	0.103
Reproduction				
Days Open	204	179	35	0.550
Conception Rate	49	51	12	0.887
Second Lactation				
Cows Freshening	11	15		
Cows Finishing	11	14		
Cows finishing lactation open	1	2		
Transition Disorders				
Displaced Abomasum	1	0		
Uterine Infection	2	1		
Production , 305 d ME				
Milk, kg	13,720	14,539	749	0.379
4% FCM, kg	12,338	14,104	742	0.067
Fat, kg	515	587	33	0.094
Protein, kg	424	449	23	0.390
Fat, %	3.79	4.03	0.21	0.367
Protein, %	3.10	3.09	0.06	0.957
Reproduction				
Days Open	92	165	25	0.045
Conception Rate	60	44	12	0.304

2.2.2 Body composition

The extent that altering the level of forage and concentrate affects body composition of dairy heifers is not completely clear. Fortin et al. (1980) determined that the proportional accretion rates of water, protein, ash, and fat were not altered by two different ADG for Holstein heifers, similar to the conclusions of Old and Garrett (1987) for Herford and Charolais steers given three feeding levels. In contrast, Davis Rincker et al. (2008) observed greater fat concentration in the carcass, as estimated from rib composition, which increased linearly and quadratically with increasing energy intake; although the composition of these animals was determined at different slaughter weights. Waldo et al. (1997) observed greater proportion of the empty body was fat at the conclusion of the feeding trial when Holstein heifers were given diets for high levels of ADG and lower when ADG was lower. When comparing the two diets offered in the experiment of Waldo et al. (1997), heifers fed the the corn silage diet had a greater proportion of the empty body as fat than when heifers were fed an alfalfa silage diet. Bailey (1989) observed greater fat and lower protein accretion in Holstein steers fed low forage diets than the control steers fed a high forage diet across a large range of body weight. In contrast to these results, others (Jesse et al., 1976; Kim et al., 2003; Scollan et al., 2003) have shown that diet forage level had limited effects on body composition.

When fed for equal ADG between dietary groups, the experiments of Carson et al. (2000), Zanton and Heinrichs (2007), and Hoffman et al. (2007) also did not observe differences in growth of the skeletal measurements evaluated. Body condition score was also not different between dietary groups in the experiments of Carson et al. (2000) and

Hoffman et al. (2007). However, the results of Zanton and Heinrichs (2007) showed an increase in the paunch girth of heifers fed the high concentrate diet, which the authors attributed to greater rates of visceral fat accretion for heifers fed this diet. The recent results that nutrient utilization for fat synthesis (Baldwin, VI et al., 2007) and the greater rate of fat deposition in the omental fat depot (McLeod et al., 2007) may be affected by site and source of carbohydrate absorption combined with the results of portal and mesenteric flux differences between high forage and concentrate fed steers (Reynolds and Huntington, 1988a; Huntington, 1989) may give additional support for this hypothesis. Additionally, adipocyte cellularity and lipogenic enzyme activity may be altered by feeding level and carbohydrate source (Schoonmaker et al., 2004). Ultimately, it appears that increasing ADG and increasing the concentration of concentrate in the diet can increase fat accretion, but does not do so consistently. Differences in site of absorption and composition of absorbed nutrients likely contribute to the discrepancy in the literature. As noted previously, however, if fat accretion was enhanced for heifers fed the higher concentrate diets, it did not translate into reduced first lactation milk production.

2.3 NUTRITIONAL EFFECTS OF LIMIT-FEEDING

2.3.1 Metabolic Adaptations

Limit-fed animals are animals that are given energy or nutrients at levels below the genetic capacity for growth, although this would include intake levels down to

fasting, in practice the level is generally above maintenance and at a level required to affect a productivity benefit for the animal operation. For dairy heifers to be raised efficiently with minimal reductions in future productivity, the growth of the animal must be restricted to a level of optimal ADG (Zanton and Heinrichs, 2005). Aside from the effects that may be associated with the development of the mammary gland there are additional factors that are altered to enhance the thriftiness of the animal during periods of energy restriction.

The overall effect of limit-feeding is to shift nutrient utilization away from energy storing, toward those that are associated with maintenance and increase the efficiency of remaining biological processes (Owens et al., 1995). Much of the adaptation in nutrient utilization result from the mobilization or reduced deposition of body reserves (Jarrett et al., 1976) and a characteristically reduced size of many of the visceral tissues (Ferrell et al., 1986; Burrin et al., 1990; Johnson et al., 1990; Wester et al., 1995; McLeod and Baldwin, 2000). Energy consumption in the whole body is reduced as intake of feed is reduced (Ferrell et al., 1986; Eisemann and Nienaber, 1990; Reynolds et al., 1991a) and the proportional energy utilization by different tissues may change differentially based on level of nutrition (Eisemann and Nienaber, 1990; Ortigues and Durand, 1995).

The metabolic demand for digestion and absorption of consumed nutrients is very high (Reynolds and Huntington, 1988a; Burrin et al., 1990; Eisemann and Nienaber, 1990; Reynolds et al., 1991b; Ortigues and Durand, 1995) since the consumption of oxygen by the portal drained viscera typically exceeds 25% of whole body oxygen consumption. Assimilation and distribution of absorbed nutrients is also an energetically expensive process with the liver contributing an additional 25% of whole body oxygen

consumption (Reynolds et al., 1991a). Limit-feeding produces a reduction in the size of these metabolically active organs, which is the sole contributor to their reduced energy requirement because the mass specific oxygen utilization (Burrin et al., 1990; Johnson et al., 1990; Wester et al., 1995; McLeod and Baldwin, 2000) and substrate utilization (Baldwin and McLeod, 2000) is unaffected.

Nutrient utilization by non-visceral tissues is also affected by level of nutrition. Harris et al. (1992) determined that as dietary intake decreased the proportion of protein that was synthesized and retained in the body increased and amino acid utilization for oxidative purposes declined. Hindquarter oxygen consumption has been shown to decrease (Eisemann and Nienaber, 1990) or stay constant (Ortigues and Durand, 1995) with altered level of intake although the proportional contribution to whole body oxygen utilization is often increases due to a proportionally greater reduction in visceral tissue oxygen consumption. Thus, restricting nutrient intake results in reduced energy utilization for components of the viscera, however as the utilization of energy for maintenance purposes becomes an increasing proportion of whole body energy utilization, the efficiency of energy utilization for gain is often reduced (Reynolds et al., 1991a; Waldo et al., 1997). Depending on the extent of nutrient restriction and degree of reductions of maintenance requirements, as well as the potential for improved digestibility, the efficiency of feed utilization for gain may not be affected (Murphy and Loerch, 1994).

2.3.2 Digestive Adaptations

Limiting feed intake often improves digestibility (Tyrrell and Moe, 1975; Firkins et al., 1986; Firkins et al., 1987; Colucci et al., 1989; Zanton and Heinrichs, 2008), though many examples exist in which improvements do not occur (Reynolds et al., 1991a; Murphy et al., 1994; Waldo et al., 1997). The principle reasons for this alteration is the increased rate of passage of material from the reticulo-rumen of animals that have greater intake of feed (Blaxter et al., 1956; Colucci et al., 1990; Bhatti et al., 2008) since rate of digestion has not been reduced with increasing intake (Prigge et al., 1984; Firkins et al., 1986; Bhatti et al., 2008). The reduced rate of passage also reduces the flow of microbial protein to the small intestine (Murphy et al., 1994; Zanton and Heinrichs, 2008) and increases the bacterial energy expenditures for maintrenance (Russell, 1986). The extent in which improvements in digestibility are possible seems to depend upon the level of concentrate in the diet, which likely explains some of the discrepency in the literature. Figure 4 shows the depression in digestibility with increasing intake and the differential response with different levels of concentrate in the diet (lines calculated from the results reported in Tyrrell and Moe, 1975). This conclusion can also be made from the results of Colucci et al. (1989). From these data it would appear that the most substantial gain in digestibility response to restricted intake would be for the case in which a lower forage diet is restricted.

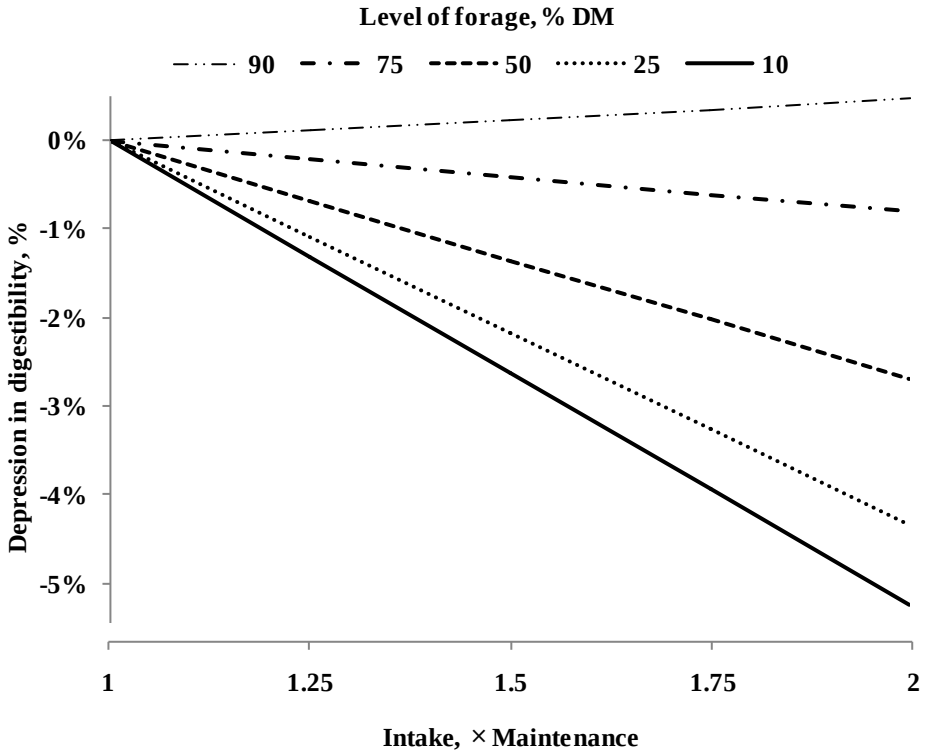

Figure 2-5: Depresion in digestibility due to intake and forage level of the diet, lines calculated from the results reported in Tyrrell and Moe (1975).

2.4 NUTRITIONAL EFFECT OF LIMIT-FEEDING LOW FORAGE/HIGH CONCENTRATE DIETS

2.4.1 Metabolic Adaptations

The efficiency of utilization of metabolizable energy has been shown to be greater for ruminants fed low as compared to high forage diets (Blaxter and Wainman, 1964). The metabolic adaptations to feeding low forage diets has been the subject of intense research for many years, particularly due to the effects that are observed with reduced milk fat production in the lactating dairy cow. The experiments that have suggested that

the production of acetate is unaffected (Davis, 1967) and propionate production increased (Bauman et al., 1971) by feeding low forage diets have had considerable influence. However, these results are not always borne out and quite often acetate absorption or production is greater for animals receiving high forage diets with propionate production unaffected (Reynolds and Huntington, 1988b; Seal et al., 1992; Reynolds et al., 1994; Huntington et al., 1996; Markantonatos et al., 2008). The partial efficiency of acetate utilization has been demonstrated to be lower in animals that have insufficient absorption of propionate (Armstrong et al., 1957; Armstrong and Blaxter, 1957a; Armstrong and Blaxter, 1957b; Tyrrell et al., 1979) and led to the conclusion that the higher acetate to propionate ratio in the rumen of high forage fed animals led to reduced metabolic efficiency.

This conclusion has not been accepted universally however (Orskov and Allen, 1966; Orskov et al., 1991; Orskov and MacLeod, 1993). Alternatively, these latter experiments focus on the reduced energy expenditure for gastrointestinal tract maintenance produced by lower tissue masses (Johnson et al., 1990; Reynolds et al., 1991a; Baldwin and McLeod, 2000) and reduced expenditures of energy for eating and digesting (Susenbeth et al., 1998; Susenbeth et al., 2004). These explanations, and potentially others, are not necessarily mutually exclusive and may combine to enhance the efficiency of utilizing digested dietary compounds.

2.4.2 Digestive Adaptations and Rumen Effects

One of the most universally observed digestive alteration with higher concentrate diets is improved dry matter digestibility (Blaxter and Wainman, 1964; Colucci et al., 1989; Reynolds et al., 1991a; Murphy et al., 1994; Moody et al., 2007; Hill et al., 2007). Additionally, in the experiment of Reynolds et al. (1991a), where ME intake was constant among differing F:C ratios fed to growing beef heifers, not only was digestibility of dry matter was increased, fecal, methane, and urinary energy lost was also significantly reduced for diets consisting of low forage. Typically high concentrate diets are composed of ingredients that are more readily fermentable than are high forage diets due to the presence of a lower concentration of neutral detergent fiber (NDF) as well as to NDF that is less extensively lignified (Van Soest, 1994). Additional factors that may contribute to a greater digestibility of feed provided as a component of a limit-fed high concentrate diets is the greater ruminal retention time (Eng et al., 1964; Goetsch and Galyean, 1982; Colucci et al., 1989; Murphy et al., 1994); although when a high concentrate diet is not limited, ruminal retention time varies with dry matter intake (Colucci et al., 1990; Poore et al., 1990). These alterations result in a greater exposure to the rumen microbial population and therefore a greater digestive potential. This potential is not always realized however, due to the rapid rate of fermentation of readily available carbohydrates resulting in a reduction in rumen pH. This is especially the case for the digestion of fibrous components of the diet when the acidity of the rumen contents becomes refractory to cellulolitic bacterial fermentation (Leedle et al., 1982; Mould et al., 1983; Russell, 1986; Russell, 1987; Mosoni et al., 2007). While there are few published reports on the

ruminal effects of limit feeding high concentrate diets compared to high forage diets, reducing the intake of a high concentrate or high forage diet both reduce pH and VFA concentrations (Murphy et al., 1994; Zanton and Heinrichs, 2008). Volatile fatty acid production (Herbein et al., 1978; Sutton et al., 2003) are generally greater (especially propionate) for high concentrate diets. Moody et al. (2007) did not observe significant reductions in mean or minimum rumen pH , although the amount of time that was spent above 6 and the maximum pH tended to be higher for heifers limit fed the high forage diet. The levels of pH reduction in that experiment, and potentially other factors, did produce a reduction in NDF digestibility without any impact on VFA concentration. In summary, compared to high forage diets, high concentrate diets are more rapidly fermentable due to their chemical composition, tend to be retained in the rumen longer when limit-fed, but may result in suboptimal rumen conditions that may limit digestibility of some feed components.

2.5 EFFECTS OF LIMIT FEEDING HIGH CONCENTRATE DIETS OF PROTEIN UTILIZATION AND EFFICIENCY

Reynolds et al. (1991a; 1991b) investigated the effects of differing the proportions of forage and concentrate in rations fed to growing beef heifers on energy metabolism at the level of the whole animal as well as for the portal drained viscera tissues and the liver. Glucose release to the periphery was significantly increased when feeding a low forage ration, possibly due to the decreased glucose metabolism by the PDV as glucose output by the liver was not significantly different between diets (Reynolds et al., 1991b). While nitrogen dynamics were discussed, the responses are difficult to resolve or to ascribe to a

particular forage-to-concentrate ratio due to differences in nitrogen intake between treatments. Of note however, is that, while nitrogen intake was greater for the high forage ration, tissue retention of nitrogen was the greatest for the low forage ration. Relative to intake, heifers fed the high forage ration excreted more fecal dry matter, nitrogen, and energy and more urinary nitrogen. While it is unclear if the improved nitrogen efficiencies are due to differences in nitrogen intake, the flow of some nitrogen containing compounds (ammonia, α-amino nitrogen, and urea) across the PDV were not significantly affected by the treatment rations fed, indicating that post-absorptive nitrogen efficiency may be improved by low forage rations.

Reynolds et al. (1991b) also found that the maximal contribution of amino acid to gluconeogenesis tended ($P<0.10$) to be reduced and significantly less ($P<0.05$) α-amino acid N was removed by the liver in the heifers fed the high concentrate ration. Similarly, Huntington et al. (1996) fed iso-nitrogenous and iso-energetic diets to six multi-catheterized beef steers to investigate the dynamics of nitrogen when fed varying proportions of forage and concentrate. In a comparison of diets containing 63 or 37% forage, significantly more urea nitrogen and glucose was released by the splachnic tissues to the periphery when fed 37% forage, while acetate release was significantly reduced. Amino acid release by the splachnic tissues was greater for the low forage diet, however statistical significance was not attained.

Several studies have recently been published investigating the level of nitrogen is required for dairy heifers (Hoffman et al., 2001; Marini and Van Amburgh, 2003; Gabler and Heinrichs, 2003; Marini et al., 2004). The combined results of these experiments and others from across a large range of dietary nitrogen intake and body weights indicates

that nitrogen was utilized at a maximum gross efficiency at 1.8 g N/kg $BW^{0.75}$ (Zanton and Heinrichs, 2007). However, none of these experiments directly compared the protein requirements of heifers with varying levels of forages. The results previously cited (Reynolds et al., 1991a; Reynolds et al., 1991b; Huntington et al., 1996), however, lead to the hypothesis that nitrogen may be utilized more efficiently when included in a high concentrate ration as opposed to a high forage ration. Several hypotheses have been posited on the mechanisms responsible for these altered efficiencies: sparing amino acids from gluconeogenesis, greater efficiency of microbial protein synthesis, and reduced urea synthesis from hepatic detoxification of ammonia (Lobley, 1992; Reynolds and Maltby, 1994; Lobley et al., 1996; Wray-Cahen et al., 1997).

A further factor that may affect the nitrogen efficiency for heifers fed high concentrate diets are the hormonal alterations that may occur. For instance, Thorp et al. (2000) observed a linear increase in the plasma concentrations of both insulin and insulin-like growth factor-1 when the levels of dietary concentrate were increased from 0 to 60% of ration dry matter at constant level of metabolizable energy intake. Janes et al. (1985) determined in sheep, using a hyperinsulinemic-euglycemic clamp, that tissue responsiveness to glucose (non-insulin dependent glucose disposal) was enhanced at higher levels of concentrate feeding, however ME intake was also higher for the sheep fed in this group. In a different experiment, when sheep maintained under thermoneutral conditions were fed increasing level of dietary protein at a constant intake of ME, the tissue responsiveness to insulin increased and the insulin sensitivity tended to increase (Sano and Terashima, 2001).

If N efficiency is also improved by reducing the proportions of forage in ruminant diets, then an opportunity may exist to reduce feed costs and the environmental impact associated with raising dairy heifers through reducing the provision of feed protein in a high concentrate, limit-feeding management system.

2.6 CONCLUSIONS

Studies have shown that feeding higher concentrate rations in a restricted manner to growing dairy heifers from 4 to 22 mo of age leads to similar growth performance with respect to weight gains and structural growth. First and second lactation performance has not been altered by the alteration of dietary concentrates in the rearing period,provided that the level of feeding is restricted to allow for optimal ADG for maximum milk production. These results also lead to the overall conclusion that provided the level of intake is restricted to allow for an optimal level of ADG, high concentrate/limit fed rations can be fed to dairy heifers successfully and can reduce feed costs and nutrient waste. Finally, feeding limited amounts of highly digestible, high concentrate rations may reduce energy requirements of the heifer through increased digestive and metabolic efficiency.

2.7 REFERENCES

Abeni, F., L. Calamari, L. Stefanini, and G. Pirlo. 2000. Effects of daily gain in pre- and postpubertal replacement dairy heifers on body condition score, body size, metabolic profile, and future milk production. J. Dairy Sci. 83:1468–1478.

Akers, R. M. 1985. Lactogenic Hormones - Binding-Sites, Mammary Growth, Secretory-Cell Differentiation, and Milk Biosynthesis in Ruminants. J. Dairy Sci. 68:501–519.

Armstrong, D. G., and K. L. Blaxter. 1957a. The Heat Increment of Steam-Volatile Fatty Acids in Fasting Sheep. British Journal of Nutrition. 11:247–&.

Armstrong, D. G., and K. L. Blaxter. 1957b. The Utilization of Acetic, Propionic and Butyric Acids by Fattening Sheep. British Journal of Nutrition. 11:413–425.

Armstrong, D. G., K. L. Blaxter, and N. M. Graham. 1957. The Heat Increments of Mixtures of Steam-Volatile Fatty Acids in Fasting Sheep. British Journal of Nutrition. 11:392–408.

Bailey, C. B. 1989. Rate and Efficiency of Gain, from Weaning to Slaughter, of Steers Given Hay, Hay Supplemented with Ruminal Undegradable Protein, Or Concentrate. Canadian Journal of Animal Science. 69:691–705.

Baldwin, R. L., and K. R. McLeod. 2000. Effects of diet forage:concentrate ratio and metabolizable energy intake on isolated rumen epithelial cell metabolism in vitro. J. Anim Sci. 78:771–783.

Baldwin, R. L., VI, K. R. McLeod, J. P. McNamara, T. H. Elsasser, and R. G. Baumann. 2007. Influence of abomasal carbohydrates on subcutaneous, omental, and mesenteric adipose lipogenic and lipolytic rates in growing beef steers. J. Anim Sci. 85:2271–2282.

Bauman, D. E., C. L. Davis, and H. F. Bucholtz. 1971. Propionate Production in the Rumen of Cows Fed Either a Control or High-Grain, Low-Fiber Diet. J. Dairy Sci. 54:1282–1287.

Berry, S. D., T. B. McFadden, R. E. Pearson, and R. M. Akers. 2001. A local increase in the mammary IGF-1: IGFBP-3 ratio mediates the mammogenic effects of estrogen and growth hormone. Domestic Animal Endocrinology. 21:39–53.

Bhatti, S. A., J. G. Bowman, J. L. Firkins, A. V. Grove, and C. W. Hunt. 2008. Effect of intake level and alfalfa substitution for grass hay on ruminal kinetics of fiber digestion and particle passage in beef cattle. J. Anim Sci. 86:134–145.

Blaxter, K. L., N. M. Graham, and F. W. Wainman. 1956. Some Observations on the Digestibility of Food by Sheep, and on Related Problems. British Journal of Nutrition. 10:69–91.

Blaxter, K. L., and F. W. Wainman. 1964. Utilization of Energy of Different Rations by Sheep + Cattle for Maintenance + for Fattening. Journal of Agricultural Science. 63:113–&.

Brown, E. G., M. J. Vandehaar, K. M. Daniels, J. S. Liesman, L. T. Chapin, J. W. Forrest, R. M. Akers, R. E. Pearson, and M. S. W. Nielsen. 2005. Effect of increasing energy and protein intake on mammary development in heifer calves. J. Dairy Sci. 88:595–603.

Burrin, D. G., C. L. Ferrell, R. A. Britton, and M. Bauer. 1990. Level of Nutrition and Visceral Organ Size and Metabolic-Activity in Sheep. British Journal of Nutrition. 64:439–448.

Capuco, A. V., G. E. Dahl, D. L. Wood, U. Moallem, and R. E. Erdman. 2004. Effect of bovine somatotropin and rumen-undegradable protein on mammary growth of prepubertal dairy heifers and subsequent milk production. J. Dairy Sci. 87:3762–3769.

Capuco, A. V., J. J. Smith, D. R. Waldo, and C. E. Rexroad, Jr. 1995. Influence of Prepubertal Dietary Regimen on Mammary Growth of Holstein Heifers. J. Dairy Sci. 78:2709–2725.

Carson, A. F., A. R. G. Wylie, J. D. G. Mc Evoy, M. Mc Coy, and L. E. R. Dawson. 2000. The effects of plane of nutrition and diet type on metabolic hormone concentrations, growth and milk production in high genetic merit dairy herd replacements. Anim. Sci. 70:349–362.

Colucci, P. E., G. K. MacLeod, W. L. Grovum, L. W. Cahill, and I. McMillan. 1989. Comparative Digestion in Sheep and Cattle Fed Different Forage to Concentrate Ratios at High and Low Intakes. J. Dairy Sci. 72:1774–1785.

Colucci, P. E., G. K. MacLeod, W. L. Grovum, I. McMillan, and D. J. Barney. 1990. Digesta Kinetics in Sheep and Cattle Fed Diets with Different Forage to Concentrate Ratios at High and Low Intakes. J. Dairy Sci. 73:2143–2156.

Davis, C. L. 1967. Acetate Production in the Rumen of Cows Fed Either Control or Low-Fiber, High-Grain Diets. J. Dairy Sci. 50:1621–1625.

Eisemann, J. H., and J. A. Nienaber. 1990. Tissue and Whole-Body Oxygen-Uptake in Fed and Fasted Steers. British Journal of Nutrition. 64:399–411.

Eng, K. S., J. C. Smith, J. H. Craig, and M. E. Riewe. 1964. Rate of Passage of Concentrate and Roughage Through Digestive Tract of Sheep. J. Anim Sci. 23:1129–1132.

Ettema, J. F., and J. E. P. Santos. 2004. Impact of Age at Calving on Lactation, Reproduction, Health, and Income in First-Parity Holsteins on Commercial Farms. J. Dairy Sci. 87:2730–2742.

Ferrell, C. L., L. J. Koong, and J. A. Nienaber. 1986. Effect of previous nutrition on body-composition and maintenance energy costs of growing lambs. Br. J. Nutr. 56:595–605.

Firkins, J. L., L. L. Berger, N. R. Merchen, G. C. Fahey, and D. R. Nelson. 1986. Effects of feed-intake and protein degradability on ruminal characteristics and site of digestion in steers. J. Dairy Sci. 69:2111–2123.

Firkins, J. L., S. M. Lewis, L. Montgomery, L. L. Berger, N. R. Merchen, and G. C. Fahey, Jr. 1987. Effects of feed intake and dietary urea concentration on ruminal dilution rate and efficiency of bacterial growth in steers. J. Dairy Sci. 70:2312–2321.

Foldager, J., and K. Sejrsen. 1987. Mammary gland development and milk production in dairy cows in relation to feeding and hormone manipulation during rearing. Pages 102–116 in Research in Cattle Production. 1 ed. Landhusholdningsselskabets Forlag, Copenhagen, Denmark.

Fortin, A., S. Simpfendorfer, J. T. Reid, H. J. Ayala, R. Anrique, and A. F. Kertz. 1980. Effect of Level of Energy Intake and Influence of Breed and Sex on the Chemical Composition of Cattle. J. Anim Sci. 51:604–614.

Gabler, M. T., and A. J. Heinrichs. 2003. Effects of increasing dietary protein on nutrient utilization in heifers. J. Dairy Sci. 86:2170–2177.

Gabler, M. T., P. R. Tozer, and A. J. Heinrichs. 2000. Development of a cost analysis spreadsheet for calculating the costs to raise a replacement dairy heifer. J. Dairy Sci. 83:1104–1109.

Gardner, R. W., J. D. Schuh, and L. G. Vargus. 1977. Accelerated Growth and Early Breeding of Holstein Heifers. J. Dairy Sci. 60:1941–1948.

Gardner, R. W., L. W. Smith, and R. L. Park. 1988. Feeding and Management of Dairy Heifers for Optimal Lifetime Productivity. J. Dairy Sci. 71:996–999.

Goetsch, A. L., and M. L. Galyean. 1982. Effect of Dietary Concentrate Level on Rumen Fluid Dilution Rate. Canadian Journal of Animal Science. 62:649–652.

Harris, P. M., P. A. Skene, V. Buchan, E. Milne, A. G. Calder, S. E. Anderson, A. Connell, and G. E. Lobley. 1992. Effect of Food-Intake on Hindlimb and Whole-Body Protein-Metabolism in Young Growing Sheep - Chronic Studies Based on Arteriovenous Techniques. British Journal of Nutrition. 68:389–407.

Heinrichs, A. J. 1993. Raising Dairy Replacements to Meet the Needs of the 21St-Century. J. Dairy Sci. 76:3179–3187.

Heinrichs, A. J. 1996. Nutrition and management of replacement cattle. Animal Feed Science and Technology. 59:155–166.

Herbein, J. H., R. W. Van Maanen, A. D. McGilliard, and J. W. Young. 1978. Rumen propionate and blood glucose kinetics in growing cattle fed isoenergetic diets. J. Nutr. 108:994–1001.

Hill, S. R., K. F. Knowlton, R. E. James, R. E. Pearson, G. L. Bethard, and K. J. Pence. 2007. Nitrogen and phosphorus retention and excretion in late-gestation dairy heifers. J. Dairy Sci. 90:5634–5642.

Hof, G., and P. J. Lenaers. 1984. The importance of roughage in the rearing period on the feed-intake and performance of adult dairy-cows. Livest. Prod. Sci. 11:287–302.

Hoffman, P. C. 1997. Optimum body size of Holstein replacement heifers. J. Anim Sci. 75:836–845.

Hoffman, P. C., N. M. Brehm, S. G. Price, and A. Prill-Adams. 1996. Effect of Accelerated Postpubertal Growth and Early Calving on Lactation Performance of Primiparous Holstein Heifers. J. Dairy Sci. 79:2024–2031.

Hoffman, P. C., N. M. Esser, L. M. Bauman, S. L. Denzine, M. Engstrom, and H. Chester-Jones. 2001. Short communication: Effect of dietary protein on growth and nitrogen balance of Holstein heifers. J. Dairy Sci. 84:843–847.

Hoffman, P. C., and D. A. Funk. 1992. Applied Dynamics of Dairy Replacement Growth and Management. J. Dairy Sci. 75:2504–2516.

Hoffman, P. C., C. R. Simson, and M. Wattiaux. 2007. Limit feeding of gravid Holstein heifers: Effect on growth, manure nutrient excretion, and subsequent early lactation performance. J. Dairy Sci. 90:946–954.

Huntington, G. B. 1989. Hepatic Urea Synthesis and Site and Rate of Urea Removal from Blood of Beef Steers Fed Alfalfa Hay Or A High Concentrate Diet. Canadian Journal of Animal Science. 69:215–223.

Huntington, G. B., E. J. Zetina, J. M. Whitt, and W. Potts. 1996. Effects of dietary concentrate level on nutrient absorption, liver metabolism, and urea kinetics of beef steers fed isonitrogenous and isoenergetic diets. J. Anim Sci. 74:908–916.

Janes, A. N., T. E. Weekes, and D. G. Armstrong. 1985. Insulin action and glucose metabolism in sheep fed on dried-grass or ground, maize-based diets. Br. J. Nutr. 54:459–471.

Jarrett, I. G., O. H. Filsell, and F. J. Ballard. 1976. Utilization of Oxidizable Substrates by Sheep Hind Limb - Effects of Starvation and Exercise. Metabolism-Clinical and Experimental. 25:523–531.

Jesse, G. W., G. B. Thompson, J. L. Clark, H. B. Hedrick, and K. G. Weimer. 1976. Effects of Ration Energy and Slaughter Weight on Composition of Empty Body and Carcass Gain of Beef-Cattle. J. Anim Sci. 43:418–425.

Johnson, D. E., K. A. Johnson, and R. L. Baldwin. 1990. Changes in Liver and Gastrointestinal Tract Energy Demands in Response to Physiological Workload in Ruminants. J. Nutr. 120:649–655.

Kim, E. J., N. D. Scollan, M. S. Dhanoa, and P. J. Buttery. 2003. Effects of supplementary concentrates on growth and partitioning of nutrients between different body components in steers fed on grass silage at similar levels of metabolizable energy intake. J. Agric. Sci. 141:103–112.

Lammers, B. P., A. J. Heinrichs, and R. S. Kensinger. 1999. The effects of accelerated growth rates and estrogen implants in prepubertal Holstein heifers on estimates of mammary development and subsequent reproduction and milk production. J. Dairy Sci. 82:1753–1764.

Leedle, J. A., M. P. Bryant, and R. B. Hespell. 1982. Diurnal variations in bacterial numbers and fluid parameters in ruminal contents of animals fed low- or high-forage diets. Appl. Environ. Microbiol. 44:402–412.

Little, W., and R. M. Kay. 1979. Effects of Rapid Rearing and Early Calving on the Subsequent Performance of Dairy Heifers. Animal Production. 29:131–142.

Lobley, G. E. 1992. Control of the metabolic fate of amino acids in ruminants: a review. J. Anim Sci. 70:3264–3275.

Lobley, G. E., P. J. Weijs, A. Connell, A. G. Calder, D. S. Brown, and E. Milne. 1996. The fate of absorbed and exogenous ammonia as influenced by forage or forage-concentrate diets in growing sheep. Br. J. Nutr. 76:231–248.

Marini, J. C., J. D. Klein, J. M. Sands, and M. E. Van Amburgh. 2004. Effect of nitrogen intake on nitrogen recycling and urea transporter abundance in lambs. J. Anim Sci. 82:1157–1164.

Marini, J. C., and M. E. Van Amburgh. 2003. Nitrogen metabolism and recycling in Holstein heifers. J. Anim. Sci. 81:545–552.

Markantonatos, X., M. H. Green, and G. A. Varga. 2008. Use of compartmental analysis to study ruminal volatile fatty acid metabolism under steady state conditions in Holstein heifers. Animal Feed Science and Technology. 143:70–88.

McLeod, K. R., and R. L. Baldwin. 2000. Effects of diet forage:concentrate ratio and metabolizable energy intake on visceral organ growth and in vitro oxidative capacity of gut tissues in sheep. J. Anim Sci. 78:760–770.

McLeod, K. R., R. L. Baldwin, VI, M. B. Solomon, and R. G. Baumann. 2007. Influence of ruminal and postruminal carbohydrate infusion on visceral organ mass and adipose tissue accretion in growing beef steers. J. Anim Sci. 85:2256–2270.

Meyer, M. J., A. V. Capuco, D. A. Ross, L. M. Lintault, and M. E. Van Amburgh. 2006a. Developmental and nutritional regulation of the prepubertal bovine mammary gland: II. Epithelial cell proliferation, parenchymal accretion rate, and allometric growth. J. Dairy Sci. 89:4298–4304.

Meyer, M. J., A. V. Capuco, D. A. Ross, L. M. Lintault, and M. E. Van Amburgh. 2006b. Developmental and nutritional regulation of the prepubertal heifer mammary gland: I. Parenchyma and fat pad mass and composition. J. Dairy Sci. 89:4289–4297.

Moody, M. L., G. I. Zanton, J. M. Daubert, and A. J. Heinrichs. 2007. Nutrient utilization of differing forage-to-concentrate ratios by growing Holstein heifers. J. Dairy Sci. 90:5580–5586.

Mosoni, P., F. Chaucheyras-Durand, C. Bera-Maillet, and E. Forano. 2007. Quantification by real-time PCR of cellulolytic bacteria in the rumen of sheep after supplementation of a forage diet with readily fermentable carbohydrates: effect of a yeast additive. J. Appl. Microbiol. 103:2676–2685.

Mould, F. L., E. R. Orskov, and S. O. Mann. 1983. Associative effects of mixed feeds. 1. Effects of type and level of supplementation and the influence of the rumen fluid pH on cellulolysis in vivo and dry-matter digestion of various roughages. Anim. Feed Sci. Technol. 10:15–30.

Murphy, T. A., and S. C. Loerch. 1994. Effects of restricted feeding of growing steers on performance, carcass characteristics, and composition. J. Anim Sci. 72:2497–2507.

Murphy, T. A., S. C. Loerch, and B. A. Dehority. 1994. The influence of restricted feeding on site and extent of digestion and flow of nitrogenous compounds to the duodenum in steers. J. Anim Sci. 72:2487–2496.

Nennich, T. D., J. H. Harrison, L. M. VanWieringen, D. Meyer, A. J. Heinrichs, W. P. Weiss, N. R. St-Pierre, R. L. Kincaid, D. L. Davidson, and E. Block. 2005. Prediction of Manure and Nutrient Excretion from Dairy Cattle. J. Dairy Sci. 88:3721–3733.

Old, C. A., and W. N. Garrett. 1987. Effects of Energy Intake on Energetic Efficiency and Body Composition of Beef Steers Differing in Size at Maturity. J. Anim Sci. 65:1371–1380.

Orskov, E. R., and D. M. Allen. 1966. Utilization of Salts of Volatile Fatty Acids by Growing Sheep .1. Acetate Propionate and Butyrate As Sources of Energy for Young Growing Lambs. British Journal of Nutrition. 20:295–&.

Orskov, E. R., and N. A. MacLeod. 1993. Effect of Level of Input of Different Proportions of Volatile Fatty-Acids on Energy-Utilization in Growing Ruminants. British Journal of Nutrition. 70:679–687.

Orskov, E. R., N. A. MacLeod, and Y. Nakashima. 1991. Effect of different volatile fatty acids mixtures on energy metabolism in cattle. J. Anim Sci. 69:3389–3397.

Ortigues, I., and D. Durand. 1995. Adaptation of Energy-Metabolism to Undernutrition in Ewes - Contribution of Portal-Drained Viscera, Liver and Hindquarters. British Journal of Nutrition. 73:209–226.

Owens, F. N., D. R. Gill, D. S. Secrist, and S. W. Coleman. 1995. Review of some aspects of growth and development of feedlot cattle. J. Anim Sci. 73:3152–3172.

Peri, I., A. Gertler, I. Bruckental, and H. Barash. 1993. The Effect of Manipulation in Energy Allowance During the Rearing Period of Heifers on Hormone Concentrations and Milk-Production in 1St Lactation Cows. J. Dairy Sci. 76:742–751.

Poore, M. H., J. A. Moore, and R. S. Swingle. 1990. Differential passage rates and digestion of neutral detergent fiber from grain and forages in 30, 60 and 90% concentrate diets fed to steers. J. Anim Sci. 68:2965–2973.

Prigge, E. C., M. J. Baker, and G. A. Varga. 1984. Comparative digestion, rumen fermentation and kinetics of forage diets by steers and wethers. J. Anim Sci. 59:237–245.

Radcliff, R. P., M. J. Vandehaar, L. T. Chapin, T. E. Pilbeam, D. K. Beede, E. P. Stanisiewski, and H. A. Tucker. 2000. Effects of diet and injection of bovine somatotropin on prepubertal growth and first-lactation milk yields of Holstein cows. J. Dairy Sci. 83:23–29.

Radcliff, R. P., M. J. Vandehaar, A. L. Skidmore, L. T. Chapin, B. R. Radke, J. W. Lloyd, E. P. Stanisiewski, and H. A. Tucker. 1997. Effects of diet and bovine somatotropin on heifer growth and mammary development. J. Dairy Sci. 80:1996–2003.

Reynolds, C. K., D. L. Harmon, R. L. Prior, and H. F. Tyrrell. 1994. Effects of mesenteric vein L-alanine infusion on liver metabolism of organic acids by beef heifers fed diets differing in forage: concentrate ratio. J. Anim Sci. 72:3196–3206.

Reynolds, C. K., and G. B. Huntington. 1988b. Partition of Portal-Drained Visceral Net Flux in Beef Steers .2. Net Flux of Volatile Fatty-Acids, D-Beta-Hydroxybutyrate and L-Lactate Across Stomach and Post-Stomach Tissues. British Journal of Nutrition. 60:553–562.

Reynolds, C. K., and G. B. Huntington. 1988a. Partition of Portal-Drained Visceral Net Flux in Beef Steers .1. Blood-Flow and Net Flux of Oxygen, Glucose and Nitrogenous Compounds Across Stomach and Post-Stomach Tissues. British Journal of Nutrition. 60:539–551.

Reynolds, C. K., and S. A. Maltby. 1994. Regulation of nutrient partitioning by visceral tissues in ruminants. J. Nutr. 124:1399S–1403S.

Reynolds, C. K., H. F. Tyrrell, and P. J. Reynolds. 1991b. Effects of Diet Forage-To-Concentrate Ratio and Intake on Energy-Metabolism in Growing Beef Heifers - Net Nutrient Metabolism by Visceral Tissues. J. Nutr. 121:1004–1015.

Reynolds, C. K., H. F. Tyrrell, and P. J. Reynolds. 1991a. Effects of diet forage-to-concentrate ratio and intake on energy-metabolism in growing beef heifers - Whole-body energy and nitrogen-balance and visceral heat-production. J. Nutr. 121:994–1003.

Rincker, L. E. D., M. S. W. Nielsen, L. T. Chapin, J. S. Liesman, K. M. Daniels, R. M. Akers, and M. J. Vandehaar. 2008. Effects of feeding prepubertal heifers a high-energy diet for three, six, or twelve weeks on mammary growth and composition. J. Dairy Sci. 91:1926–1935.

Russell, J. B. 1987. Effect of extracellular pH on growth and proton motive force of Bacteroides succinogenes, a cellulolytic ruminal bacterium. Appl. Environ. Microbiol. 53:2379–2383.

Russell, J. B. 1986. Heat production by ruminal bacteria in continuous culture and its relationship to maintenance energy. J. Bacteriol. 168:694–701.

Sano, H., and Y. Terashima. 2001. Effects of dietary protein level and cold exposure on tissue responsiveness and sensitivity to insulin in sheep. J. Anim Physiol Anim Nutr. (Berl). 85:349–355.

Schoonmaker, J. P., F. L. Fluharty, and S. C. Loerch. 2004. Effect of source and amount of energy and rate of growth in the growing phase on adipocyte cellularity and lipogenic enzyme activity in the intramuscular and subcutaneous fat depots of Holstein steers. J. Anim Sci. 82:137–148.

Scollan, N. D., M. S. Dhanoa, E. J. Kim, J. M. Dawson, and P. J. Buttery. 2003. Effects of diet and stage of development on partitioning of nutrients between fat and lean deposition in steers. Anim. Sci. 76:237–249.

Seal, C. J., D. S. Parker, and D. P. J. Avery. 1992. The Effect of Forage and Forage Concentrate Diets on Rumen Fermentation and Metabolism of Nutrients by the Mesenteric-Drained and Portal-Drained Viscera in Growing Steers. British Journal of Nutrition. 67:355–370.

Sejrsen, K., J. T. Huber, and H. A. Tucker. 1983. Influence of Amount Fed on Hormone Concentrations and Their Relationship to Mammary Growth in Heifers. J. Dairy Sci. 66:845–855.

Sejrsen, K., J. T. Huber, H. A. Tucker, and R. M. Akers. 1982. Influence of nutrition on mammary development in pre- and postpubertal heifers. J. Dairy Sci. 65:793–800.

Sejrsen, K., and S. Purup. 1997. Influence of prepubertal feeding level on milk yield potential of dairy heifers: a review. J. Anim Sci. 75:828–835.

Sejrsen, K., S. Purup, M. Vestergaard, and J. Foldager. 2000. High body weight gain and reduced bovine mammary growth: physiological basis and implications for milk yield potential. Domest. Anim. Endocrinol. 19:93–104.

Sejrsen, K., J. Foldager, M. T. Sorensen, R. M. Akers, and D. E. Bauman. 1986. Effect of Exogenous Bovine Somatotropin on Pubertal Mammary Development in Heifers. J. Dairy Sci. 69:1528–1535.

Silva, L. F. P., B. E. Etchebarne, M. S. Weber Nielsen, J. S. Liesman, M. Kiupel, and M. J. Vandehaar. 2008. Intramammary Infusion of Leptin Decreases Proliferation of Mammary Epithelial Cells in Prepubertal Heifers. J. Dairy Sci. 91:3034–3044.

Silva, L. F. P., J. S. Liesman, B. E. Etchebarne, M. S. W. Nielsen, and M. J. Vandehaar. 2005. Short Communication: Intramammary Infusion of IGF-I Increases Bromodeoxyuridine Labeling in Mammary Epithelial Cells of Prepubertal Heifers. J. Dairy Sci. 88:2771–2773.

Silva, L. F. P., M. J. Vandehaar, M. S. Weber Nielsen, and G. W. Smith. 2002a. Evidence for a Local Effect of Leptin in Bovine Mammary Gland. J. Dairy Sci. 85:3277–3286.

Silva, L. F. P., M. J. Vandehaar, B. K. Whitlock, R. P. Radcliff, and H. A. Tucker. 2002b. Short Communication: Relationship Between Body Growth and Mammary Development in Dairy Heifers. J. Dairy Sci. 85:2600–2602.

Susenbeth, A., T. Dickel, K. H. Sudekum, W. Drochner, and H. Steingass. 2004. Energy requirements of cattle for standing and for ingestion, estimated by a ruminal emptying technique. J. Anim Sci. 82:129–136.

Susenbeth, A., R. Mayer, B. Koehler, and O. Neumann. 1998. Energy requirement for eating in cattle. J. Anim Sci. 76:2701–2705.

Sutton, J. D., M. S. Dhanoa, S. V. Morant, J. France, D. J. Napper, and E. Schuller. 2003. Rates of production of acetate, propionate, and butyrate in the rumen of lactating dairy cows given normal and low-roughage diets. J. Dairy Sci. 86:3620–3633.

Swanson, E. W. 1960. Effect of rapid growth with fattening of dairy heifers on their lactational ability. J. Dairy Sci. 43:377–387.

Thibault, C., D. Petitclerc, R. Spratt, M. Leonard, K. Sejrsen, and P. Lacasse. 2003. Effect of Feeding Prepubertal Heifers with a High Oil Diet on Mammary Development and Milk Production. J. Dairy Sci. 86:2320–2326.

Thorp, C. L., A. R. G. Wylie, R. W. J. Steen, C. Shaw, and J. D. Mcevoy. 2000. Effects of incremental changes in forage: concentrate ratio on plasma hormone and metabolite concentrations and products of rumen fermentation in fattening beef steers. Anim. Sci. 71:93–109.

Tozer, P. R., and A. J. Heinrichs. 2001. What affects the costs of raising replacement dairy heifers: a multiple-component analysis. J. Dairy Sci. 84:1836–1844.

Tyrrell, H. F., and P. W. Moe. 1975. Effect of intake on digestive efficiency. J. Dairy Sci. 58:1151–1163.

Tyrrell, H. F., P. J. Reynolds, and P. W. Moe. 1979. Effect of Diet on Partial Efficiency of Acetate Use for Body Tissue Synthesis by Mature Cattle. J. Anim Sci. 48:598–606.

Van Amburgh, M. E., D. M. Galton, D. E. Bauman, R. W. Everett, D. G. Fox, L. E. Chase, and H. N. Erb. 1998. Effects of three prepubertal body growth rates on performance of Holstein heifers during first lactation. J. Dairy Sci. 81:527–538.

Van Soest, P. J. 1994. Nutritional Ecology of the Ruminant. 2 ed. Cornell University Press, Ithaca, NY.

vis Rincker, L. E., M. S. Weber Nielsen, L. T. Chapin, J. S. Liesman, and M. J. Vandehaar. 2008. Effects of Feeding Prepubertal Heifers a High-Energy Diet for Three, Six, or Twelve Weeks on Feed Intake, Body Growth, and Fat Deposition. J. Dairy Sci. 91:1913–1925.

Waldo, D. R., A. V. Capuco, and C. E. Rexroad. 1998. Milk production of Holstein heifers fed either alfalfa or corn silage diets at two rates of daily gain. J. Dairy Sci. 81:756–764.

Waldo, D. R., H. F. Tyrrell, A. V. Capuco, and C. E. Rexroad, Jr. 1997. Components of Growth in Holstein Heifers Fed Either Alfalfa or Corn Silage Diets to Produce Two Daily Gains. J. Dairy Sci. 80:1674–1684.

Wester, T. J., R. A. Britton, T. J. Klopfenstein, G. A. Ham, D. T. Hickok, and C. R. Krehbiel. 1995. Differential effects of plane of protein or energy nutrition on visceral organs and hormones in lambs. J. Anim Sci. 73:1674–1688.

Whitlock, B. K., M. J. Vandehaar, L. F. P. Silva, and H. A. Tucker. 2002. Effect of Dietary Protein on Prepubertal Mammary Development in Rapidly Growing Dairy Heifers. J. Dairy Sci. 85:1516–1525.

Wray-Cahen, D., J. A. Metcalf, F. R. Backwell, B. J. Bequette, D. S. Brown, J. D. Sutton, and G. E. Lobley. 1997. Hepatic response to increased exogenous supply of plasma amino acids by infusion into the mesenteric vein of Holstein-Friesian cows in late gestation. Br. J. Nutr. 78:913–930.

Zanton, G. I., and A. J. Heinrichs. 2008. Rumen digestion and nutritional efficiency of dairy heifers limit-fed a high forage ration to four levels of dry matter intake. J. Dairy Sci. 91:3579–3588.

Zanton, G. I., and A. J. Heinrichs. 2005. Meta-analysis to assess effect of prepubertal average daily gain of Holstein heifers on first-lactation production. J. Dairy Sci. 88:3860–3867.

Zanton, G. I., and A. J. Heinrichs. 2007. The Effects of Controlled Feeding of a High-Forage or High-Concentrate Ration on Heifer Growth and First-Lactation Milk Production. J. Dairy Sci. 90:3388–3396.

Chapter 3

DIGESTION AND NITROGEN UTILIZATION IN DAIRY HEIFERS LIMIT-FED A LOW OR HIGH FORAGE RATION AT FOUR LEVELS OF NITROGEN INTAKE

3.1 ABSTRACT

The hypothesis of this experiment is that a low forage (LF) ration will be utilized with a greater efficiency than a high forage ration (HF) by dairy heifers and that the response will be affected by level of N intake. To test this hypothesis, 8 Holstein heifers (beginning at 362 ± 7 kg and 12.3 ± 0.4 mo) were fed eight rations according to a split-plot, 4 x 4 Latin square design. Treatments were formulated to contain 25% or 75% forage (corn silage and chopped wheat straw) and fed at 4 levels of N intake [0.94 (Low), 1.62 (MLow), 2.30 (MHigh), 2.96 (High) g N/kg $BW^{0.75}$ per d]. Diets were limit-fed to maintain equal ME intake. Blood samples were collected over d 19-20 and feces and urine were collected for 8 d/ 28 d period. Organic matter intake was greater for heifers fed HF, but, due to increased OM digestibility of LF (74.0 vs 67.6% ± 0.9; P <0.01), digestible OMI was unaffected by forage level (P > 0.50). OM digestibility was affected by an interaction between forage level and N intake (P < 0.01); increasing to a plateau of 78.01% at 18.43 %CP for LF and 68.78% at 13.90 %CP for HF fed heifers. Apparent N digestibility was greater for heifers fed LF and increased from 47.7% to 80.8% between Low and High N intake. Less N appeared in the feces of heifers fed LF than HF (45.56 vs 52.60 g per d). Urea-N excretion was not different between forage levels, but increased

linearly with N intake. Concentration of PUN was significantly higher for LF and with increasing N intake. Urea clearance rate (L/h) did not differ between forage levels and increased, but at a decreasing rate, as N intake increased. A significant interaction resulted from urea clearance increasing at a greater rate and obtaining higher values for HF, whereas clearance of urea for heifers fed LF obtained significantly lower maximal values. Like urea-N excretion, daily urinary N excretion was affected only by N intake. Retained N responded linearly to increased levels of NI. The significant reduction observed in fecal N excretion for LF was counterbalanced by numerical increases in urinary N excretion so that total N excretion and retention were not different between forage levels. The percent of N intake that was retained only tended to be affected by an interaction and was not significantly affected by forage level. It is concluded that increasing N intake increases the digestibility of OM, the magnitude of which depends on the level of dietary forage provided. Furthermore, differences in N utilization between LF and HF in this trial were small and were not evident until N intake increased to impractical levels.

3.2 INTRODUCTION

Utilization of dietary nitrogen is very inefficient in growing ruminants when compared to growing non-ruminant farm animals. Reasons for this observation are varied and include utilization of absorbed amino acids for gluconeogenesis (Reynolds et al., 1991a), utilization of amino acids to support gastrointestinal tract (**GIT**) protein turnover (Attaix et al., 1988) and energy requirements (Lobley et al., 2003), and deamination of dietary amino acids by the ruminal microflora (Eschenlauer et al., 2002). However, the presence of the ruminal microflora does provide the ruminant with an opportunity to consume non-protein nitrogen to meet some, and potentially all, of amino acid requirements through AA synthesis from NH_3 and dietary carbohydrate (Virtanen, 1966); the extent of incorporation of NH_3 to microbial AA depending on preformed AA availability (Atasoglu et al., 2004) and the source of carbohydrate (Hristov et al., 2005).

When low forage (**LF**) diets are fed to ruminants at energy intakes equal to high forage (**HF**) diets, N retention and efficiency of N utilization are often improved with the LF diets (Murphy et al., 1994; Driedger and Loerch, 1999; Moody et al., 2007), although the improvements do not attain statistical significance in all cases (Reynolds et al., 1991b; Kirkpatrick et al., 1997; Hill et al., 2007). However, in many of the experiments in which forage to concentrate ratios have been altered and N utilization evaluated, the rations either differed in N intake (g/d), energy intake (Mcal ME/d), or both; although altering the levels of N and energy intake impacts the partitioning of N between retention

and excretion in growing cattle (Schroeder and Titgemeyer, 2008; Zanton and Heinrichs, 2008a).

Improved feed efficiency and unaltered growth and first lactation characteristics have been observed from dairy heifers raised with reduced proportions of dietary forage provided at restricted intakes (Hoffman et al., 2007; Zanton and Heinrichs, 2007). If N efficiency is also improved by reducing the proportions of forage in ruminant diets, then an opportunity may exist to reduce feed costs and the environmental impact associated with raising dairy heifers through reducing the provision of feed protein in a LF, limit-feeding management system. The extent of alterations in N utilization for limit-fed LF and HF diets across a large range of N intakes is unavailable. Therefore, the objective of this experiment was to evaluate efficiency of nutrient and N utilization of dairy heifers fed LF and HF diets at equal ME intakes and 4 levels of N intake. The hypothesis is that N will be utilized with improved efficiency when provided as a component of a LF diet compared to a HF diet.

3.3 MATERIALS AND METHODS

3.3.1 Animals, Diets, and Experimental Design

All procedures involving the use of animals were approved by the Pennsylvania State University Institutional Animal Care and Use Committee. Eight Holstein heifers (12.3 ± 0.4 mo; 362 ± 7 kg initial age and weight, respectively) were randomly assigned to 2 forage levels: LF (25% forage) and HF (75% forage) and to a N intake sequence

within forage level administered according to a split-plot, 4 × 4 Latin square design with 28 d periods. The whole plot factor is the proportion of forage in the diet and the subplot is the level of N intake and all diets were provided at a level calculated to provide equal intakes of ME. The forage components of the diet, corn silage and wheat straw, were chosen to provide minimal levels of N to the diet so as to enable a greater range of N intakes allowable and held constant within forage level to reduce the impact that variation in forage N concentration would have on the N intake between forage levels. The levels of N intake chosen were based on the range of N intakes observed in the analysis of literature data by Zanton and Heinrichs (2008a) and were formulated to be 1.00 (**Low**), 1.67 (**MLow**), 2.33 (**MHigh**), 3.00 (**High**) g N/kg $BW^{0.75}$ per d. Because energy concentration of the formulated rations differed between forage levels, DMI was greater for the HF group and, consequently, the concentration of N was required to be lower for heifers fed the HF diet to maintain equal N intakes between forage levels. Alterations in N concentration were made by exchanging high protein by-products and urea for starch in both forage levels without changing the forage component of the diet (Table 1). Rations were balanced to maintain a consistent chemical composition across N intakes (within forage level) and equivalent DMI within forage level.

Heifers were weighed on d 0 and 1, 7 and 8, 14 and 15 of each period; the amount of feed offered for the following interval being based on the average of the previous 2 weights and provided at a level formulated to allow for 800 g/d. Rations were mixed daily (Super Data Ranger, American Calan, Northwood, NH) prior to feeding at 1200 h by preparing the LF, high and low N rations and the HF, high and low N rations and by mixing the appropriate proportion of the two rations to prepare the intermediate N

Table. **3-1**: Ingredient and chemical composition of treatment rations offered containing low or high levels of forage and 4 levels of N intake

	LF				HF			
Ingredients, % DM	Low	MLow	MHigh	High	Low	MLow	MHigh	High
Corn Silage[1]	12.92	12.93	12.94	12.95	51.57	51.59	51.61	51.63
Wheat Straw[2]	11.76	11.77	11.78	11.80	23.49	23.50	23.51	23.52
Cracked Corn	70.40	62.10	53.79	45.49	19.44	12.96	6.48	0.00
Soy Expellers	0.00	1.67	3.33	5.00	0.00	1.30	2.60	3.89
SBM	0.00	1.82	3.64	5.46	0.00	0.00	0.00	0.00
Fish Meal	0.00	2.41	4.82	7.23	0.20	2.95	5.69	8.44
Blood Meal	0.00	1.61	3.22	4.83	0.90	2.91	4.91	6.92
Cane Molasses	1.92	1.92	1.92	1.93	1.92	1.92	1.92	1.92
Vegetable Oil[3]	0.21	0.14	0.07	0.00	0.88	0.73	0.59	0.44
Urea	0.17	0.74	1.32	1.90	0.00	0.47	0.94	1.40
$NaHCO_3$	0.80	1.00	1.19	1.39	0.00	0.21	0.41	0.62
Limestone	0.76	0.66	0.56	0.46	0.26	0.17	0.09	0.00
Salt	0.00	0.00	0.00	0.00	0.63	0.46	0.29	0.12
CaSO4	0.16	0.25	0.35	0.44	0.00	0.08	0.16	0.24
$K_2SO_4 \cdot MgSO_4$	0.16	0.25	0.35	0.44	0.05	0.11	0.18	0.24
TM Salt[4]	0.19	0.18	0.18	0.17	0.16	0.15	0.15	0.15
Vitamins A,D,E[5]	0.28	0.27	0.26	0.26	0.25	0.25	0.24	0.24
Vitamin E[6]	0.28	0.27	0.26	0.26	0.25	0.25	0.24	0.24
Chemical Composition								
CP, %DM	7.87	13.70	19.53	25.36	7.45	12.60	17.76	22.91
MP[7], %DM	6.00	10.02	13.12	15.18	5.32	8.80	12.45	14.99
NPN, % N	34.32	35.12	35.92	36.72	50.47	47.13	43.78	40.44
Soluble CP, % CP	45.61	44.11	42.62	41.12	51.47	47.87	44.27	40.67
RDP, % CP[7]	66.01	62.83	61.71	60.29	56.48	52.38	51.60	50.33
RUP, % CP[7]	33.99	37.17	38.29	39.71	43.52	47.62	48.40	49.67
NDF, %DM	24.48	23.80	23.11	22.43	42.34	41.69	41.03	40.37
ADF, %DM	14.25	14.08	13.92	13.76	27.33	27.11	26.88	26.66
Hemicellulose[8], %DM	10.23	9.71	9.19	8.67	15.01	14.58	14.15	13.72
Starch, %DM	58.36	52.24	46.13	40.01	34.67	29.83	25.00	20.17
Ca, %DM	0.32	0.46	0.59	0.72	0.31	0.45	0.60	0.74
P, %DM	0.21	0.29	0.37	0.45	0.17	0.25	0.33	0.42
ME[7], Mcal/kg	2.69	2.72	2.72	2.73	2.39	2.40	2.40	2.41
ME[9], Mcal/kg	2.36	2.46	2.58	2.55	2.16	2.33	2.33	2.30
Na, %DM	0.27	0.34	0.41	0.48	0.29	0.31	0.33	0.34
K, %DM	0.57	0.66	0.75	0.85	0.87	0.91	0.96	1.00
Cl, %DM	0.26	0.29	0.31	0.33	0.74	0.67	0.60	0.53
S, %DM	0.18	0.26	0.35	0.43	0.15	0.23	0.30	0.38
N:S	6.92	7.76	8.59	9.42	7.82	8.46	9.10	9.74

[1] Corn silage contained 7.58% CP, 39.50 % NDF, 24.80 % ADF, 32.75 % Starch
[2] Wheat straw contained 3.40% CP, 82.17 % NDF, 56.77 % ADF, 0.67 % Starch
[3] 50% corn oil and 50 % soybean oil
[4] TM Salt contained Na, 33.81%; Cl, 52.13%; Co, 70; Cu, 400; I, 70; Fe, 1750; Mn, 2800; Zn, 3500 ppm
[5] Vitamins A, D, E: 4,000 kIU of vitamin A/kg; 1,000 kIU of vitamin D/kg; 4,000 IU of vitamin E/kg
[6] Vitamin E contained 20,000 IU of vitamin E/kg
[7] MP: metabolizable protein, RDP: rumen degradable protein, RUP: rumen undegradable protein; ME: metabolizable energy; calculated according to NRC, 2001 using observed BW, DMI, and ingredients.
[8] Hemicellulose = NDF – ADF
[9] ME: calculated as digestible OM × 0.82 × 4.409 / DMI

treatment rations. Corn silage DM was evaluated thrice weekly and ration alterations were made as required; the DM concentration of the LF ration was reduced to 65% (from approximately 75%) by the addition of water to minimize dustiness and within-meal sorting. Refusals, if present, and feed samples were collected daily during sample collection periods immediately prior to feeding. Heifers were housed in a ventilated, environmentally controlled tie stall barn with rubber mattress bedding. Heifers were allowed access to an exercise lot for 2 h prior to feeding on days that sampling was not occurring. Water was available for ad libitum consumption and the volume of water consumed daily was monitored using unidirectional flow meters (Sensus Metering Systems, Uniontown, PA).

3.3.2 Sample Collection and Analysis

Adaptation to treatment rations were made over the first 18 d of each period followed by 10 d of sampling. Between 0600 and 0800 on d 18, heifers were catheterized in the right jugular vein (16 GA, 13.3 cm; BD Angiocath, Franklin Lakes, NJ) and sampled at times relative to feeding of 0, 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 16, 20, 24 h. Immediately after sampling, blood was centrifuged at 4,000 × g at 4°C for 15 min, plasma was aspirated and stored at -20°C until analysis for urea N (procedure no. 0580; Stanbio Laboratory Inc., San Antonio, TX).

Feces and urine was completely collected from d 20 immediately after feeding to d 28 immediately prior to feeding for 8 days of total collection. Urine was collected through the use of the urine cup collection method of Fellner et al. (1988) modified to

enable floor-level urine collection and attachment without gluing the cups to the heifer. Urine was weighed and subsampled daily after feeding. Urine pH was monitored and acidified to pH < 2 by the addition of 12 N HCl as required; realized equivalents of H^+ added was 1.30, 1.36, 1.52, and 1.61 for N intake levels Low to High, respectively (SE: ±0.08; Forage effect *P* >0.35). These levels of acidification would be sufficient to capture at least 10% of digested N (assuming no N retention) where NH_3 typically comprises <10% of urine N (Szanyiova et al., 1995). Feces were collected as deposited into covered containers, which were weighed and subsampled daily after feeding. On 2 days per period a subsample of feces and unacidified, chilled (4°C) urine was subsampled from 4 heifers (1 observation per heifer per period) and ammonia volatilization was analyzed using a bench-top, steady-state (dynamic) flux chamber under laboratory conditions as detailed by Lascano et al. (2008).

Feed ingredients (dry basis) and feces (wet basis) were composited by period. Samples were dried in a forced air oven at 55°C for 48 h, ground through a 1-mm screen (Wiley mill, Arthur Thomas, Philadelphia, PA), and analyzed for analytical DM by drying a 102°C for 48 h and ash by combustion at 600°C for 6 h (Association of Official Analytical Chemists, 1990), NDF (with the inclusion of α-amylase and sodium sulfite) and ADF (Van Soest et al., 1991). Starch was analyzed on samples reground to pass through a 0.5 mm screen by a modification of the procedure reported by Bach Knudsen (1997; modification included 150 mg of sample, 45 units of amyloglucosidase, and analysis of released glucose monomers with procedure no. 1075, Stanbio Laboratory Inc., San Antonio, TX). Feed N was analyzed on dried samples and fecal and urine N was analyzed on wet samples by the Kjeldahl method (Association of Official Analytical

Chemists, 1990). Urine was also analyzed for urea N (procedure no. 0580; Stanbio Laboratory Inc., San Antonio, TX) and creatinine (procedure no. 0400; Stanbio Laboratory Inc., San Antonio, TX). Fractionation of feed and fecal N were determined on dried samples as non-protein N [TCA method; feed only], acid detergent insoluble N, and neutral detergent insoluble N by the procedures of Lictra et al. (1996). Metabolizable energy intake was calculated for each heifer within each period using the observed digestible OM intake × 4.409 × 0.82 (National Research Council, 2001) assuming digestible OM intake and TDN intake are equal.

3.3.3 Statistical Analysis

Data were analyzed as a split-plot, Latin square design with fixed effects of period, forage, N intake level, and forage × N intake interaction and a random effect of heifer(forage). Adaptation to treatment lasted 18 d based on the results of Biddle et al. (1975) in which stabilization of urine N excretion occurred at approximately 3 wks of consuming a N depletion ration; responses during repletion were less consistent. Given this consideration, N level treatment sequences were balanced for carryover with respect to previous N level such that all treatments followed every other treatment once; therefore, the fixed effect of previous treatment was included in the statistical analysis. For responses in which multiple observations occur within a period the fixed effect of time and fixed effect interactions with time were included in the statistical analysis. Correlation between residuals was modeled using the first-order autoregressive covariance structure when multiple observations were made over time within heifer

within period. The effect of forage was evaluated with the denominator degrees of freedom and the error term associated with whole plot error of heifer(forage) while the effect of N intake and the interaction evaluated against the pooled residual error. Variance homogeneity was evaluated for the main effects of forage and N intake and time relative to feeding when repeated measures were analyzed; evidence of significant heterogeneity was determined using a Levene test for equality of variance. Normality of the residuals were evaluated by the Shapiro-Wilk test for normality. Differential responses between forage levels to N intake were assessed for some variables through mixed model regression analysis; output from this analysis is displayed in figures as the adjusted (for random effect of heifer) response against N intake. Least squares means are presented in tables and evidence for statistical significance was declared at $P < 0.05$.

3.4 RESULTS AND DISCUSSION

3.4.1 Dietary Intakes

Actual intakes of N (g/kg $BW^{0.75}$, Table 2) were lower than the formulated levels due to a greater rate of gain than anticipated for all groups, lower levels of N analyzed in period composited samples than in pretrial samples used for formulation, and the presence of refusals for some heifers fed the HF diet. However the differences in formulated intakes and observed N intakes do little to change the interpretation of the data because the profile of N intake increased linearly and the maximum difference in N intake between forage level, within N intake level was 0.02 g N/kg $BW^{0.75}$.

Intake of other nutrients and chemical components were also affected by the alterations in the N composition of the ration (Table 2); the primary differences were

Table. **3-2**: Daily feed component and water intake by heifers fed low or high forage diets at 4 levels of N intake.

							Contrasts, *P*-value				
Item	Forage	N Intake				SE	Forage	N Intake		Interaction	
		Low	MLow	MHigh	High			L	Q	L	Q
BW, kg	LF	408	410	410	405	10	0.73	0.56	0.10	0.74	0.78
	HF	412	413	415	411						
N Intake, g/kg $BW^{0.75}$	LF	0.95	1.63	2.30	2.97	0.01	0.47	<0.01	0.29	0.13	0.23
	HF	0.93	1.61	2.30	2.96						
Intake, kg/d											
As Fed	LF	9.96	10.01	10.02	9.93	0.29	<0.01	0.90	0.40	0.78	0.71
	HF	13.82	13.95	14.06	13.86						
Dry Matter	LF	6.61	6.64	6.61	6.56	0.14	<0.01	0.36	0.04	0.05	0.22
	HF	7.23	7.39	7.46	7.37						
Organic Matter	LF	6.30	6.23	6.11	5.96	0.14	<0.01	<0.01	0.07	0.03	0.27
	HF	6.88	6.95	6.93	6.77						
Ash	LF	0.31	0.41	0.51	0.60	0.01	0.17	<0.01	0.17	0.04	0.53
	HF	0.35	0.44	0.53	0.60						
NDF	LF	1.46	1.43	1.41	1.37	0.08	<0.01	0.52	0.33	0.30	0.40
	HF	2.98	3.06	3.06	3.00						
Starch	LF	3.86	3.47	3.06	2.63	0.08	<0.01	<0.01	0.75	0.33	0.99
	HF	2.59	2.23	1.90	1.50						
CP	LF	0.54	0.93	1.31	1.67	0.03	0.89	<0.01	0.18	0.43	0.80
	HF	0.53	0.93	1.32	1.68						
MP[1]	LF	0.40	0.67	0.87	0.99	0.02	0.13	<0.01	<0.01	<0.01	0.09
	HF	0.38	0.65	0.93	1.10						
ME, Mcal[2]	LF	15.60	16.31	17.05	16.71	0.43	0.50	<0.01	<0.01	0.95	0.14
	HF	15.63	17.26	17.34	16.98						
CP:ME, g/Mcal	LF	34.71	56.83	76.57	100.02	1.00	0.25	<0.01	0.07	0.59	0.39
	HF	33.92	53.66	76.20	99.19						

[1] MP: metabolizable protein; calculated according to NRC, 2001 using observed BW, DMI, and ingredients.
[2] ME: calculated as digestible OM × 0.82 × 4.409

observed for OM and starch intake varying negatively with increasing N intake and ash intake varying positively and ME intake varying positively and quadratically with N intake. Importantly, NDF intake was not affected by N intake and ME and CP intake was not different between forage levels ($P > 0.10$). Sources of dietary N were different for different levels of forage and N intake, which will be the case for natural

diets. These differences in N source are likely realized through the differences observed in calculated metabolizable protein intakes. The extent to which differences or lack of differences observed in this experiment are due to differences in sources of N or metabolizable protein intake are not completely distinguishable from other differences in the main factors altered in this experiment. Regression analysis of the responses against metabolizable protein intake (results not shown), do not result in a different interpretation than that reached by analysis of the independent variables experimentally manipulated, however.

Increased intake in CP requires another macronutrient intake to be reduced for isoenergetic conditions to prevail. The typical choice for this replacement in ruminant and nonruminant nutritional experiments is starch, as was the case in this experiment. The principle difference between this experiment and others is that, since there were 2 levels of NDF fed, starch intake changed linearly over a considerably greater range (2.36 kg) than either CP (1.15 kg) or NDF (1.65 kg). The sum total of these alterations is that heifers were fed diets containing 2 levels of NDF, 4 levels of linearly increasing CP intake, and 7 levels of linearly decreasing starch intake. Inferences will focus on forage level and CP intake; but pertinent relationships (or lack thereof) with starch will also be highlighted when relevant.

3.4.2 Diet Digestibility

Digestibility of dietary components is shown in Table 3. Dry matter and OM digestibility were improved by reducing the forage component of the diet and with

Table. **3-3**: Nutrient digestibility by heifers fed low or high forage diets at 4 levels of N intake

							Contrasts, *P*-value				
		N Intake						N Intake		Interaction	
Digestibility, %	Forage	Low	MLow	MHigh	High	SE	Forage	L	Q	L	Q
DM	LF	67.89	71.92	76.60	77.28	0.99	<0.01	<0.01	<0.01	<0.01	0.26
	HF	62.10	67.82	68.41	68.68						
OM	LF	68.46	72.47	77.25	77.73	1.04	<0.01	<0.01	<0.01	0.01	0.30
	HF	62.90	68.64	69.23	69.47						
Ash	LF	56.56	63.57	68.95	72.94	1.42	<0.01	<0.01	0.04	0.22	0.41
	HF	46.39	54.81	57.72	59.94						
NDF	LF	34.82	39.11	48.11	49.03	1.89	<0.01	<0.01	0.05	0.17	0.40
	HF	41.18	50.67	51.13	53.01						
ADF	LF	45.02	51.71	57.86	52.28	2.90[1]	0.40	0.07	0.01	0.10	0.45
	HF	46.91	54.26	49.14	49.28	2.13					
Hemicellulose[2]	LF	16.28	14.49	28.08	42.58	—	<0.01	<0.01	0.49	0.74	0.12
	HF	29.40	42.58	54.05	60.67						
	SE	7.44	5.20	3.60	2.29						
Starch	LF	89.53	92.52	95.84	96.82	1.11	0.07	<0.01	0.39	0.11	0.56
	HF	93.53	95.57	96.63	98.23						
CP											
Apparent	LF	51.29	67.88	78.03	82.51	1.31	0.02	<0.01	<0.01	0.10	0.20
	HF	44.17	64.98	73.65	79.12						
True[3]	LF	98.87	95.74	97.67	97.74	1.51	0.22	0.80	0.19	0.88	0.53
	HF	95.66	95.16	94.89	95.61						

[1] Variance heterogeneity was observed across forage or N intake levels for indicated items.
[2] Hemicellulose = NDF – ADF
[3] True CP digestibility is predicted as [N intake – (fecal N – 6.217 × DMI)]/N intake based on the results of linear regression analysis.

increasing intake of N. Comparable observations have been made previously with beef cattle (Reynolds et al., 1991b), sheep (Murphy et al., 1994), dairy heifers (Moody et al., 2007; Hill et al., 2007), and dry dairy cows (Driedger and Loerch, 1999) when LF diets are compared to HF diets at restricted intakes. Likewise, increasing dietary N intake for dairy heifers has been shown in several experiments to enhance the digestibility of DM and OM (Hoffman et al., 2001; Marini and Van Amburgh, 2005). As observed in Figure 1, the relationship between the response to additional N intake depended on the forage level in that the level of improvement in apparent OM digestibility was greater for LF than for

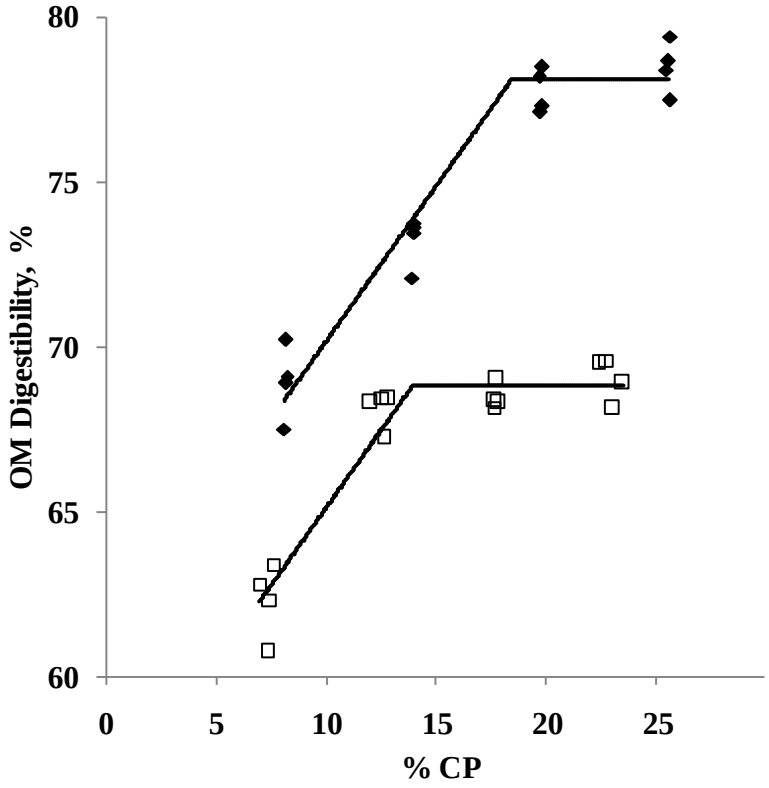

Figure. 3-1: Piecewise regression analysis of the response in OM digestibility to altered levels of dietary CP concentration for heifers fed low forage (LF, ♦) or high forage (HF, □) treatment rations at 4 levels of CP intake.

Response functions were significantly different for the different forage levels, but the slope component did not differ ($P > 0.297$) between the forage levels and was fixed at a constant value for both forage levels. Response for LF is OM digestibility = 60.73 + 0.94 × %CP when %CP < 18.43; when %CP > 18.43 OM digestibility = 78.01. Response for HF is OM digestibility = 55.69 + 0.94 × %CP when %CP < 13.90; when %CP > 13.90 OM digestibility = 68.78.

HF. The response in OM digestibility to additional N intake was also maximized at different levels of dietary CP concentration (18.43 vs. 13.90 for LF and HF, respectively). This relationship indicates that dietary CP concentration limited apparent OM digestibility over a greater range for LF than for HF and that after the limitation had been removed, apparent OM digestibility was limited by the chemical composition of the OM.

Digestibility of NDF increased in a manner consistent with OM with increasing N intake, however the significant reduction in NDF digestibility for the LF diet was primarily due to significant reductions in hemicellulose digestibility since digestibility of ADF did not differ between forage levels. Significant linear and quadratic trend for increased NDF digestibility with increasing N intake resulted from the combination of increased linear digestibility of hemicellulose and quadratic effects to increased N intake on the digestibility of ADF. The reduction in NDF digestibility was consistent with the results reported by Moody et al. (2007) and Kirkpatrick et al. (1997) as well as that which would be expected based on the anticipated reduction in rumen pH associated with the consumption of the LF diet; although the presence and extent of pH alterations is unknown for the current experiment. The results for NDF digestibility for the current experiment conflict with the results of several other reports (Colucci et al., 1989; Reynolds et al., 1991b; Murphy et al., 1994) in which lower forage diets resulted in significantly higher NDF digestibility when fed at restricted intakes, likely due to enhanced retention times and more digestible sources of NDF offsetting the negative consequences of reduced ruminal pH. Digestibility of ADF in the current experiment corresponds to neither of these results in that there were no differences between forage levels. The extent to which the level of intake and forages fed affect the differences in responses observed between the different experiments cannot be evaluated from available data; although in the current experiment the ADF from the most indigestible feedstuff offered (wheat straw) comprised a similar proportion of the ADF across all diets (48% of ADF for LF vs. 49% of ADF for HF), which may blunt the impact of the potential

improvements in ruminal retention time and highly digestible ADF sources for LF rations.

Starch digestibility was improved as the level of N intake increased for both LF and HF rations. Starch digestibility also tended to be higher for the HF ration. Viera et al. (1980) determined that a linear increase in starch digestibility was associated with increased N intake, though these results have not been confirmed in subsequent experiments where N was fed (Streeter and Mathis, 1995) or infused abomasally (Richards et al., 2002). The extent that alterations observed in total tract starch digestibility is due to ruminal or post-ruminal digestibility changes are unknown from the current experiment. It has been observed previously that increasing rates of dry matter degradation were observed in situ when rumen NH_3-N concentration was increased up to 6.1 or 12.5 mg/dl for barley or corn, respectively; increases beyond these levels did not result in significant increases in degradation rate (Odle and Schaefer, 1987). Several experiments with lactating dairy cows, where rumen NH_3-N concentration was unlikely to be limiting to rumen starch digestion, have demonstrated that greater intake of starch resulted in greater levels of rumen starch digestibility and lower levels of post-ruminal starch digestibility, the reverse occurring with lower starch intake, although total tract starch digestibility was unaffected in either experiment (Cameron et al., 1991; Ipharraguerre et al., 2005). When casein and starch were infused into the rumen or abomasum of steers according to a factorial experiment, starch infused into the rumen was highly digested in the total tract and unaffected by the site of casein infusion although when starch was infused into the abomasum, total tract digestibility was lower when infused into the rumen and higher when casein was co-infused into the abomasum

(Taniguchi et al., 1995). The trend toward lower starch digestibility for heifers fed LF and improvements realized with increasing N intake are consistent with the observations of Taniguchi et al. (1995).

Reduced digestibility of starch observed for LF has not been observed in other experiments in which forage level was altered in limit-fed sheep (Colucci et al., 1989; Murphy et al., 1994) or cattle (Colucci et al., 1989). Considering that starch intake increased linearly over a combination of forage and N intake levels (Table 2), the relationship between starch intake and digested starch could be evaluated by regression analysis in the current experiment. Using mixed model regression, a significant quadratic response was observed between starch intake (x) and and starch digested in the total tract (y): $y = 1.054$ (± 0.016; $P < 0.01$) x -0.046 (± 0.008; $P < 0.01$) x^2 with the intercept not differing from 0 ($P > 0.10$); responses were not affected by forage level. This response would indicate a continuously increasing, but at a decreasing rate, digestion of dietary starch. Although the quadratic coefficient was highly significant, the absolute reduction in rate of increase in digested starch with increasing starch intake was very minimal in the range of intake observed in the current experiment (1.50 – 3.86 kg/d). From the foregoing discussion it can be inferred that starch intake is more closely related to its disappearance in the total tract than N intake or forage level individually.

Finally, it can be concluded that digestibility of the major carbohydrate components of the diets (NDF and starch) were individually greater for the HF diets. Since the more highly digestible component (starch) comprises a greater proportion of carbohydrate for the LF ration, the greater OM digestibility for the LF diet is due to the composition of the diet. Enhanced digestibility of OM was not due to a greater level of

digestive efficiency for the heifers fed the LF diet since this was reduced for both carbohydrate fractions.

3.4.3 Environmental Excretion

Consistent with improvements observed in dietary digestibility, fecal wet and dry matter and fecal water excretion are significantly greater for heifers fed HF; also fecal DM and water excretion decreased with increasing N intake (Table 4). In the current

Table. 3-4: Environmental output from heifers fed low or high forage diets at 4 levels of N intake.

							Contrasts, *P*-value				
		N Intake						N Intake		Interaction	
Item, kg/d	Forage	Low	MLow	MHigh	High	SE	Forage	L	Q	L	Q
Wet Feces	LF	9.68	9.17	7.56	7.34	0.36	<0.01	<0.01	0.22	0.49	0.57
	HF	15.77	13.90	13.71	12.57						
Fecal DM	LF	2.12	1.86	1.55	1.50	0.08	<0.01	<0.01	<0.01	0.01	0.40
	HF	2.74	2.37	2.36	2.31						
Fecal Water[1]	LF	7.56	7.31	6.01	5.84	0.31	<0.01	<0.01	0.52	0.20	0.64
	HF	13.03	11.53	11.36	10.26						
Urine	LF	10.24	10.87	14.16	15.73	2.75	0.38	<0.01	0.44	0.89	0.74
	HF	6.62	8.25	9.40	13.21						
Manure	LF	19.92	20.03	21.70	23.07	2.74	0.56	0.02	0.26	0.99	0.62
	HF	22.39	22.14	23.09	25.76						

[1] Weight lost on drying at 55°C.

experiment, heifers fed LF produced an average of 60% of the mass of wet feces as produced by heifers fed HF. Although the magnitude of the changes in wet fecal output differs between experiments as a result of the composition of the diet fed, results of previous research support these observations for limit-fed cattle (Driedger and Loerch, 1999; Moody et al., 2007; Hill et al., 2007). Decreases in wet fecal output do not carry through to decreases in manure output due to numerically greater levels of urine output

for heifers fed LF. Urine output was also significantly greater for heifers fed increasing levels of N as has been observed previously (Bannink et al., 1999; Kume et al., 2008) and greatly contributed to the increasing excretion of total manure observed as N intake increased. Urine output has also been observed to be increased by the provision of LF diets to dairy heifers (Hill et al., 2007). However, the numerical increase observed in the current experiment was much lower than the increases observed in that experiment but higher than others in which no increases were observed with limit-fed heifers (Moody et al., 2007; Zanton and Heinrichs, 2008b).

Table 3-5: Water intake and distribution in excreta from heifers fed low or high forage diets at 4 levels of N intake

							Contrasts, *P*-value				
		N Intake						N Intake		Interaction	
Item	Forage	Low	MLow	MHigh	High	SE	Forage	L	Q	L	Q
Water Intake, L/d											
Voluntary	LF	19.42	20.63	22.68	26.24	3.01	0.79	<0.01	0.23	0.20	0.74
	HF	19.34	20.24	21.32	23.62						
Total[1]	LF	22.76	24.00	26.07	29.60	3.00	0.63	<0.01	0.26	0.20	0.75
	HF	25.93	26.79	27.91	30.11						
Total, /kg DMI	LF	3.48	3.60	4.00	4.53	0.46	0.83	<0.01	0.14	0.09	0.91
	HF	3.59	3.62	3.73	4.12						
Total, /kg CP Intake	LF	42.53	25.59	20.73	17.35	3.75	0.55	<0.01	<0.01	0.16	0.68
	HF	49.14	28.96	21.57	17.65						
Water excreted per consumed, %[2]											
Feces	LF	35.95	33.46	24.19	20.57	3.81	0.02	<0.01	0.93	0.74	0.79
	HF	50.40	43.74	40.65	34.56						
Urine	LF	41.31	43.38	51.38	51.26	5.51	0.07	<0.01	0.80	0.40	0.49
	HF	25.24	29.52	33.80	42.91						
Manure	LF	77.26	76.73	75.46	71.73	3.00	0.95	0.52	0.77	0.17	0.26
	HF	75.64	73.15	74.34	77.37						

[1] Total water intake = voluntary water intake + feed water

[2] Consumption is total water intake and excretion includes fecal water calculated as weight lost on drying at 55°C and total weight of urine output.

The inverse relationship between fecal water excretion and urine excretion in response to alterations of N intake as well as to forage level is shown in Table 4 and the distribution relative to water consumption is detailed in Table 5. Fecal water excretion

(kg) was determined to be most highly correlated (r = 0.989) to fecal NDF excretion (kg) among the fecal excretion variables evaluated. The relationship between fecal water excretion (y) and fecal NDF (x) was not differentially affected by level of forage ($P > 0.50$) and could be represented with a common equation as y = 1.28 (±0.45; $P < 0.01$) + 6.69 (±0.43; $P < 0.01$) x. Water intake was not different between forage levels, regardless of means of expression, while water intake increased with greater levels of N intake. The proportion of water intake that was excreted did not depend upon any of the dietary factors altered and averaged 75.21%, while the proportion of intake excreted in feces and urine were affected by both forage level and N intake. These observation reflect the well coordinated balance of water through alterations in excreta water output and voluntary water intake, but the strong relationship between NDF excretion and fecal water excretion indicate the importance of the water holding capacity of undigested fiber or an enhanced rate of passage through the lower GIT in affecting the regulation of partitioning of consumed water. Overall, the responses observed for water partitioning contribute substantially to the lack of significant differences observed in total manure output and limit the effects of improved dietary digestibility observed for LF and with increased N intake.

3.4.4 Fecal N and N Digestibility

Apparent digestibility of N increased with increasing N intake and for heifers fed the LF diet (Table 3). Considering that a significant proportion of fecal N excreted does

not directly originate from the diet (Mason, 1969), true digestibility of dietary N and non-dietary fecal N (**NDFN**) was evaluated by regression analysis (Figure 2). Response of

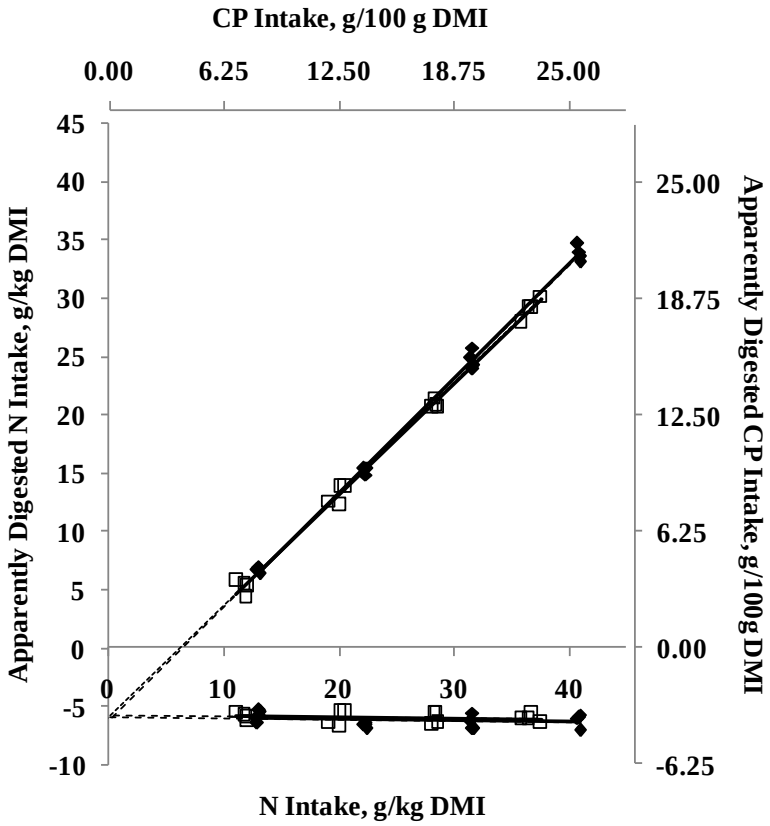

Fig. **3-2**: Relationship between dietary N intake (g/kg DMI) and N that was apparently digested for heifers fed low forage (LF ,♦) or high forage (HF, □) treatment rations at 4 levels of CP after adjustment for random heifer effects.

Extrapolations are indicated by broken lines. Parenthetical values associated with the equation are SE. Lines are not significantly different ($P > 0.20$) and individual coefficients are not different ($P > 0.20$) and a common equation representing both forage levels is Apparently digested N = - 6.217 (±0.217) + 0.970 (±0.008) × N Intake. Individual equations are LF: Apparently digested N = - 6.306 (±0.312) + 0.980 (±0.010) × N Intake and HF: Apparently digested N = - 6.105 (±0.312) + 0.958 (±0.011) × N Intake. Non-dietary fecal N is represented by negative values to correspond with NDFN determined by extrapolation and determined using acid detergent soluble N. The common equation of NDFN = - 5.843 (±0.291; $P < 0.01$) - 0.011 (±0.011; $P > 0.33$) × N Intake; lines were not different between forage groups ($P > 0.76$)

apparently digested N (y; g/kg DMI) to N intake (x; g/kg DMI) was not different between LF and HF diets ($P > 0.35$) and can be represented by the common equation y = -6.217 (±0.217) + 0.970 (±0.008) x. Results indicate that, when expressed relative to

DMI, no dietary differences in NDFN and true digestibility exist between HF and LF, limit-fed heifers (Table 3). Fecal N was also partitioned using neutral and acid detergent solutions (table 6; Mason, 1969). Insoluble N (presumably of feed origin) was higher in

Table **3-6**: Fecal N partitioning using detergent solutions in heifers fed low or high forage diets at 4 levels of N intake

								Contrasts, *P*-value			
		N Intake						N Intake		Interaction	
Item	Forage	Low	MLow	MHigh	High	SE	Forage	L	Q	L	Q
Neutral Detergent											
Insoluble N, g	LF	7.19	11.83	10.69	13.61	0.60	<0.01	0.02	<0.01	0.07	0.13
	HF	13.61	16.64	14.64	14.78						
Soluble N, g	LF	34.90	35.82	34.93	36.72	1.27	0.21	<0.01	0.99	<0.01	0.44
	HF	33.73	34.96	40.85	41.18						
Soluble N, g/kg DMI	LF	5.28	5.38	5.26	5.57	0.20	0.32	<0.01	0.50	<0.01	0.68
	HF	4.67	4.74	5.45	5.58						
Soluble N, % fecal N	LF	82.95	74.99	76.51	78.43	1.19	<0.01	0.91	<0.01	<0.01	0.05
	HF	71.05	67.53	73.33	73.40						
True N Digestibility, %	LF	91.66	92.04	94.67	96.20	0.52	<0.01	<0.01	0.08	<0.01	<0.01
	HF	83.86	88.71	92.91	94.43						
Acid Detergent											
Insoluble N, g	LF	3.88	2.69	2.74	4.96	1.32	<0.01	0.04	0.57	0.12	0.29
	HF	5.18	6.18	9.92	9.96						
Soluble N, g	LF	38.22	44.80	42.95	42.21	2.01	0.16	0.07	0.08	0.81	0.34
	HF	42.16	45.27	45.64	46.32						
Soluble N, g/kg DMI	LF	5.78	6.74	6.47	6.37	0.32	0.46	0.13	0.14	0.89	0.23
	HF	5.83	6.16	6.09	6.28						
Soluble N, % fecal N	LF	90.95	94.09	93.72	89.48	2.50	<0.01	0.08	0.46	0.20	0.18
	HF	89.21	87.72	81.99	82.65						
True N Digestibility, %	LF	95.53	97.81	98.57	98.42	0.85	<0.01	0.02	0.74	0.03	0.08
	HF	93.80	95.46	95.17	96.53						

feces for heifers fed HF diets and with increasing intake of N when determined using either acid or neutral detergent solutions. Detergent soluble N was not different between forage levels for both detergent solutions, but increased as N intake increased—although this response was strongest for heifers fed the HF diet and determined using neutral detergent. Whereas results from the regression analysis extrapolate a single level of NDFN for all N intakes within forage level, analysis with detergents can yield individual NDFN for all diets. When related to DMI and extracted with neutral detergent,

excretion of NDFN increased with increasing N intake and at a greater rate for heifers fed the HF ration, although differences between forage levels were not significant. Excretion of NDFN extracted with acid detergent (g/kg DMI) was unaffected by dietary treatment.

Regardless of extraction solution, the proportion of fecal N that was NDFN was greater when the LF diet was fed, a result explained by the lower excretion of dietary N for heifers fed these diets. As a result of this observation, true N digestibility was determined to be greater for heifers fed LF and increased significantly with increasing levels of N intake.

The discrepancies observed in the current experiment between detergent solutions have also been observed by Mason (1969) when describing the technique; although the divergence of values determined in this experiment were greater than the ones observed in that experiment. Explanations may relate to the inability of neutral detergent solution to completely extract the mucopeptide fraction of the bacterial cell wall, alterations occurring during the storage of feces being more evident in the neutral detergent extraction, the analysis of dried feces in the current experiment compared to fresh feces, or a combination of the previous explanations or an as yet unidentified factor (Mason, 1969). Although the regression equations or regression coefficients were not significantly different between forage levels (Figure 2), results of acid detergent extraction agree most closely with the results determined for the regression analysis of apparently digested N (evaluated with fecal N excretion determined on fresh feces) against N intake when the results of the equations are considered separately. As such, when evaluated with acid detergent, NDFN averaged 6.34g/kg DMI and true digestibility averaged 97.58 % for heifers fed the LF diet compared to 6.217 g/kg DMI and 97.0 % determined by regression

analysis and heifers fed the HF diet with NDFN of 6.09 g/kg DMI and 95.24 % determined with acid detergent compared to 6.105 g/kg DMI and 95.82 % determined by regression analysis.

Previous experiments have observed that when starch (Orskov et al., 1970) or glucose (Thornton et al., 1970) was infused post-ruminally that there was greater excretion of fecal N than when no fermentable carbohydrate was infused. In the current experiment, no relationship between starch intake, starch digested in the total tract, or fecal starch excretion and NDFN could be identified. There was also no relationship between NDFN excretion (g/kg DMI) and forage level (thus NDF intake; Figure 2), in agreement with the results obtained by Ouellet et al. (2002) using ^{15}N dilution. There was also no change in NDFN excretion with changing N intake when analyzed with acid detergent which conflicts results from several other sources wherein NDFN excretion increases as N intake increases where the specific endogenous N losses increase (Stein et al., 2007); although the complexity of the N dynamics in the ruminant GIT is a likely explanation for this difference as the former results were derived from primarily non-ruminant experiments. Thus, from the results of this experiment, NDFN losses were not different between forage levels and comprised a large proportion of fecal N. Also the true N digestibility was greater for the LF diets than the HF diets due to greater levels of dietary N excreted in the feces for HF.

The combination of unaltered NDFN and increased levels of dietary N excretion into the feces resulted in significantly greater levels of fecal N excretion for heifers fed HF compared to LF at all levels of N intake (Table 7). Increasing N intake resulted in

Table 3-7: Nitrogen distribution in heifers fed low or high forage diets at 4 levels of N intake

								Contrasts, *P*-value			
		N Intake						N Intake		Interaction	
Item, g/d	Forage	Low	MLow	MHigh	High	SE	Forage	L	Q	L	Q
Intake	LF	86.65	148.99	209.39	267.25	4.04	0.89	<0.01	0.18	0.43	0.80
	HF	84.81	148.70	211.97	269.27						
Fecal	LF	42.09	47.64	45.63	46.88	1.49	<0.01	<0.01	0.01	0.01	0.85
	HF	47.35	51.59	55.50	55.98						
Apparently Digested	LF	44.40	101.35	163.76	220.37	4.26	0.23	<0.01	0.72	0.85	0.77
	HF	37.47	97.10	156.46	213.29						
Urinary	LF	28.99	66.96	109.22	131.85	—[1]	0.89	<0.01	0.74	0.28	0.28
	HF	24.10	58.90	103.78	146.73						
	SE	3.19	7.17	7.22	11.74						
Excreted	LF	71.08	114.77	155.51	178.67	—	0.35	<0.01	0.43	0.20	0.27
	HF	71.45	110.67	159.95	202.65						
	SE	3.62	3.34	7.71	12.81						
Retained											
g/d	LF	15.41	33.31	53.55	88.39	—	0.16	<0.01	0.55	0.11	0.07
	HF	13.37	37.11	51.69	66.43						
	SE	1.21	2.15	3.68	8.22						
% N Intake	LF	17.88	22.08	25.57	33.36	—	0.17	<0.01	0.40	0.18	0.06
	HF	15.54	24.57	24.39	24.87						
	SE	1.19	1.13	1.96	3.33						
% N Apparently Digested	LF	34.81	32.21	32.53	40.25	—	0.67	0.89	0.49	0.18	0.08
	HF	34.51	37.41	33.06	31.38						
	SE	2.64	1.18	2.54	4.09						

[1] Variance heterogeneity was observed across N intake levels for indicated items

significant increases in fecal N excretion, with the greatest increases occurring for HF due to the greater excretion of feed N as discussed previously. These numerical differences between LF and HF were, for the most part, maintained in the N that was apparently digested, although the differences were not significant (P >0.23).

3.4.5 Plasma Urea, Urine Components, and Renal Responses

Urine N increased linearly as N intake increased and was not significantly different between forage levels (Table 7). As would be expected based on a number of experiments (Hoffman et al., 2001; Gabler and Heinrichs, 2003; Marini and Van

Amburgh, 2003), plasma urea-N levels increased significantly and linearly as N intake increased (Table 8), although the postprandial concentration profiles are similar for all

Table **3-8**: Urea-N and creatinine responses in heifers fed low or high forage diets at 4 levels of N intake

								Contrasts, *P*-value			
		N Intake						N Intake		Interaction	
Item	Forage	Low	MLow	MHigh	High	SE	Forage	L	Q	L	Q
PUN, mg/dl	LF	4.10	10.39	13.83	19.35	0.99	0.04	<0.01	0.35	0.17	0.34
	HF	3.60	8.04	12.12	16.53						
UUN, mg/dl	LF	208	621	806	768	145	0.74	<0.01	<0.01	0.16	0.99
	HF	151	619	924	944						
UUN/PUN	LF	52	63	51	43	15	0.55	0.62	0.03	0.12	0.23
	HF	38	72	81	59						
UUN, g/d	LF	14.83	54.64	87.45	109.12	5.33	0.38	<0.01	0.14	0.10	0.13
	HF	7.80	41.45	81.18	114.55						
UUN/N Intake	LF	17.08	36.12	42.14	41.07	2.46	0.04	<0.01	<0.01	0.03	0.37
	HF	8.92	27.16	38.68	42.88						
UUN, % Urine N	LF	50.79	79.96	82.83	85.42	—[2]	0.08	<0.01	<0.01	0.29	0.64
	HF	32.86	68.19	81.20	81.99						
	SE	7.46	3.68	2.38	8.62						
Urea Clearance, L/h	LF	14.61	21.05	23.08	23.97	1.43	0.20	<0.01	0.02	<0.01	0.35
	HF	8.21	22.31	29.69	31.69	3.17					
Urine Creatinine											
mg/dl	LF	141	105	92	75	25	0.60	<0.01	0.68	0.93	0.62
	HF	152	132	109	87						
g/d	LF	9.95	9.94	10.35	9.84	0.69	0.68	0.26	0.54	0.25	0.95
	HF	9.03	9.59	10.02	10.16						
g/kg BW	LF	24.47	24.24	25.25	24.04	1.51	0.50	0.32	0.68	0.27	0.91
	HF	22.03	23.23	24.02	24.65						

[1] PUN: plasma urea-N; UUN: urine urea-N
[2] Variance heterogeneity was observed across N intake levels

levels of N intake and forage level (Figure 3). Levels of PUN were also significantly greater for heifers fed LF despite the constant intake of N and lower levels of soluble protein intake. Since PUN concentration represents the balance between entrance and removal of urea-N, assuming a constant plasma volume, forage level dependent alterations in these fluxes likely occurred independent of N intake. Several sources have observed that as the level of forage in the diet increases, the absorption of NH_3 and therefore the PUN concentration and net hepatic urea flux increases

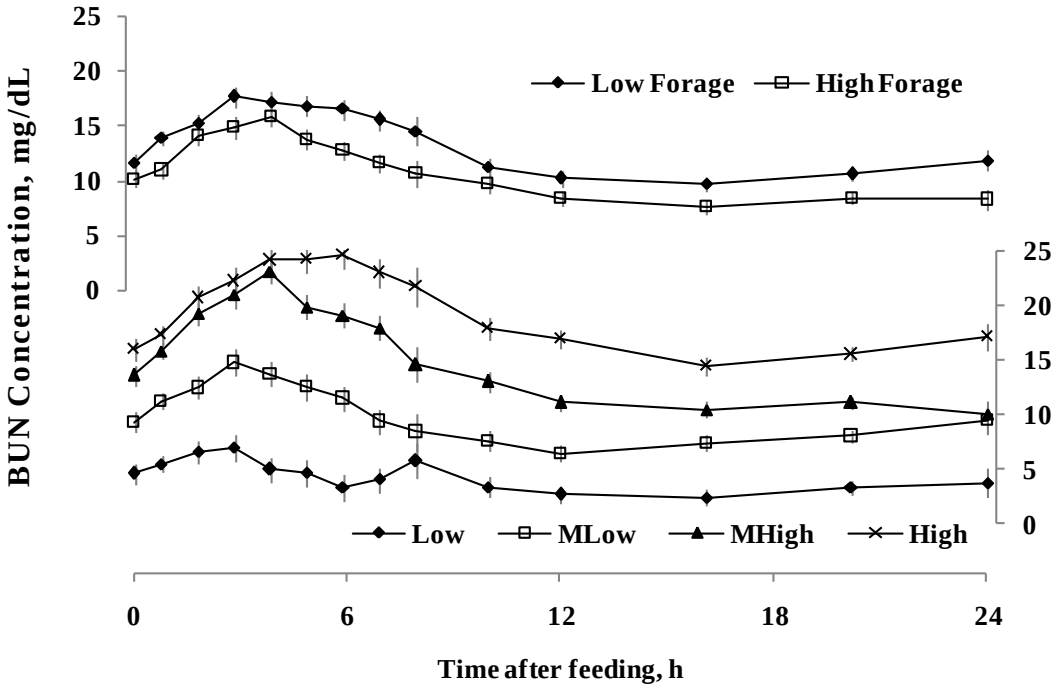

Figure 3-3: Profile of alterations in BUN after feeding for heifers fed low forage or high forage treatment rations at 4 levels of CP intake.

The forage × CP level × time interaction was not significant, so only the main effects are presented. Standard errors at each time point are indicated by vertical lines (|).

(Huntington, 1989; Reynolds et al., 1991a; Seal et al., 1992). However caution must be exercised when ascribing the results of the afore-mentioned experiments to the proportion of forage in the diet, because in each case N intake was greater for the animals fed HF. When N intake is similar (Lobley et al., 1996) or equal (Huntington et al., 1996), NH_3 absorption is unaffected by forage level of the diet in contrast to the previous experiments in which forage level and N intake were altered simultaneously. Results of this latter experiment did however obtained greater plasma urea concentrations as well as higher liver urea flux for animals fed higher concentrate, lower forage diets despite equivalent liver NH_3 and α-amino N net fluxes (Huntington et al., 1996).

Plasma urea has two sites of exit from the body: recycled to the GIT and excretion in urine. Sunny et al. (2007) concluded that the transfer of PUN to the GIT was limited by the capture of the urea within the GIT and not by transport/diffusion into the GIT. Transfer of urea to the rumen has been increased by infusion of sucrose ruminally (Kennedy, 1980), the inclusion of rapidly fermentable carbohydrates in the diet (Norton et al., 1982; Lobley et al., 2000). Greater transfer of urea to the GIT was also observed through greater provision of dietary N in sheep (Cocimano and Leng, 1967; Bunting et al., 1987; Marini et al., 2004) and cattle (Bunting et al., 1989) in spite of the reduced urease activity of bacteria adherent to the surface of various sections of the GIT (Marini et al., 2004). Few experiments exist, however, in which urea-N recycling to the GIT is examined with animals receiving different forage levels but with isnitrogenous and isoenergetic intakes. The results of several available experiments indicate that recycling of urea N to the rumen is greater for animals fed LF diets (Norton et al., 1982; Al-Dehneh et al., 1997; Ouellet et al., 2002), but due to greater levels of recycling to the lumen of post-ruminal tissues (Norton et al., 1982; Reynolds and Huntington, 1988; Huntington, 1989), total tract urea-N recycling may be equal or greater for animals fed HF diets (Norton et al., 1982; Huntington et al., 1996). In reviewing urea recycling data from a number of sources with steers fed similar levels of digestible N intake, Lapierre and Lobley (2001) observed average net urea transfer to the portal-drained viscera was 103 vs. 101 mmol/h for forage or concentrate diets , respectively. Ultimately, alterations in urea production and recycling cannot be evaluated from the results of this experiment, however from the greater concentration of PUN maintained and the numerically increased excretion of urinary urea-N (**UUN**) for heifers fed LF, particularly at lower N

intakes, it could be inferred that there is less GIT recycling of urea, greater production of urea, or both for heifers fed LF.

The fraction of N intake or urea entry rate that is recycled as urea to the GIT has been increased with increasing concentrations of forage in the diet (Norton et al., 1982; Huntington et al., 1996) or with lower levels of dietary N intake in both cattle (Bunting et al., 1989; Marini and Van Amburgh, 2003; Wickersham et al., 2008) and sheep (Cocimano and Leng, 1967; Bunting et al., 1987; Marini and Van Amburgh, 2005). The alternative mode of removal of urea from the plasma pool is through excretion in urine. As shown in Table 8, although the mass of UUN excreted did not differ significantly between forage levels, the proportion of N intake that is excreted as UUN is greater for heifers fed LF than HF. This ratio also increased linearly and quadratically with increasing N intake such that at the highest levels of N intake, 42% of N intake is excreted as UUN.

The observation that a plateau occurs in the UUN excretion to N intake ratio, may indicate that the maximum capacity for the kidney to concentrate UUN had been exceeded. This could also be the inference from the plateau in the UUN concentration, UUN clearance, and the quadratic response in the concentration ratio of UUN:PUN (mg/dl:mg/dl; Table 8) to increasing N intake. Thus, although the response was rather variable, the urea concentration gradient between urine and plasma was maximized between MLow and MHigh N intake levels for both forage level groups beyond which PUN concentrations increased at a greater (linear) rate than UUN concentrations (quadratic/plateauing). A quadratic response in the UUN:PUN ratio has been observed previously in sheep offered different levels of N intake (Cocimano and Leng, 1967), and

coinciding alterations in UUN excretion and urine flow has been attributed to UUN becoming a progressively more important osmotic compound as its need for excretion increases (Cocimano and Leng, 1967; Thornton, 1970). This interpretation also seems to be valid for the current experiment; thus as N intake increases and greater quantities of UUN need to be removed from circulation, greater quantities of urine were required to be excreted. This interpretation is also consistent with the tendency of increased kidney mass for sheep fed greater quantities of N without significant effect on kidney urea transport protein abundance (Marini et al., 2004).

Kohn et al. (2005) determined from regression analysis that urinary N clearance was 1.3 L of blood cleared/(d·kg BW). Results from the current experiment, when combined and forced though the origin (forage level differences and intercept were not different from 0), yield a urinary N clearance of 1.87 (±0.13) L of plasma cleared/(d·kg BW). A constant level of clearance would not be expected as it has been known for some time that urea clearance varies to some extent with the dietary protein intake (Schmidt-Nielsen, 1958). The principle of clearance, while potentially useful to aid urinary N prediction from a readily accessible sample, applies to specific chemical compounds and equals the product of the concentration of the component in the urine and urine volume divided by the concentration of the component in plasma. All of these measures increase with increasing N intake, but with differing rates and response functions. As such, due to the increasing proportion of urinary N excreted as UUN, when urea clearance is measured in the current experiment the result is not a constant as determined through regression for total urinary N, but a volume of plasma that increases linearly and quadratically with increasing N intake (Table 8). This type of response profile has been

observed previously in sheep (Cocimano and Leng, 1967; Sunny et al., 2007) and also would indicate that a limit to UUN concentrating ability had been reached with insufficient increases in urine flow to excrete the additional urea produced.

Urinary creatinine excretion did not differ due to dietary alterations whether expressed as total output or per kg BW, suggesting a constant muscle mass for heifers fed different dietary treatments (Table 8). Creatinine concentration varied inversely with increases in N intake, but was not different between forage levels. Due to the large range in urine excretion by heifers in this experiment, a relationship between creatinine concentration, BW, and urine volume was evaluated for potential predictive purposes (Figure 4), comparable to relationships defined for dairy cows (Valadares et al., 1999). A close relationship between these variables was confirmed in the current experiment with dairy heifers and indicated an excretion of 24.2 mg of creatinine per kg BW. This value is lower than that determined in lactating dairy cows by Valadares et al. (1999), but corresponds closely to values determined with nonlactating cattle (Orskov and MacLeod, 1982; Jones et al., 1990).

3.4.6 Ammonia Volatilization from Manure

Daily volatilization of NH_3 from collected feces and urine is shown in Table 9. Consistent with the results of several experiments (James et al., 1999; Frank and Swensson, 2002; Misselbrook et al., 2005), response in NH_3 volatilization to additional.

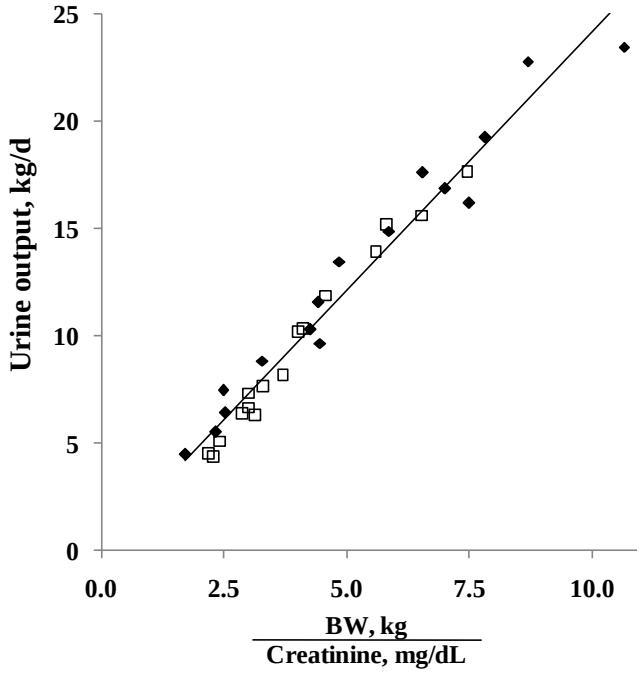

Figure 3-4: Relationship between BW (kg), urine creatinine concentration (mg/dl), and urine output (kg) for heifers low forage (♦) or high forage (□) treatment rations at 4 levels of CP intake.

Lines were not different between heifers offered different forage levels ($P > 0.27$) or protein levels ($P > 0.13$) and the intercept did not differ significantly from 0 ($P > 0.93$) when data from all nutritional groups were represented by one linear regression equation: Urine output (kg/d) = 2.42 × BW (kg)/urine creatinine (mg/dl).

intake of N are highly significant. For heifers fed the Low N diets, NH_3 volatilization was barely detectable throughout the 24 h period of measurement and these means were not found to differ significantly from 0. In the current experiment, the proportion of total excreted N that was volatilized as NH_3-N increased as the N intake increased due to the greater excretion of UUN; the proportion of UUN volatilized did not differ between diets. Although UUN will be rapidly hydrolyzed in the presence of urease, in the current experiment only 33% of UUN was observed to be volatilized as NH_3-N.

Urea-N

Table **3-9**: Ammonia volatilization from heifers fed low or high forage diets at 4 levels of N intake

NH_3 Volatilization	Forage	N Intake				SE	Contrasts, *P*-value				
		Low	MLow	MHigh	High		Forage	N Intake L	N Intake Q	Interaction L	Interaction Q
mg/g Manure	LF	0.29*	1.07	1.90	1.94	0.26	0.57	<0.01	0.99	0.16	0.42
	HF	0.15*	0.97	1.75	1.70						
g/d	LF	4.90*	20.09	35.73	43.31	4.18	0.90	<0.01	0.06	0.88	0.45
	HF	3.21*	20.28	40.62	41.36						
NH_3-N/ N Excreted, %	LF	5.43	13.93	18.86	19.11	—	0.69	<0.01	<0.01	0.85	0.21
	HF	3.63*	14.29	21.06	16.09						
	SE	1.35	3.33	0.70	1.42						
NH_3-N/UUN Excreted, %	LF	25.30	28.86	36.12	31.63	—	0.34	0.63	0.18	0.57	0.53
	HF	31.99	38.26	44.27	29.53						
	SE	10.49	9.60	2.66	3.15						

[1] Volatilization was determined from a daily composite of manure slurry for 24 h using a bench-top, steady-state (dynamic) flux chamber. Values marked with * are not different from 0.

concentration of the slurry was not measured at the conclusion of the 24 h procedure, however the low levels of recovery of UUN as volatilized NH_3-N may indicate that urease activity was limiting, an additional chemical or methodological factor limited NH_3 volatilization from the manure slurries, or that defecated bacteria present in the feces were capturing the NH_3-N from UUN into microbial protein. The possibility or extent of this latter factor is unknown. Ammonia volatilization did not differ between forage levels in the current experiment, regardless of the means of expression. Lascano et al. (2008) determined that NH_3 volatilization was unaffected by forage or concentrate diets fed to dairy heifers whether measured from the barn floor or in the laboratory procedure used in the current experiment. Likewise, Hill et al. (2007) observed no significant differences in the manure characteristics measured in that experiment between heifers limit-fed or fed HF. Although wet fecal excretions were lower for heifers fed LF, the similarities in total manure and N excretion resulted in similar levels of NH_3 volatilization between forage levels. Thus, altering levels of dietary forage proved

ineffective in reducing NH_3 emissions from dairy heifer manure, however emissions were reduced through reducing dietary N intake.

3.4.7 Retained N and N Efficiency

Since both fecal and urinary N excretion increased with increasing N intake, total N excretion increased at a greater rate than either response individually, although differences in average N excretion or in the excretion response to additional N intake were not significant between forage levels. Consequently, and contrary to the hypothesis of this experiment, retained N was not different for heifers receiving the different forage levels; retained N also responded linearly to increasing levels of N intake. Zanton and Heinrichs (2008a) indicated that over a large range of N intake, retained N could be represented as a first-order exponential plateau function with the maximum level of N retention could be limited by energy level of the diet or the genetic potential of the animal for protein deposition. From the lack of significant quadratic effect due to the increase in N intake it can be inferred that, for the range of N intake and level of energy intake used in this experiment, that N intake was limiting N retention and not energy intake or genetic potential. Given that these heifers were limit-fed diets for ADG (802 g/d for LF and 787 g/d for HF; SE: 39 g/d, $P > 0.78$) that are well lower than maximum attainable ADG, it would not be expected that genetic potential would limit the retention of N. However, the divergence of retained N response at High N intake resulted in a tendency for a differential quadratic response ($P < 0.07$), possibly indicating a movement toward an energy limitation for heifers fed HF at this level of N intake that does not occur for

heifers fed LF. However, the variability in response at this level of N intake without greater levels of N intake to confirm the accuracy of the value, the lack of significant effect, and the impractically high level of N intake required to achieve this divergence in response substantially reduce the confidence and potential importance of this inference.

Although the proportion of N excreted in the feces was greater for heifers fed HF, and as this difference was largely counterbalanced by the increased excretion of urine N in heifers fed LF, the efficiency of intake N retention did not differ between forage levels (Table 5). The efficiency of intake N retention increased with increasing N intake, however. This efficiency was maximized for heifers fed HF at ~24.5% when N intake was at or greater than the MLow level. This level of efficiency approximates the level of efficiency observed by Zanton and Heinrichs (2008b) when a HF diet was limit-fed at levels of DMI greater or equal to 1.50% BW as well as the average maximum level of efficiency observed in a literature analysis by Zanton and Heinrichs (2008a), although individual experiment and diet combinations obtained greater levels of efficiency. In contrast, heifers fed LF retained intake N at similar levels of efficiency as HF until N intake increased to the High level of N intake where the efficiency of retaining N intake continued to increase numerically. The tendency for a quadratic interaction resulted from the plateau observed for heifers fed HF with essentially no nonlinearity observed for LF. No differences in apparently digested N retention efficiency were observed in the current experiment, which averaged 34.52%. Consequently, under the conditions of the current experiment with heifers limit-fed LF or HF over a range on N intakes, N retention and the efficiency of N retention did not differ between heifers fed diets containing different forage levels.

The lack of response in N retention and efficiency to altered levels of forage observed in this experiment, is contrary to the responses observed in several other experiments (Murphy et al., 1994; Driedger and Loerch, 1999; Moody et al., 2007). The cause of this discrepancy cannot be confidently determined, however the provision of isoenergetic and isonitrogenous diets in the current experiment are likely a significant contributor. Additional factors that may contribute to the differences between experiments are the quality, proportion, and type of the forage included in the diets, level and quality of rumen undegradable protein, as well as animal factors such as age and growth potential. While different combinations of forage and concentrates may alter the absolute levels of N retention and efficiencies observed, under the conditions of the current experiment, in which HF and LF diets were given under isoenergetic and isonitrogenous conditions across a wide range of N intakes, no alterations in N retention or efficiency of N retention were observed.

3.5 CONCLUSION

When LF or HF diets are limit-fed to growing dairy heifers, OM digestibility was greater for heifers fed LF due to the inclusion of more digestible dietary constituents since the digestibility of the constituents individually were equal or lower for LF than HF. Organic matter digestibility was improved by greater N intake, but the maximum level of OM digestibility required greater intake of N for heifers fed LF than HF. Manure excretion and NH_3 volatilization were not different between forage levels, but were increased with increasing N intake due to greater levels of urine and total N excretion.

Differences in N partitioning observed between forage levels were limited to differences in apparent and true digestibility. Thus, the significant reduction observed in fecal N excretion for LF were counterbalanced by numerical increases in urinary N excretion so that total N excretion and retention were not different between forage levels. As N intake increased beyond MLow, there was evidence for maximized UUN concentrating ability, which may contribute to increased urine output. Increasing N intake increased all aspects of N partitioning and efficiency evaluated except for the proportion of apparently digested N that was retained, which was affected by none of the dietary alterations made in this experiment. There is no evidence that would recommend increasing N intake above MLow (1.67 g N/kg $BW^{0.75}$ or approximately 13% CP), because improvements in N retention do not come at increased levels of efficiency and manure and N output as well as NH_3 emissions increase. In conclusion, contrary to the hypothesis of this experiment, N efficiency was not increased for dairy heifers fed a LF diet compared to HF when intakes of N and energy were held constant between groups. Thus limit-fed dairy heifers should receive the same quantity of N regardless of the forage level in the ration.

3.6 REFERENCES

Al-Dehneh, A., J. T. Huber, R. Wanderley, C. B. Theurer, M. Pessarakli, and D. DeYoung. 1997. Incorporation of recycled urea-N into ruminal bacteria flowing to the small intestine of dairy cows fed a high-grain or high-forage diet. Anim. Feed Sci. Technol. 68:327–338.

Association of Official Analytical Chemists. 1990. Official methods of analysis. 15 ed. AOAC, Arlington, VA.

Atasoglu, C., A. Y. Guliye, and R. J. Wallace. 2004. Use of stable isotopes to measure de novo synthesis and turnover of amino acid-C and -N in mixed micro-organisms from the sheep rumen in vitro. Br. J. Nutr. 91:253–261.

Attaix, D., E. Aurousseau, G. Bayle, D. Rosolowskahuszcz, and M. Arnal. 1988. Respective influences of age and weaning on skeletal and visceral muscle protein-synthesis in the lamb. Biochem. J. 256:791–795.

Bach Knudsen, K. E. 1997. Carbohydrate and lignin contents of plant materials used in animal feeding. Anim. Feed Sci. Technol. 67:319–338.

Bannink, A., H. Valk, and A. M. Van Vuuren. 1999. Intake and excretion of sodium, potassium, and nitrogen and the effects on urine production by lactating dairy cows. J. Dairy Sci. 82:1008–1018.

Biddle, G. N., J. L. Evans, and J. R. Trout. 1975. Labile nitrogen reserves in the ruminant. Metabolic changes in growing cattle employing a nitrogen depletion-repletion treatment. J. Nutr. 105:1578–1583.

Bunting, L. D., J. A. Boling, and C. T. MacKown. 1989. Effect of dietary protein level on nitrogen metabolism in the growing bovine: I. Nitrogen recycling and intestinal protein supply in calves. J. Anim. Sci. 67:810–819.

Bunting, L. D., J. A. Boling, C. T. MacKown, and R. B. Muntifering. 1987. Effect of dietary protein level on nitrogen metabolism in lambs: Studies using ^{15}N-Nitrogen. J. Anim. Sci. 64:855–867.

Cameron, M. R., T. H. Klusmeyer, G. L. Lynch, J. H. Clark, and D. R. Nelson. 1991. Effects of urea and starch on rumen fermentation, nutrient passage to the duodenum, and performance of cows. J. Dairy Sci. 74:1321–1336.

Cocimano, M. R., and R. A. Leng. 1967. Metabolism of urea in sheep. Br. J. Nutr. 21:353–371.

Colucci, P. E., G. K. MacLeod, W. L. Grovum, L. W. Cahill, and I. McMillan. 1989. Comparative digestion in sheep and cattle fed different forage to concentrate ratios at high and low intakes. J. Dairy Sci. 72:1774–1785.

Driedger, L. J., and S. C. Loerch. 1999. Limit-feeding corn as an alternative to hay reduces manure and nutrient output by Holstein cows. J. Anim. Sci. 77:967–972.

Eschenlauer, S. C. P., N. McKain, N. D. Walker, N. R. McEwan, C. J. Newbold, and R. J. Wallace. 2002. Ammonia production by ruminal microorganisms and enumeration, isolation, and characterization of bacteria capable of growth on peptides and amino acids from the sheep rumen. Appl. Environ. Microbiol. 68:4925–4931.

Fellner, V., M. F. Weiss, A. T. Belo, R. L. Belyea, F. A. Martz, and A. H. Orma. 1988. Urine cup for collection of urine from cows. J. Dairy Sci. 71:2250–2255.

Frank, B., and C. Swensson. 2002. Relationship between content of crude protein in rations for dairy cows and milk yield, concentration of urea in milk and ammonia emissions. J. Dairy Sci. 85:1829–1838.

Gabler, M. T., and A. J. Heinrichs. 2003. Effects of increasing dietary protein on nutrient utilization in heifers. J. Dairy Sci. 86:2170–2177.

Hill, S. R., K. F. Knowlton, R. E. James, R. E. Pearson, G. L. Bethard, and K. J. Pence. 2007. Nitrogen and phosphorus retention and excretion in late-gestation dairy heifers. J. Dairy Sci. 90:5634–5642.

Hoffman, P. C., N. M. Esser, L. M. Bauman, S. L. Denzine, M. Engstrom, and H. Chester-Jones. 2001. Short communication: Effect of dietary protein on growth and nitrogen balance of Holstein heifers. J. Dairy Sci. 84:843–847.

Hoffman, P. C., C. R. Simson, and M. Wattiaux. 2007. Limit feeding of gravid Holstein heifers: Effect on growth, manure nutrient excretion, and subsequent early lactation performance. J. Dairy Sci. 90:946–954.

Hristov, A. N., J. K. Ropp, K. L. Grandeen, S. Abedi, R. P. Etter, A. Melgar, and A. E. Foley. 2005. Effect of carbohydrate source on ammonia utilization in lactating dairy cows. J. Anim Sci. 83:408–421.

Huntington, G. B. 1989. Hepatic urea synthesis and site and rate of urea removal from blood of beef steers fed alfalfa hay or a high concentrate diet. Can. J. Anim. Sci. 69:215–223.

Huntington, G. B., E. J. Zetina, J. M. Whitt, and W. Potts. 1996. Effects of dietary concentrate level on nutrient absorption, liver metabolism, and urea kinetics of beef steers fed isonitrogenous and isoenergetic diets. J. Anim Sci. 74:908–916.

Ipharraguerre, I. R., J. H. Clark, and D. E. Freeman. 2005. Varying protein and starch in the diet of dairy cows. I. Effects on ruminal fermentation and intestinal supply of nutrients. J. Dairy Sci. 88:2537–2555.

James, T., D. Meyer, E. Esparza, E. J. Depeters, and H. Perez-Monti. 1999. Effects of dietary nitrogen manipulation on ammonia volatilization from manure from Holstein heifers. J. Dairy Sci. 82:2430–2439.

Jones, S. J., D. L. Starkey, C. R. Calkins, and J. D. Crouse. 1990. Myofibrillar protein-turnover in feed-restricted and realimented beef-cattle. J. Anim Sci. 68:2707–2715.

Kennedy, P. M. 1980. Effects of dietary sucrose and the concentrations of plasma urea and rumen ammonia on the degradation of urea in the gastrointestinal-tract of cattle. Br. J. Nutr. 43:125–140.

Kirkpatrick, D. E., R. W. J. Steen, and E. F. Unsworth. 1997. The effect of differing forage:concentrate ratio and restricting feed intake on the energy and nitrogen utilization by beef cattle. Livest. Prod. Sci. 51:151–164.

Kohn, R. A., M. M. Dinneen, and E. Russek-Cohen. 2005. Using blood urea nitrogen to predict nitrogen excretion and efficiency of nitrogen utilization in cattle, sheep, goats, horses, pigs, and rats. J. Anim Sci. 83:879–889.

Kume, S., K. Nonaka, T. Oshita, T. Kozakai, and H. Hirooka. 2008. Effects of urinary excretion of nitrogen, potassium and sodium on urine volume in dairy cows. Livest. Sci. 115:28–33.

Lapierre, H., and G. E. Lobley. 2001. Nitrogen recycling in the ruminant: A review. J. Dairy Sci. 84:E223–E236.

Lascano, G. J., G. I. Zanton, M. L. Moody, P. A. Topper, E. F. Wheeler, and A. J. Heinrichs. 2008. Effect of changing the ratio of forage to concentrate on ammonia emissions by dairy heifers. J. Dairy Sci. 91:4301–4306.

Licitra, G., T. M. Hernandez, and P. J. Van Soest. 1996. Standardization of procedures for nitrogen fractionation of ruminant feeds. Anim. Feed Sci. Technol. 57:347–358.

Lobley, G. E., D. M. Bremner, and G. Zuur. 2000. Effects of diet quality on urea fates in sheep as assessed by refined, non-invasive [$^{15}N^{15}N$]urea kinetics. Br. J. Nutr. 84:459–468.

Lobley, G. E., X. Z. Shen, G. W. Le, D. M. Bremner, E. Milne, A. G. Calder, S. E. Anderson, and N. Dennison. 2003. Oxidation of essential amino acids by the ovine gastrointestinal tract. Br. J. Nutr. 89:617–629.

Lobley, G. E., P. J. M. Weijs, A. Connell, A. G. Calder, D. S. Brown, and E. Milne. 1996. The fate of absorbed and exogenous ammonia as influenced by forage or forage-concentrate diets in growing sheep. Br. J. Nutr. 76:231–248.

Marini, J. C., J. D. Klein, J. M. Sands, and M. E. Van Amburgh. 2004. Effect of nitrogen intake on nitrogen recycling and urea transporter abundance in lambs. J. Anim Sci. 82:1157–1164.

Marini, J. C., and M. E. Van Amburgh. 2003. Nitrogen metabolism and recycling in Holstein heifers. J. Anim. Sci. 81:545–552.

Marini, J. C., and M. E. Van Amburgh. 2005. Partition of nitrogen excretion in urine and the feces of Holstein replacement heifers. J. Dairy Sci. 88:1778–1784.

Mason, V. C. 1969. Some observations on distribution and origin of nitrogen in sheep faeces. J. Agric. Sci. 73:99–111.

Misselbrook, T. H., J. M. Powell, G. A. Broderick, and J. H. Grabber. 2005. Dietary manipulation in dairy cattle: Laboratory experiments to assess the influence on ammonia emissions. J. Dairy Sci. 88:1765–1777.

Moody, M. L., G. I. Zanton, J. M. Daubert, and A. J. Heinrichs. 2007. Nutrient utilization of differing forage-to-concentrate ratios by growing Holstein heifers. J. Dairy Sci. 90:5580–5586.

Murphy, T. A., S. C. Loerch, and F. E. Smith. 1994. Effects of feeding high-concentrate diets at restricted intakes on digestibility and nitrogen metabolism in growing lambs. J. Anim. Sci. 72:1583–1590.

National Research Council. 2001. Nutrient Requirements of Dairy Cattle. 7 ed. National Academy of Science, Washington, D.C.

Norton, B. W., J. B. MacKintosh, and D. G. Armstrong. 1982. Urea synthesis and degradation in sheep given pelleted grass diets containing flaked barley. Br. J. Nutr. 48:249–264.

Odle, J., and D. M. Schaefer. 1987. Influence of rumen ammonia concentration on the rumen degradation rates of barley and maize. Br. J. Nutr. 57:127–138.

Orskov, E. R., C. Fraser, V. C. Mason, and S. O. Mann. 1970. Influence of starch digestion in large intestine of sheep on caecal fermentation, caecal microflora and faecal nitrogen excretion. Br. J. Nutr. 24:671–682.

Orskov, E. R., and N. A. MacLeod. 1982. The determination of the minimal nitrogen excretion in steers and dairy cows and its physiological and practical implications. Br. J. Nutr. 47:625–636.

Ouellet, D. R., M. Demers, G. Zuur, G. E. Lobley, J. R. Seoane, J. V. Nolan, and H. Lapierre. 2002. Effect of dietary fiber on endogenous nitrogen flows in lactating dairy cows. J. Dairy Sci. 85:3013–3025.

Reynolds, C. K., and G. B. Huntington. 1988. Partition of portal-drained visceral net flux in beef steers. 1. Blood flow and net flux of oxygen, glucose and nitrogenous compounds across stomach and post stomach tissues. Br. J. Nutr. 60:539–551.

Reynolds, C. K., H. F. Tyrrell, and P. J. Reynolds. 1991a. Effects of diet forage-to-concentrate ratio and intake on energy metabolism in growing beef heifers - Net nutrient metabolism by visceral tissues. J. Nutr. 121:1004–1015.

Reynolds, C. K., H. F. Tyrrell, and P. J. Reynolds. 1991b. Effects of diet forage-to-concentrate ratio and intake on energy-metabolism in growing beef heifers - Whole-body energy and nitrogen balance and visceral heat-production. J. Nutr. 121:994–1003.

Richards, C. J., A. F. Branco, D. W. Bohnert, G. B. Huntington, M. Macari, and D. L. Harmon. 2002. Intestinal starch disappearance increased in steers abomasally infused with starch and protein. J. Anim. Sci. 80:3361–3368.

Schmidt-Nielsen, B. 1958. Urea excretion in mammals. Physiol. Rev. 38:139–168.

Schroeder, G. F., and E. C. Titgemeyer. 2008. Interaction between protein and energy supply on protein utilization in growing cattle: A review. Livest. Sci. 114:1–10.

Seal, C. J., D. S. Parker, and D. P. J. Avery. 1992. The effect of forage and forage-concentrate diets on rumen fermentation and metabolism of nutrients by the mesenteric-drained and portal-drained viscera in growing steers. Br. J. Nutr. 67:355–370.

Stein, H. H., B. Seve, M. F. Fuller, P. J. Moughan, and C. F. M. de Lange. 2007. Invited review: Amino acid bioavailability and digestibility in pig feed ingredients: terminology and application. J. Anim Sci. 85:172–180.

Streeter, M. N., and M. J. Mathis. 1995. Effect of supplemental fish meal protein on site and extent of digestion in beef steers. J. Anim. Sci. 73:1196–1201.

Sunny, N. E., S. L. Owens, R. L. Baldwin, S. W. El-Kadi, R. A. Kohn, and B. J. Bequette. 2007. Salvage of blood urea nitrogen in sheep is highly dependent on plasma urea concentration and the efficiency of capture within the digestive tract. J. Anim. Sci. 85:1006–1013.

Szanyiova, M., L. Leng, and S. Faix. 1995. Partition of nitrogenous substances in the urine of sheep on different dietary protein intakes. Vet. Res. 26:27–31.

Taniguchi, K., G. B. Huntington, and B. P. Glenn. 1995. Net nutrient flux by visceral tissues of beef steers given abomasal and ruminal infusions of casein and starch. J. Anim. Sci. 73:236–249.

Thornton, R. F. 1970. Factors affecting urinary excretion of urea nitrogen in cattle. 2. Plasma urea nitrogen concentration. Aust. J. Agric. Res. 21:145–152.

Thornton, R. F., P. R. Bird, M. Somers, and R. J. Moir. 1970. Urea excretion in ruminants. 3. Role of hind-gut (caecum and colon). Aust. J. Agric. Res. 21:345–354.

Valadares, R. F. D., G. A. Broderick, S. C. V. Filho, and M. K. Clayton. 1999. Effect of replacing alfalfa silage with high moisture corn on ruminal protein synthesis estimated from excretion of total purine derivatives. J. Dairy Sci. 82:2686–2696.

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

Veira, D. M., G. K. Macleod, J. H. Burton, and J. B. Stone. 1980. Nutrition of the weaned Holstein calf. 2. Effect of dietary protein level on nitrogen balance, digestibility and feed intake. J. Anim. Sci. 50:945–951.

Virtanen, A. I. 1966. Milk production of cows on protein-free feed. Science. 153:1603–1614.

Wickersham, T. A., E. C. Titgemeyer, R. C. Cochran, and E. E. Wickersham. 2008. Effect of undegradable intake protein supplementation on urea kinetics and microbial use of recycled urea in steers consuming low-quality forage. Br. J. Nutr. 23:1–8.

Zanton, G. I., and A. J. Heinrichs. 2007. The effects of controlled feeding of a high-forage or high-concentrate ration on heifer growth and first-lactation milk production. J. Dairy Sci. 90:3388–3396.

Zanton, G. I., and A. J. Heinrichs. 2008a. Analysis of nitrogen utilization and excretion in growing dairy cattle. J. Dairy Sci. 91:1519–1533.

Zanton, G. I., and A. J. Heinrichs. 2008b. Rumen digestion and nutritional efficiency of dairy heifers limit-fed a high forage ration to four levels of dry matter intake. J. Dairy Sci. 91:3579–3588.

VITA

Geoffrey I. Zanton

Education

The Pennsylvania State University, University Park, PA

May, 2009 — *Ph.D. in Dairy and Animal Science*
Minor: Statistics

University of Wisconsin—Madison, Madison, WI

May, 2002 — *Bachelor of Science in Dairy Science*
Option : Natural Science

December, 2000 — *Bachelor of Science in Animal Science*
Option : Business

Employment

Research Assistant:
Department of Dairy and Animal Science, University Park, PA: June 2002— Present

Laboratory Assistant:
Livestock Laboratory, Madison, WI: February 1999—May 2002

Dairy Production:
Zanton Farms, Avalon, WI

Peer Reviewed Publications

Lascano, G. J., **G. I. Zanton**, M. L. Moody, P. A. Topper, E. F. Wheeler, and A. J. Heinrichs. 2008. Short Communication: Effect of changing the ratio of forage to concentrate on ammonia emissions by dairy heifers. *J. Dairy Sci.* 91:4301-4306.

Zanton, G. I. and A. J. Heinrichs. 2008.
Rumen digestion and nutritional efficiency of dairy heifers limit-fed a high forage ration to four levels of dry matter intake. *J. Dairy Sci.* 91:3579-3588.

Zanton, G. I. and A. J. Heinrichs. 2008.
Analysis of nitrogen utilization and excretion in growing dairy cattle. *J. Dairy Sci.* 91:1519-1533.

Moody, M. L., **G. I. Zanton**, J. M. Daubert, and A. J. Heinrichs. 2007.
Nutrient utilization of differing forage-to-concentrate ratios by growing Holstein heifers. *J. Dairy Sci.* 90:5580-5586.

Zanton, G. I. and A. J. Heinrichs. 2007.
The effects of controlled feeding of a high-forage or high-concentrate ration on heifer growth and first-lactation milk production. *J. Dairy Sci.* 90: 3388-3396.

Zanton, G. I., M. T. Gabler, and A. J. Heinrichs. 2007.
Manipulation of soluble and rumen-undegradable protein in diets fed to postpubertal dairy heifers. *J. Dairy Sci.* 90: 978-986.

Zanton, G. I. and A. J. Heinrichs. 2005.
Meta-analysis to assess effect of prepubertal average daily gain of Holstein heifers on first-lactation production. *J. Dairy Sci.* 88: 3680-3867.