THE EFFECT OF ABOMASAL INFUSION OF HISTIDINE AND PROLINE ON MILK COMPOSITION AND MAMMARY AMINO ACID UTILIZATION IN HIGH PRODUCING LACTATING DAIRY COWS

by Megan Wiles Hofherr

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A Thesis

Presented to the Faculty of the Graduate School

of Cornell University

In Partial Fulfillment of the Requirements for the Degree of

Master of Science

by

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August 2010

ABSTRACT

The high cost of feed and increasing necessity to reduce nitrogen (N) waste in dairy production systems has driven research in the area of improving milk protein synthesis and overall efficiency of N utilization in lactating dairy cows. One strategy that has been investigated is reducing the total crude protein (CP) level of the diet while supplementing the ration with limiting amino acids (AA) for milk production. However, currently there is not enough information on the effects of increasing absorptive supply of certain individual AA on productive performance and mammary metabolism in high producing lactating dairy cows. Specifically, histidine (His) has been shown to be a limiting amino acid in grass fed lactating dairy cows and to alter fat secretion under certain conditions. In one published study in which the nonessential AA proline (Pro) was infused into the duodenum of two cows, a significant increase in milk protein output and a reduction in arginine (Arg) uptake by the mammary gland were observed. The objective of this study was to determine the effect of abomasal infusion of His and Pro, separately and in combination, on productive performance and mammary amino acid utilization in high producing lactating dairy cows.

Four rumen-fistulated Holstein cows (52 ± 16 DIM) with indwelling intercostal arterial catheters were used in a 4 × 4 Latin square experiment. Experimental treatments were continuous abomasal infusion of water (Control), His (H, 10g/d), Pro (P, 20 g/d), and His (10 g/d) + Pro (20 g/d)(H+P), with 7-d treatment periods. Cows were fed a TMR (15.6 % CP, 2.7 Mcals/kg ME) once per day for ad libitum intake, and refusals were measured and analyzed. The CNCPS v6.1 was used to formulate a diet to exceed the metabolizable energy requirement, provide 95% of the predicted

metabolizable protein requirement, and supply adequate amounts of all essential amino acids, except Arg.

Compared to the Control treatment, abomasal infusion of Pro decreased dry matter intake (DMI) by 1.8 kg/d and improved feed efficiency ($P \leq 0.05$) by 0.16 kg 3.5% FCM per kg dry matter. Fat corrected milk (FCM) yields were not affected by treatment (51.8 kg/d, TRT C; 50.6 kg/d, TRT H; 49.0 kg/d TRT H+P; 52.4 kg/d TRT P). Abomasal infusion of His resulted in no difference in milk yield or composition, and there was no effect of Pro infusion on protein and fat contents and yields. Pro infusion increased lactose percentage ($P \leq 0.05$) but not yield. The lactose response suggests that longer infusions might have resulted in increased milk yield. Mammary blood flow, expressed as L plasma/L milk, was not significantly different among treatments; though, Pro infusion increased blood flow by 14% relative to the control treatment (694.8 vs. 606.8 L plasma/L milk for P and C, respectively). Arterial concentration of His tended to be higher for His infusion than for both water and Pro infusions. The AV differences for all EAA were not affected by AA infusion; however, AV differences for Asp, Cys, Glu, and Cit were numerically lowest for Pro infusion, with no changes for other NEAA. Compared to the Control infusion, His infusion decreased extraction efficiency of His by the mammary gland. Although the P treatment did not significantly affect arterial concentration, AV difference, or extraction rate of Pro or Arg when compared to values for the control, it appears that Pro infusion tended to alter extraction efficiency and mammary uptake of Cit and Val.

Results of this experiment suggest that His does not limit milk production or milk protein synthesis in high producing lactating dairy cows fed corn silage based rations. Lactation performance and feed efficiency were not improved by abomasal infusion of His and Pro, simultaneously. Unlike results of other studies, increased absorptive supply of both His and Pro did not increase milk protein synthesis in this experiment.

Further, abomasal infusion of Pro did not reduce Arg uptake by the mammary gland, which is not consistent with other experiments in which Pro was infused postruminally in lactating cows and goats. However, this work does suggest that postruminal supplementation of Pro might improve feed efficiency and alter milk fat secretion in high producing dairy cows in early lactation.

BIOGRAPHICAL SKETCH

Megan Wiles Hofherr was born in Seoul, South Korea and was adopted when she was three months old into a loving and supportive family living in Maryland. Her parents, Bob and Jane, raised her to appreciate the simple things, to cherish family and friends, and to find a balance in working hard and having fun. Megan grew up with the best of siblings, Ryan, Tara, and Justine, with whom she spent much time playing board games, eating at Etta's, walking dogs, and going on day trips. At a young age, Megan developed an interest in biology and animal care and husbandry. She was an active member of the Harford County 4-H Dog Club and enjoyed training and showing her dog Jerry Maguire for several years. She enjoys playing piano, Irish step dancing, eating sweets (and most other foods), and playing Scrabble and many other games.

She attended the University of Delaware in Newark, DE, where she majored in Animal Science and minored in Biology. During undergrad, Megan took a bunch of hands-on animal production courses. She immediately developed a passion for working with and learning about a variety of food animal species, especially dairy cows. She spent three years working under the supervision of Dr. Limin Kung, Jr. as an undergraduate research assistant in a ruminant nutrition and microbiology lab.

Megan decided to continue on with her interests in animal nutrition and research by pursuing a Master's degree in Animal Science at Cornell University. With the guidance and support of her research advisor Dr. Mike Van Amburgh, she led an interesting study focusing on histidine and proline supplementation in lactating dairy cows. Upon completing this degree, Megan will attend the University of Pennsylvania School of Veterinary Medicine in Philadelphia, PA.

ACKNOWLEDGMENTS

First, I would like to thank Dr. Mike Van Amburgh for being such an enthusiastic and understanding adviser throughout this process. He has taught me so much about research and the dairy industry. A special thanks to Debbie Ross for helping me during the planning stages and for assisting me with both my animal and lab work. I would also like to thank Deb Dwyer for her expertise in intercostal artery catheterization techniques and willingness to offer time and advice while my study was starting. I thank my committee members, Dr. Boisclair and Dr. Nydam, for their input throughout various stages of my program. I also thank Dr. Bauman and Dr. Austic for their time and guidance. Thanks to Dr. Kung for being a great undergraduate research adviser and for teaching and challenging me in the lab.

Many thanks to Gladys Birdsall, Ray Axtell, Bob Metzler, and the rest of the farm crew at the Cornell T&R. Also, I would like to thank the students that helped me with my experiment, especially Chris and Keenan (thanks for having long arms!), Corey, Christina, Fernanda, Ashley, Lauren, Eric, and Matt, for spending a bunch of time (all hours of the day) at the farm and for putting up with me. Thank you to Dr. Barbano, Chass, and Michelle for helping with some of the milk analysis.

Thank you to my loving family members, who have always motivated me to work hard and to keep things in perspective. Thank you to my Mom and Dad for their love and generosity, and for supporting me every step of the way. Many thanks to Ryan, Tara, and Justine for being the best siblings a person could ask for. Special thanks to Kevin for the never-ending love, support, and patience throughout this experience.

I would like to thank my friends, both at Cornell and elsewhere, for supporting me during my time as a Master's student. Thank you to Lindsey Reich, with whom I have shared a close friendship and the graduate school experience despite being miles apart.

I am grateful to Julie, Anne, Manolo, Katie, Shannon, Maris, Melanie, and other Cornell friends who made my time in Ithaca easier, more memorable, and more fun. Thanks to my lab mates Fernando, Erin, Ryan, and Emiliano for answers to my many questions and for generously giving up time to help me.

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LIST OF ABBREVIATIONS

AA	Amino acid
Arg	Arginine
AV	Arteriovenous
BCAA	Branched chain amino acid
BW	Body weight
Cit	Citrulline
CNCPS	Cornell Net Carbohydrate and Protein System
CP	Crude protein
Cys	Cysteine
DIM	Days in milk
DMI	Dry matter intake
FCM	Fat corrected milk
His	Histidine
Ile	Isoleucine
Leu	Leucine
Lys	Lysine
MBF	Mammary blood flow
ME	Metabolizable energy
Met	Methionine
MP	Metabolizable protein
MUN	Milk urea nitrogen
N	Nitrogen
NPN	Non protein nitrogen
OAT	Ornithine aminotransferase

Orn	Ornithine
P5C	Δ^1-pyrolline-5-carboxylate
Phe	Phenylalanine
Pro	Proline
PUN	Plasma urea nitrogen
SCC	Somatic cell count
TMR	Total mixed ration
Tyr	Tyrosine
Val	Valine

CHAPTER ONE: LITERATURE REVIEW

Introduction

Milk protein has become a topic of significant interest due to its high nutritive and economic value, relative to other milk components. It is essential to the manufacturing of cheese and contributes to the many healthful properties of dairy products. The implementation of milk pricing schemes that accentuate the value of milk protein is another incentive prompting research of nutritional factors influencing milk composition and regulation of milk protein synthesis. In addition to the potential economic and nutritional benefits, there exists the possibility of reducing nitrogen (N) waste by improving efficiency of conversion of feed N to milk protein. Improving this efficiency by determining appropriate feeding strategies could reduce the environmental impact of the dairy industry, which remains under scrutiny by government agencies, research groups, and the media.

Current literature in the area of milk protein emphasizes a positive response in production of milk protein yield associated with increased intake of energy or crude protein (CP) (DePeters and Cant, 1992; Emery, 1978; Jenkins and McGuire, 2006). Although amount and source of dietary protein have been shown to affect the protein content of milk, this effect is modest and variable (Jenkins and McGuire, 2006). According to the review written by Emery, milk protein content increases approximately 0.02% for each 1% increase in dietary protein (Emery, 1978). Nonetheless, overfeeding N to dairy cows is costly for the producer and harmful for the environment. Therefore, it is no longer feasible to increase milk production or milk protein synthesis by feeding diets with high levels of CP.

Current approaches to reduce or optimize N inputs without decreasing milk production involve careful formulation of diets in which not only metabolizable

protein (MP) requirements are met, but also individual amino acid (AA) requirements for milk protein synthesis (Lapierre et al., 2007). Amino acids that are taken up by the lactating mammary gland are the building blocks of milk protein as well as other structural and enzymatic proteins. Incomplete understanding of how they influence metabolism within the mammary gland and production of milk components presents a serious challenge to nutritionists whose research is focused on the identification of AA that limit milk protein synthesis. If certain limiting AA are not being supplied to the mammary gland in sufficient amounts, postruminal delivery of these nutrients might be the most effective strategy to optimize AA supply for milk production without overfeeding N (Rogers et al., 1987). This strategy could be beneficial only if the microbial protein contribution to MP supply is not compromised and energy supply supports high levels of milk production (Doepel et al., 2004).

Results of experiments designed to determine effects of postruminal administration of individual or mixtures of AA are difficult to interpret, as the response observed when correcting a limitation might resemble one imposed by creating an imbalance. Although abomasal or duodenal infusion of casein has been used extensively to demonstrate a positive response in milk and milk protein production by providing more AA to the small intestine, the results are inconsistent and appear to depend on diet composition and production level of the animals (Clark et al., 1977; Derrig et al., 1974; Mackle et al., 1999; Spires et al., 1975). It is likely that postruminally infused casein or essential amino acid (EAA) mixtures are effective in supplying some AA that are limiting while providing the small intestine with others that were already in adequate supply.

Essential amino acids, individually and as a group, have been the common focus of experiments in which AA supply in lactating dairy cows is perturbed by postruminal supplementation of nutrients (Schwab et al., 1992; Bequette et al., 2000; Misciatelli et

al., 2003). Histidine (His) has received attention as an EAA found to be limiting for milk protein production when grass silage-based diets are supplemented with cereal concentrates (Huhtanen et al., 2002; Kim et al., 2001; Korhonen et al., 2000; Vanhatalo et al., 1999). Additionally, His has been the subject of experiments in which removal of the AA from an infused mixture resulted in decreased milk protein yield, increased milk fat yield and a reduced concentration of His in plasma (Bequette et al., 2000; Weekes et al., 2006). Further, a novel rumen bacterium *Allisonella histaminiformans*, isolated in ruminal fluid of lactating dairy cows fed a diet with 9.8 kg grain, has been shown to utilize His as its sole energy source to produce histamine, a potent vasodilator involved in local immune responses (Garner et al., 2002). Conversion of His to histamine in the rumen might reduce the amount of His contributing to absorbable protein in the small intestine and could subsequently limit milk protein synthesis in animals being fed diets supplemented heavily with soluble protein.

Studies designed to determine the effects of postruminal supplementation of nonessential amino acids (NEAA) are not well documented. Casein, the main protein found in milk, is rich in the NEAA proline (Pro), glutamate (Glu), and glutamine (Gln) (Swaisgood, 1995). The mammary gland extracts these nutrients in deficit of the amount that is secreted in milk (Mepham, 1982). In order to meet the Pro requirement for milk protein synthesis, intracellular arginine (Arg) and ornithine (Orn) are metabolized to produce Pro, which is accompanied by a considerable loss of N (Alumot et al., 1983). Pro synthesis via Arg metabolism might represent one or more rate-limiting steps that affect the efficiency and/or maximum capacity of milk protein synthesis.

More research focused on changes in mammary AA metabolism and milk component synthesis in response to increased postruminal supply of individual and

combined AA is needed. In theory, it would be beneficial to decrease total protein intake and supplement limiting AA to improve efficiency of N utilization in dairy cows. However, current literature is lacking conclusive information on the effects of postruminal supplementation of EAA aside from Lys and Met, and NEAA supplementation on productive performance and AA utilization. Therefore, the focus of this thesis will be observed responses to increasing abomasal supply of one EAA His and one NEAA Pro.

Overview of Milk Protein Synthesis

On average, bovine milk contains between 3 to 3.5% of protein. Milk-specific proteins include caseins, which constitute approximately 80% of bovine true protein, and whey proteins, which represent the remaining 20% (Bordin et al, 2001). Caseins are phosphoproteins that precipitate from skim milk at a pH of 4.6 at 20^{o}C and comprise proteins from four gene expressions: α_{s1}-, α_{s2}-, β-, and κ-caseins (Swaisgood, 1995). Whey proteins, which correspond to the serum fraction of milk protein, include β-lactoglobulin, α-lactalbumin, serum albumin, immunoglobulins, lactoferrin, and proteose-peptone. The high quality of casein and whey proteins is indispensable to the nutritional, growth-stimulating, and immunological benefits of milk consumption (De Wit, 1998). The AA composition of milk protein is relatively constant among many variables, including diet (Clark, 1975).

Biosynthesis of milk proteins is a complex metabolic process that occurs in mammary secretory cells. The overall mechanism of milk protein synthesis is nearly the same as protein synthesis that occurs in other body tissues. Full expression of milk protein genes is the result of efficient transcriptional, translational, and secretory components, along with an optimal supply of AA and energy. Rate of synthesis of milk proteins largely depends on the concentration of pertinent mRNAs, which are

affected by rate of transcription and stability (Mepham et al., 1998). The onset of transcription, regulated by the hormones dexamethasone and prolactin, drives mRNA production to make proteins. Additionally, it has been suggested that steps involved in translation initiation, regulated by insulin and AA, are rate-limiting for milk protein production (Cant et al., 1999).

Other factors that can influence milk protein yield and content in dairy cows include stage of lactation, breed, parity, disease, environmental temperature, and nutrition (DePeters and Cant, 1992). Nutritional factors that have been researched most extensively consist of energy intake and amount and source of dietary protein or AA (Jenkins and McGuire, 2006). The AA supply to the mammary gland is crucial to support synthesis of not only milk proteins, but also structural and enzymatic proteins that affect the production of other milk components. The mammary gland appears to be able to regulate its supply of nutrients through changes in blood flow, extraction efficiency of AA, and other regulatory mechanisms (Lacasse et al., 1996; Bequette et al., 1998; Bequette et al., 2000).

Amino Acid Uptake and Utilization in Mammary Epithelial Cells

Milk proteins are synthesized from AA that are either delivered to the mammary gland via the bloodstream or produced within secretory cells. Uptake of AA, which is influenced by extraction rate of the AA and mammary blood flow (MBF), and the intracellular metabolic conversions of these substrates are essential to the production of milk proteins. As Mepham (1982) has described in detail, upon being taken up into a mammary secretory cell, AA might pass unaltered into milk, blood, or lymph, undergo polymerization to form milk proteins that are secreted via exocytosis, be retained to form structural proteins or enzymes, or enter into one of many metabolic reactions that yield CO_2, urea, NEAA, and other substrates.

The high level of protein synthesis and AA metabolism that occurs in the lactating mammary gland is supported by active AA transport systems. Several key AA transport systems have been identified in bovine mammary tissue (Baumrucker, 1984; Baumrucker, 1985; Shennan et al., 1997). Transport of cationic AA, Arg and Lys, is carried out through a shared saturable mediated system, which has been shown to operate independent of other AA transport systems and to be inhibited competitively by these AA or Orn (Baumrucker, 1984). Most of the other AA transport systems, including A and ASC that transport most of the neutral AA, have been shown to rely on the transmembrane sodium gradient, which is maintained by sodium-potassium ATPase activity, to concentrate AA inside the cell (Baumrucker, 1985). Therefore, many of the AA transport systems require an energy source to establish and maintain concentration gradients of AA (Hanigan et al., 2001). The presence of the N system, which transports Gln, His, and Asn into mammary cells, and the Anionic AA system in bovine mammary tissue have been postulated due to strong indirect evidence (Baumrucker, 1985). Amino acid transport across the cell membrane is considered bi-directional (Baumrucker, 1984, 1985), and it appears that efflux of AA from mammary cells can be driven by intracellular concentrations of AA (Bequette et al., 2000). The extent to which nutrient availability for milk synthesis is limited by the lack of or competition of specific AA transport systems within the mammary gland remains unclear.

The AA have been categorized according to their metabolic fate within the mammary gland, which corresponds to the amount taken up by mammary epithelial cells relative to the amount that is secreted in milk protein (Mepham, 1982; Lapierre et al., 2005). The EAA, which cannot be synthesized within the body, are taken up by the mammary gland in amounts that are approximately equal to or greater than the amounts that are secreted in milk. Amino acids such as Arg, Lys, and the BCAA Ile,

Leu, and Val, are often grouped together because they are extracted in quantities that exceed their output in milk. In addition, hepatic extraction of Lys and the BCAA is low compared to that of other AA (Lapierre et al., 2005). Results from bovine mammary explant experiments using [^{14}C] Ile, Leu, and Val have shown that the carbon skeletons of these AA are catabolized to produce TCA cycle intermediates, CO_2, and NEAA (Wohlt et al., 1977). There is sufficient evidence to support that BCAA are involved in several key functions in mammary epithelial cells, including providing residues for milk protein, being oxidized to produce CO_2 and energy, and donating carbon to be incorporated into NEAA (Mepham, 1982; Bequette et al., 2006).

Other EAA, notably Met, His, Phe, Tyr, and Trp, are catabolized mainly by the liver and are extracted by the mammary gland in amounts approximately equal to what is secreted in milk (Lapierre et al., 2007). These AA, especially Phe and Tyr, are often used as markers to estimate mammary blood flow (MBF) indirectly via the Fick Principle technique because they are assumed to be incorporated directly from arterial plasma into milk protein with very little intracellular metabolism occurring (Verbeke et al., 1972; Davis et al., 1988).

The NEAA are taken up by the mammary gland in insufficient quantities to account for their output in milk. This deficit is corrected mainly by intracellular synthesis of these AA by carbon, sulfur, and N sources, including some EAA (Bequette and Backwell, 1997; Wohlt et al., 1977). Arg, along with Orn and Cit, which are extracted by the mammary gland but are not incorporated into milk protein, have been shown in perfused sheep and goat gland experiments to contribute approximately 20% of Pro residues in casein (Verbeke et al., 1968; Roets et al., 1974). Individually, Glu, Gln, and Pro account for approximately 10% or more of bovine milk protein (Swaisgood, 1995). Therefore, it seems plausible to assume that the

intracellular synthesis of these AA constitutes an important element of milk protein secretion.

Efficiency of AA Utilization for Milk Protein Synthesis in Lactating Dairy Cows

Lactating dairy cows utilize AA to support several important functions. The animal first partitions AA to fulfill a maintenance requirement. The remaining supply of digested and absorbed AA is used to support deposition of body protein, fetal growth, gluconeogenesis, or synthesis of milk protein (Tamminga and Oldham, 1980). Nonruminant animals rely mostly on AA present in the diet to meet these requirements. The profile of AA that is absorbed from the gastrointestinal tract closely resembles the profile in the diet consumed. Therefore, it is relatively simple to correct a deficiency of a single AA in most nonruminant species by supplementing it directly into the diet (Lapierre et al., 2006).

Ruminant species, however, possess a unique environment in their rumen, in which dietary rumen degradable protein (RDP) and free AA are subject to rapid degradation by action of microbial peptidases and deaminases (Leng and Nolan, 1984). Rumen bacteria and protozoa can use these products for growth and incorporation into new proteins if sufficient energy is available to support growth and other functions of rumen microbes. The breakdown of peptides and free AA in the rumen also causes ammonia production and loss from the rumen (Wallace, 1996).

Microbial protein synthesized in the rumen continues through the gastrointestinal tract and can contribute up to 66% of the nonammonia nitrogen (NAN) that is absorbed in the small intestine (Clark et al., 1992). Duodenal AA flow consists of mainly rumen undegradable protein (RUP) and microbial protein; however, endogenous secretions might contribute up to 30% of this flow (Lapierre et al., 2006, Marini et al., 2008). Absorbed AA are transported to the liver where some are

removed from the bloodstream, and a new profile of AA is carried through general circulation and is utilized by tissues for maintenance or productive purposes. Alterations in the pattern of AA supply in the digestive tract and liver challenge the ability to estimate the efficiency at which individual dietary AA are utilized for milk protein synthesis (Doepel et al., 2004).

Better prediction of milk protein secretion given a known metabolizable protein (MP) supply is necessary for balancing a ration that will effectively meet the targeted level of performance without overfeeding nutrients (Lapierre et al., 2007). It is currently understood that the efficiency of converting dietary N into milk is approximately 25 to 35% (Bequette et al., 1998; Doepel et al., 2004). Both the National Research Council (NRC, 2001) model and the Cornell Net Carbohydrate and Protein System (CNCPS, Fox et al., 2004) use fixed efficiencies of conversion of MP supply for milk production. The NRC uses a single transfer coefficient for milk production of 67% and recommends that the two EAA Lys and Met are included in the diet at 7.2% and 2.4% of MP supply, respectively (NRC, 2001). The CNCPS calculates MP requirements from milk yield and milk protein content, and MP is converted to milk protein with a fixed efficiency of 67%, which matches the NRC value (Tylutki et al., 2008). Metcalf et al. (2008) tested the accuracy of this transfer coefficient and found that 0.68, the value used by the Agricultural Food Research Council (1993), is a reasonable estimate for the efficiency of conversion of MP to milk protein for diets limiting in MP supply. However, the authors suggest that an efficiency value of 0.63 be used when MP supply is close to matching requirements (Metcalf et al., 2008). Transfer coefficients for individual AA for maintenance or lactation used by CNCPS are shown in Table 1. The efficiencies of utilization of individual AA for lactation are based on the uptake to output ratio of each AA across the mammary gland (Fox et al., 2004).

Table 1. Efficiencies of utilization of absorbed AA for physiological functions used by CNCPS. (Fox et al., 2004)

Amino acid	Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Val
Maintenance	0.85	0.85	0.66	0.66	0.85	0.85	0.85	0.85	0.76
Pregnancy	0.38	0.32	0.32	0.42	0.53	0.35	0.48	0.57	0.32
Lactation	0.35	0.96	0.66	0.66	0.82	1.00	0.98	0.78	0.62

An incomplete understanding of ruminant amino acid metabolism in mammary and extramammary tissues presents a difficult task to researchers that are working to enhance models that predict nutrient requirements for optimum milk yield and composition of lactating dairy cows. Recently it has been proposed to incorporate variable factors for transfer efficiencies of absorbed AA into predictive models. This concept is based on empirical evidence suggesting a decline in marginal efficiency of conversion of AA to milk protein as AA supply approaches estimated requirement (Doepel et al., 2004; Lapierre et al., 2006).

Doepel et al. (2004) utilized data from 59 trials in 40 published studies involving postruminal or intravenous infusion of casein or free AA to fit both a segmented-linear model and a logistic model to determine estimates of efficiency of conversion of AA into milk protein. The segmented-linear model demonstrated individual fixed efficiencies of utilization of AA for lactation ranging from 0.59 (Arg) to 0.95 (His), below the breakpoint at which AA were at optimal supply. The logistic model showed that efficiency of conversion of AA for milk followed a diminishing return pattern as total digestible AA supply was increased, which is represented in Table 2. Although, the total AA supply used to estimate the efficiency of conversion of AA to milk did not account for utilization by splanchnic and hepatic tissues. According to both models, taking into account all 59 trials, Arg was the least efficiently used AA for lactation and His was the most efficiently used AA (Doepel et al., 2004). His is one of

Table 2. Efficiencies of utilization[1] of AA for lactation after discounting maintenance requirements (scurf protein, urinary protein, metabolic fecal protein, and endogenous protein) from total AA supply, using a logistic model.

	Efficiency of conversion of AA for milk			
	% of optimum supply			
AA	50%	75%	100%	125%
Arg	0.71	0.57	0.49	0.44
His	1.09	0.88	0.76	0.68
Ile	0.86	0.72	0.65	0.58
Leu	0.83	0.70	0.61	0.55
Lys	0.90	0.76	0.68	0.60
Met	0.89	0.75	0.66	0.59
Phe	0.75	0.61	0.53	0.48
Thr	0.82	0.67	0.60	0.55
Val	0.86	0.71	0.62	0.56

Adapted from Doepel et al., 2004.

[1]Calculated from AA in milk as a function of AA available for milk

the AA that is directly incorporated into milk protein with very little intracellular metabolism, and this efficiency of conversion adds to the justification of why His might limit milk protein synthesis.

Identifying Limiting Amino Acids for Milk Production in Ruminants

The need to improve efficiency of utilization of MP and to avoid overfeeding N in ruminants has driven research in the area of identifying limiting AA for production. Since AA supply to the mammary gland is dependent upon various factors including diet, absorptive capacity, metabolism within other tissues, MBF, and abundance and activity of AA transporters, it is often difficult to predict how cows will respond to increased absorptive supply of individual AA (Bequette et al., 1998; Hanigan et al., 2001; Cant et al., 2003). Although identification of AA limitations is more difficult in ruminant species than in nonruminant species, the level of understanding in the field of amino acid requirements in ruminants is growing.

Numerous AA addition studies have been performed in order to determine the effect of supplementing individual potentially limiting EAA for milk production and milk protein synthesis (Schwab et al., 1992; Pisulewski et al., 1996; Korhonen et al., 2000). The observed responses in milk and protein yields of these and similar studies are often small and highly dependent on the MP and AA supply of the experimental diet (Hanigan et al., 2001). It is important to note that when a protein sufficient diet is used, supplementation of AA usually does not result in a significant response. This concept of diminishing marginal efficiency as AA supply approaches the required amount has been well substantiated (Doepel et al., 2004).

Methionine and Lys appear to be the most commonly investigated limiting AA in conventional dairy cow rations. Studies have suggested that these two AA are the most limiting AA when cows are fed corn and alfalfa based commercial rations, and

that optimal concentrations of these AA in MP are required to increase milk protein synthesis (Schwab et al., 1976). Although Met and Lys supplementation has been shown to increase milk and milk protein yield in certain feeding and management situations (Rogers et al., 1987; Schwab et al., 1992; Pisulewski et al., 1996), the level of the response is not always consistent, especially when AA are included in the diet as rumen protected supplements (Colin-Schoellen et al., 1995; Rulquin et al., 1997; Misciatelli et al., 2003). Since all 20 AA are required for milk protein synthesis and a deficiency in any one of these AA could result in lower rates of mRNA translation if the AA is at less than saturating concentrations, it is plausible that multiple AA might be rate limiting for milk protein synthesis at any given time (Bequette et al., 2003).

Also, it has been suggested that any of the synthetic or catabolic reactions, involving various AA and energy substrates, that occur in the lactating mammary gland might represent a rate limiting step in the process of milk protein synthesis (Hanigan et al., 2001; Bequette et al., 2003). There is a need for more research on the role of specific NEAA in protein nutrition and mammary metabolism. Although these AA can be synthesized from EAA in the mammary gland and other tissues, these processes might represent rate limiting steps in milk protein synthesis (Basch et al., 1997). Additionally, adequate supply of EAA for NEAA synthesis is most likely affected by utilization in biochemical pathways that generate energy metabolites for the cell (Bequette and Nelson, 2006). For example, results of a mammary gland explant incubation study showed that approximately 55% of galactose for lactose synthesis is derived from non-glucose sources (Bequette et al., 2005). The authors estimated that between 4 to 12% of that galactose came from EAA catabolism (Bequette and Nelson, 2006). Although some of these metabolic pathways have been established, the rates at which they occur are not well defined, which further limits the

confidence with which AA can be supplemented in rations to meet requirements for synthesis of protein and other components of milk.

Histidine: A Limiting Amino Acid?

His is an EAA that is removed extensively by the liver and is characterized by a 0.98 ratio of milk output to net post-liver supply (Lapierre et al., 2005). According to the NRC (2001), His in corn silage and grass silage comprises approximately 5.7 and 5.1% of total EAA, respectively, and His in rumen bacteria and protozoa represents 4.1 and 3.6% of total EAA, respectively. Compared to the other EAA, the proportion of His in total EAA of these sources is similar to the value for Met and Trp, and these values are less than for all other EAA (NRC, 2001). His has been the subject of numerous research reports as a potential limiting AA for milk and protein production (Vanhatalo et al., 1999; Korhonen et al., 2000; Kim et al., 2001; Huhtanen et al., 2002); however the animal models and dietary conditions used for these experiments do not represent the majority of intensive dairy production systems in the US. For example, many of these experiments were conducted in Europe with cows consuming grass silage based rations and producing less milk.

It is possible that typical commercial diets in the US can provide lactating dairy cows with adequate RDP for rumen microbes and RUP to the lower GI tract to supply the animal with adequate amounts of His for high levels of milk production and protein synthesis. However, there are very few published studies in which a potential His limitation in high producing lactating cows was investigated. Furthermore, a novel rumen bacterium *Allisonella histaminiformans* has been shown to utilize His as its primary source of energy (Garner et al., 2002). This Gram-negative bacterium, which is resistant to monensin, grows rapidly by converting His to histamine and CO_2 via decarboxylation of His. Histamine is a potent vasodilator and arterial constrictor

that is naturally found in tissues and blood (Chavance, 1946). Additionally, it has received attention as one of the contributing factors in cases of bovine laminitis (Nocek, 1997; Garner et al., 2004). Histamine production in the rumen by *A. histaminiformans* appears to be diet-dependent, as conversion of His to histamine was very low in dilutions of rumen fluid from cows fed only timothy hay and was significantly higher as grain was included in the diet (Garner et al., 2002). Furthermore, extracts of both corn and alfalfa silage have been shown to stimulate growth and histamine production of *A. histaminiformans*, although alfalfa silage was most effective (Garner et al., 2004). Inclusion of high quality alfalfa and grain in the diet might lead to higher rates of histamine production by these bacteria in the rumen, which could limit absorptive supply of His.

The most recent experiments in which His was shown to be limiting for milk production or milk protein synthesis were conducted using lactating cows being fed grass silage based diets (Vanhatalo et al., 1999; Korhonen et al., 2000; Kim et al., 2001; Huhtanen et al., 2002). The cows in these studies were in early lactation, and reported averages of daily milk production ranged from 22.9 to 28.8 kg, which is less than the typical high producing dairy cow in the US. However, under these particular experimental conditions, the authors observed increases in either milk or protein yield, or both, when at least 6 g of His was continuously infused into the abomasum of the animals. The magnitude of the response in milk production varied by experiment, and researchers have reported increases of milk protein yield ranging from 26 g/d, when 6.5 g of His was infused into the abomasum (Vanhatalo et al., 1999), to 111 g/d, when 6 g of His was infused intravenously (Kim et al., 2001). Korhonen et al. (2000) reported a linear increase in daily milk, protein, and lactose yield with increasing doses of abomasal His, from 0 to 6 g. The predicted MP supply of this experimental diet relative to predicted requirement was not reported.

Correction of a His deficiency has been shown commonly in His subtraction experiments to stimulate milk and milk protein production while decreasing production of milk fat (Bequette et al., 2000; Cant et al., 2001; Weekes et al., 2006; Purdie et al., 2009). A recent study, in which mixtures of AA with or without His were infused intravenously in four Holstein cows in mid lactation, showed that milk protein yield decreased 100 g/d with His deficiency, which was accompanied by an increase in milk fat by 95 g/d (Purdie et al., 2009). Proposed mechanisms by which a His deficiency might stimulate milk fat production include faster rate of MBF to compensate for low His concentration in plasma (Bequette et al., 2000), a change in concentrations of milk fat precursors in circulation due to a more systemic response to the deficiency, and an alteration of intracellular carbon flows associated with excess AA catabolism in the mammary gland (Cant et al., 2001; Weekes et al., 2006).

Bequette et al. (2000) conducted an experiment in which four lactating goats were fed a low protein ration, and mixtures including and excluding His were infused into the abomasum of each animal. Responses to a His deficiency and subsequently low His concentration in plasma included increased MBF and capacity of the mammary gland to remove circulating His along with decreased influx and efflux of His by the mammary gland. Extraction efficiency of His increased from 24 to 83% when His supply was limiting. The authors of this study reported that the udder appeared to have the ability to regulate MBF in an attempt to overcome a single AA limitation. Also, they implicated that the mammary gland responded to the His limitation through up-regulation of His extraction processes and down-regulation of those processes that are involved in extracting other AA. Although this research does not shed light on the extent of His limitation under normal conditions, it does provide evidence to support adaptive capabilities of the mammary gland in response to a His limitation.

Proline: A Limiting Amino Acid?

Pro is one of the most abundant amino acids in casein, the major protein in most dairy products. It constitutes approximately 10% of the total AA profile of milk protein; however it is taken up by the mammary gland in deficit of its output in milk (Mepham, 1982). Measurement of AV differences of plasma free AA across the lactating mammary gland in combination with experiments involving incubation of mammary tissue slices has led to the discovery of a link between Arg metabolism and Pro synthesis. Through a series of enzymatic steps related to the urea cycle, Pro precursors are synthesized in mammary secretory cells through metabolism of Arg and ornithine (Orn), both AA taken up in excess by the mammary gland (Clark et al., 1975; Mepham and Linzell, 1966).

Though evidence in the literature refutes the presence of a complete urea cycle in mammary tissue, several key enzymes that are involved in the conversion of Arg to Pro have been identified in the lactating bovine mammary gland (Basch et al., 1996; Basch et al., 1997; Basch et al., 1995; Clark et al., 1975). A proposed mechanism of Pro synthesis in the lactating mammary gland is presented in Figure 1. Arginase, an enzyme that catalyzes the hydrolysis of Arg to Orn and urea, is expressed in lactating bovine (Basch et al., 1997; Clark et al., 1975) and rat (Glass and Knox, 1973; Yip and Knox, 1972) mammary tissue. Ornithine-δ-aminotransferase (OAT), which catalyzes the conversion of Orn and α-ketoglutarate to glutamate semialdehyde and glutamate, respectively, is coordinately expressed in the bovine lactating mammary gland (Basch et al., 1995). Glutamate semialdehyde, which is produced by activity of OAT, cyclizes to form Δ^1-pyrroline-5-carboxylate (P5C). The conversion of P5C to the final product Pro occurs via action of P5C reductase, which has been identified in both the mitochondrial and cytosolic fractions of lactating mammary gland cells (Basch et al., 1996). The enzyme P5C reductase uses NADPH, which is one of the products of

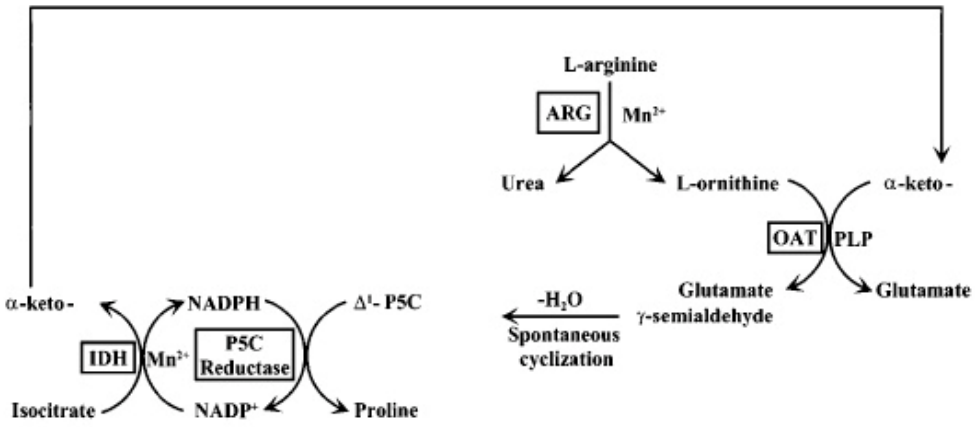

Figure 1. Proposed mechanism of proline production in the lactating bovine mammary gland. IDH = isocitrate dehydrogenase, P5C = pyrroline-5-carboxylate, OAT = ornithine aminotransferase, α-keto = α-ketoglutarate, and ARG = arginase. From (Basch et al., 1997).

isocitrate dehydrogenase, a Krebs cycle enzyme that converts isocitrate to α-ketoglutarate (Basch et al., 1997). The source of the α-ketoglutarate and NADPH required for intracellular synthesis of Pro is the Krebs Cycle; therefore, the two processes appear to be linked (Basch et al., 1997).

Uptake of Pro by the mammary gland is insufficient to account for Pro output in milk protein (Mepham, 1982), therefore intracellular Pro synthesis, which utilizes Arg and Orn, might represent a rate limiting step in milk protein synthesis (Bequette and Backwell, 1997). Arg, which is extracted in the greatest amounts relative to milk protein outputs (150 to 200% excess), is a metabolically versatile AA. A simple schematic of sources and metabolic fates of Arg is presented in Figure 2. Since approximately 20% of Pro is synthesized within mammary cells, close to half of which appears to be due to utilization of Arg (Verbeke et al., 1968), it might be important to consider other metabolic functions that require this multifunctional AA. Not only does Arg contribute as a building block of proteins, but also it serves as a precursor for synthesis of nitric oxide (NO), urea, polyamines, agmatine, creatine, and glutamate (Wu and Morris, 1998; Morris, 2006). The role of Arg in production of NO is important because NO is considered a vasorelaxant that has been shown to control blood flow to the mammary gland (Lacasse et al., 1996; Lacasse and Prosser, 2003). Although close arterial infusion of the NO donor diethylamine NONOate increased the rate of MBF by 250% in lactating goats, the short-term increase in MBF did not affect milk production (Lacasse and Prosser, 2003). Dietary supplementation of a NO donor in lactating sows was shown to enhance weight gain in nursing neonates (Kim and Wu, 2009). Polyamines, which are synthesized from Arg and Orn, are essential for normal cell growth and can regulate the function of DNA, RNA, and other proteins (Igarashi and Kashiwagi, 2000). Exploitation of the regulatory roles of Arg in

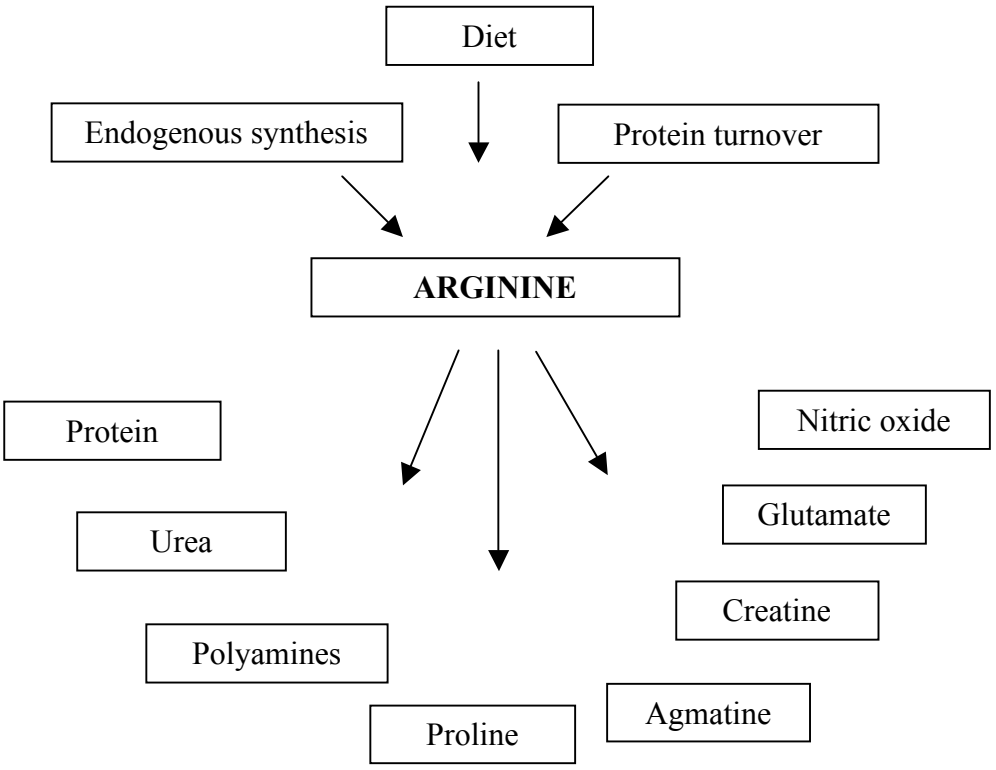

Figure 2. Sources and metabolic products of arginine. Adapted from (Morris, 2006).

mammary gland growth and milk synthesis might be an effective strategy to improve milk production (Kim and Wu, 2009).

Results from two published studies investigating the effects of duodenal Pro infusion in ruminants suggest that increasing absorptive supply of Pro might have decreased the Arg requirement of the mammary gland. To test whether or not increased Pro supply to the small intestine affects milk composition and uptake of AA by the mammary gland, Bruckental et al. (1991) performed an experiment on a small group of early and mid lactation Israeli-Fresian cows. Postruminal infusion of Pro did not affect milk protein concentration and protein yield during early lactation; however, milk fat content and 4% fat-corrected milk yield increased. Bruckental et al. also observed a significant decrease in Arg uptake by the mammary gland when Pro was infused, suggesting that the Arg requirement for milk protein synthesis was reduced with increased absorptive Pro supply (Bruckental et al., 1991). Alumot et al. (1983) observed similar responses when they infused Pro into the duodenum of two lactating goats. These authors reported a significant increase in milk fat content (from 2.8 to 3.2%) during Pro infusion (Alumot et al., 1983). Although these studies provide some evidence that postruminal infusion of Pro might increase milk protein yield or alter milk composition in lactating ruminants, the experiments used small numbers of animals, indicating that further work is required to draw practical conclusions from the results.

Conclusions and Objectives

The current literature provides a basic understanding of intracellular AA metabolism and milk protein synthesis in the mammary gland; however the effects of changing the absorptive supply of individual AA on these reactions remain unclear. The lactating mammary epithelial cell comprises a complex set of metabolic reactions

that require high levels of energy and AA, although the extent to which these reactions might limit the rate of milk protein synthesis is not well understood. More research is needed in this area in order to determine AA requirements for milk protein synthesis and to identify limiting AA in high producing lactating dairy cows. His and Pro are two AA that have not been investigated extensively, yet there is some evidence that increasing absorptive supply of each will result in higher milk protein production in lactating dairy cows.

His has been shown to increase milk production and milk protein yield in dairy cows fed grass silage based rations. Furthermore, a recently isolated rumen bacterium *A. histaminiformans*, readily converts His to histamine and utilizes His as its primary source of energy. Growth and histamine production by *A. histaminiformans* appears to be dependent on diet and seems to favor a rumen environment in which corn and alfalfa based diets that are high in grain have been fed. It has yet to be determined whether or not His limits milk production in high producing lactating dairy cows fed corn silage based commercial diets commonly found in the US. Duodenal infusion of Pro in lactating cows and goats has resulted in elevated milk protein yield and a significant decrease in Arg uptake by the mammary gland. Researchers conducting these studies suggested that increased absorptive supply of Pro might have reduced the requirement of Arg for intracellular Pro synthesis within the mammary gland.

One of the objectives of the current experiment is to determine the effect of abomasal infusion of His and Pro, together and separately, on production of protein and other components of milk. The second objective is to investigate the effect of increased absorptive supply of these AA on AV difference, uptake, and extraction efficiency of AA by the mammary gland. These questions will be answered using high producing dairy cows in early lactation being fed a corn silage based diet that is formulated to be slightly deficient in MP supply.

CHAPTER TWO: EFFECT OF ABOMASAL INFUSION OF HISTIDINE AND PROLINE ON MILK COMPOSITION AND MAMMARY AMINO ACID UTILIZATION IN HIGH PRODUCING LACTATING DAIRY COWS

ABSTRACT

Histidine (His) has been shown to be a limiting amino acid in lactating dairy cows fed grass silage based diets and to alter fat secretion under certain conditions. A significant increase in milk protein output and a reduction in arginine uptake by the mammary gland were observed when proline (Pro) was infused into the duodenum of two cows. The objective of this experiment was to determine the effects of abomasal infusion of His and Pro on lactation performance and amino acid utilization by the mammary gland in cows. Four rumen-fistulated Holstein cows (52 ± 16 DIM) with indwelling intercostal arterial catheters were used in a 4 × 4 Latin square experiment. Experimental treatments were continuous abomasal infusion of water (Control, C), His (H, 10g/d), Pro (P, 20 g/d), and His (10 g/d) + Pro (20 g/d) (H+P), with 7-d treatment periods. Cows were fed a TMR (15.6 % CP, 2.7 Mcals/kg ME) once per day for ad libitum intake, and refusals were measured and analyzed. The CNCPS v6.1 was used to formulate a diet to exceed the metabolizable energy requirement, provide 95% of the predicted metabolizable protein requirement, and supply adequate amounts of all essential amino acids, except Arg. Fat corrected milk (3.5% FCM) yields were high and not affected by treatment (51.8 kg/d, TRT C; 50.6 kg/d, TRT H; 49.0 kg/d TRT H+P; 52.4 kg/d TRT P), however abomasal infusion of Pro decreased feed intake and improved feed efficiency by 0.17 kg 3.5% FCM per kg dry matter ($P \leq 0.05$). Pro infusion increased lactose percentage ($P \leq 0.05$) but not yield. The increase in lactose content suggests that longer infusions might have resulted in increased milk yield. A

similar effect for lactose and feed efficiency was observed for the H+P treatment. Abomasal infusion of His resulted in no difference in productive performance. Mammary blood flow, expressed as L blood/L milk, was not significantly different among treatments; though, Pro infusion increased blood flow by 14% relative to the control treatment (694.8 vs. 606.8 L blood/L milk for Pro and control treatments, respectively). Arterial concentration of His tended to be higher for His infusion than for both water and Pro infusions ($P \leq 0.10$) and was not significantly different between H and H+P treatments. The AV differences for all EAA were not affected by AA infusion; however, AV differences for Asp, Cys, Glu, and Cit were numerically lowest for Pro infusion, with no changes for other NEAA. Compared to the Control infusion, His infusion decreased extraction efficiency of His by the mammary gland. Although the Pro infusion did not significantly affect arterial concentration, it appears that Pro infusion tended to alter extraction efficiency and mammary uptake of Cit and Val ($P \leq 0.10$) compared to the Control. Results show that lactation performance and feed efficiency were not improved by simultaneous abomasal infusion of His and Pro. However, this experiment indicates that postruminal supplementation of Pro might increase milk fat production and feed efficiency in high producing dairy cows in early lactation.

INTRODUCTION

Dairy products provide a unique and complete source of protein, energy, vitamins, and minerals for humans to consume. Improving conversion efficiency of dietary nutrients into milk and its beneficial components has been a research topic of significant interest for decades. Milk protein has received much attention, as it is valuable not only from a nutritional standpoint, but also from an economic one.

According to current milk pricing systems, dairy producers receive a premium for the protein their animals produce. However, concern about the detrimental environmental impact of excessive N waste on dairy farms paired with high feed costs has prompted nutritionists to investigate feeding strategies that could potentially maximize milk protein and improve efficiency of utilization of dietary nutrients. It has been suggested that the most effective strategy to reduce N losses from dairy farms is to improve the efficiency of feed N utilization by dairy cattle (Jonker et al., 2002).

Amino acid metabolism in dairy cows is complex and presents a challenge to researchers who aim to improve efficiency of utilization of consumed N for milk protein production. The NRC model (2001) predicts milk protein yield using fixed efficiencies of conversion of metabolizable protein (MP) for maintenance (67%), pregnancy (33%), and milk production (67%). The use of an MP system reflects the idea that diets can be balanced to meet requirements for absorbable protein, which are really AA. It has been postulated that efficiency of conversion of AA to milk protein is dynamic and decreases as AA supply approaches requirement (Doepel et al., 2004). However, other data suggest it is difficult to alter this efficiency under typical production conditions (Metcalf et al., 2008). Supply of AA to the mammary gland for protein synthesis is dependent upon various factors, including intracellular metabolism of nutrients within the mammary gland, local blood flow, and utilization of AA by other tissues. Once taken up by mammary secretory cells, AA are extensively synthesized and degraded via transamination, transformation, and oxidation reactions (Bequette 1998).

Several of the EAA, notably Arg and the BCAA are extracted by the mammary gland in excess of their output in milk. They can act as substrates for transamination and catabolic reactions that form intermediates for synthesis of NEAA, which are taken up by the mammary gland in deficit of their output in milk. The group of AA

for which uptake by the mammary gland is approximately equal to secretion into milk protein includes Met, His, Phe, and Tyr (Mepham, 1982). Research on identification of limiting AA for milk production in lactating dairy cows has indicated that Met and Lys are the first and second limiting AA when corn based diets are fed (Clark, 1975; Schwab et al., 1976; Schwab et al., 1992a; Schwab et al., 1992b). These studies have focused mainly on postruminal supplementation of casein or EAA, both individual and in combination, and the responses in milk and protein production are variable (Derrig et al., 1974; Schwab et al., 1975; Mackle et al., 1999; Vanhatalo et al., 1999).

Histidine is an EAA that is removed extensively by the liver of dairy cows and is characterized by a 0.98 ratio of milk output to net post-liver supply (Lapierre et al., 2005). Histidine has been shown to limit milk production in dairy cows fed grass silage based rations (Vanhatalo et al., 1999; Korhonen et al., 2000; Huhtanen et al., 2002). In these studies, a significant increase in both milk yield and milk protein yield was observed when His was infused into the abomasum of early or mid lactation dairy cows. The animals used for these experiments were producing between 22.9 and 28.8 kg of milk per day, which is typically less than the average milk yield of high producing cows being fed conventional corn silage and legume based diets. Further, a novel rumen bacterium *Allisonella histaminiformans* utilizes His as its sole energy source and readily decarboxylates this AA to form histamine, a neurotransmitter involved in local immune responses (Garner et al., 2004). Histidine degradation by these rumen bacteria might limit the amount of His that is available for absorption and utilization for productive functions.

The NEAA are taken up by mammary secretory cells in amounts that are insufficient for milk protein synthesis. These AA are synthesized within cells via catabolism of EAA that are taken up by the mammary gland in excess of their output in milk (Mepham, 1982). Although Pro accounts for approximately 10% of milk

protein (Swaisgood, 1995), it has received little attention as a potentially limiting AA for production in lactating dairy cows, mainly due to the fact that it can be synthesized within the mammary gland (Verbeke et al., 1968). Arginine and Orn, which are taken up in excess of their output in milk, contribute to intracellular synthesis of Pro (Clark et al., 1975). Several key enzymes that facilitate this process, including arginase, ornithine aminotransferase (OAT) and Δ^1-pyrroline-5-carboxylate reductase (P5C), have been identified in vitro in the lactating bovine mammary gland (Basch et al., 1995; Basch et al., 1996; Basch et al., 1997).

Bruckental et al. (1991) infused 80 g/d of Pro into the duodenum of four dairy cows being fed a 14% CP diet and observed a significant increase in milk protein yield in the two mid lactation cows. Proline infusion resulted in increased milk fat content and 4% FCM yield in all cows. Interestingly, the authors reported that Arg uptake by the mammary gland decreased by 50% during Pro infusion, and they suggested that increased postruminal supply of Pro reduced requirements for Arg by the mammary gland and improved efficiency of dietary energy utilization. In a similar experiment, two goats were fed a protein deficient diet and given a duodenal infusion of Pro. In those goats milk fat percentage increased (4.0 vs. 2.8%), and Arg uptake was decreased with Pro infusion (Alumot et al., 1983).

Currently there is not enough evidence to determine whether or not postruminal supplementation of His or Pro results in higher yields or efficiency of milk or milk protein production in high producing dairy cows fed diets conventional to the Northeastern dairy industry in the US. Additionally, information on altering AA and energy metabolism in the mammary gland or efficiency of utilization of dietary nutrients by increasing the supply of individual AA could improve current models of mammary metabolism (Maas et al., 1997; Hanigan et al., 2001). If Pro synthesis within the mammary gland represents one or more steps that might be limiting milk

protein synthesis, then supplying more Pro directly to the mammary gland could be one method of improving efficiency of milk protein production. The objective of the present study is to determine lactation performance and mammary AA utilization in response to abomasal infusion of His and Pro and to determine if infusion of these AA alters the metabolism of other EAA and NEAA.

MATERIALS AND METHODS

Animals, Design, and Treatments

All experimental procedures were approved by the Cornell University Institutional Animal Care and Use Committee. Four multiparous, rumen-fistulated Holstein cows were randomly assigned to treatments arranged in a 4 × 4 Latin square design, with consecutive 7-d periods. At the beginning of the study, cows averaged 674 ± 31 kg of BW and were 52 ± 16 DIM (mean ± SD). The cows were housed in individual tie stalls with sawdust bedding and were milked three times daily, at 0700, 1500, and 2300 h. Cows walked to the parlor for milking during the first five days of each period and were milked in their stalls with a bucket milker on the last two days of each period.

Treatments were continuous abomasal infusion of water (control; C), 10 g of His (H), 20 g of Pro (P), and 10 g of His plus 20 g of Pro (H+P). Prior to the start of the experiment, infusion cannulae were installed via the reticulo-omasal orifice into the abomasum of each cow (Mackle et al., 1999). The AA were measured and then solubilized in water, and the solutions were continuously infused using peristaltic pumps (Masterflex L/S, Model 07523-70, Cole-Parmer, Vernon Hills, IL) that were calibrated so that each cow received 4 L of the AA solution over a 24-h period. Infusates were carried via Nalgene tubing (0.5 cm i.d.), which was placed through the

rumen fistula and sulcus omasi into the abomasum. Bottles with infusates were weighed at the beginning and end of each 24-h period to ensure consistent delivery of the appropriate amounts of AA. Abomasal cannulae were checked every other day during the trial to verify proper placement. Lysine-HCl (Dyets, Inc., Bethleham, PA) was added to the experimental infusates and continuously infused at a rate of 32.5 g/d to ensure that postruminal supply of Lys would not limit milk production in the present experiment. This level of supplementation was based on estimated requirements from the CNCPS v6.1 (Tylutki et al., 2008).

Diet

Cows were fed a basal TMR (Table 3) once per day between 0700 and 0930 for ad libitum intake. The refusals were set to be 10% of the amount of feed offered and were measured each morning between 0600 and 0800. Samples of TMR, orts, forages, and concentrate were collected twice per week, dried, composited by week, and analyzed for chemical composition (Dairy One Forage Lab, Ithaca, NY). The Cornell Net Carbohydrate and Protein System (CNCPS) v6.1 (Tylutki et al., 2008) was used to formulate the ration to provide 105% of metabolizable energy requirements and to provide 95% of the metabolizable protein requirement. The diet was formulated to have a CP content of 14.4% and to provide EAA to meet requirements as shown in Table 2, with the exception of Arg, which was supplied at 95% of requirement.

Since Lys and Met are often regarded as first and second limiting AA for milk production in high producing dairy cows, Smartamine M (Adisseo, Alpharetta, GA) was included in the concentrate portion of the diet to satisfy the predicted Met requirement for the animals. Lysine was added via continuous abomasal infusion of Lys- HCl to ensure that postruminal supply of Lys would not limit milk production in

Table 3. Diet ingredients expressed as proportion of total mixed ration dry matter and formulated chemical composition.

Ingredient	**% of total ration DM**
Forage	
Corn silage, processed	46.13
Mixed mostly legume silage	11.65
Wheat straw	1.83
Concentrate	
Corn grain, steam flaked	16.28
Wheat midds by-product	6.19
Soybean hulls	6.19
Rumen bypass soy protein[1]	3.68
Whey permeate	3.24
Soybean meal, 48% CP	3.00
Rumen bypass fat	0.82
Sodium bicarbonate	0.64
Limestone, ground	0.60
Salt	0.39
Urea	0.31
Calcium sulfate	0.24
Magnesium oxide	0.10
Smartamine M	0.07
Selenium 0.60%	0.05
1100 Dairy TM	0.03
Dairy ADE-AL/MA	0.02
Chemical composition	
CP, % DM	14.4
Soluble P, % CP	38.0
NDF, % DM	32.8
Lignin, % DM	7.8
Crude fat, % DM	5.0
Calcium, % DM	0.69
Phosphorus, % DM	0.40
Magnesium, % DM	0.26
Potassium, % DM	1.32
Sodium, % DM	0.18
ME, Mcal/kg	2.65

Table 4. The formulated essential amino acid balance, requirement, and supply as predicted by Cornell Net Carbohydrate and Protein System v6.1 for a 635 kg cow consuming 22.6 kg DM/d and producing 39.9 kg milk/d at 3.65% fat and 3.01% protein.

	MP (g/d)		MP AA Supply (g/d)		
	Balance	Required	Total	Bacteria	RUP
Arg	-7.4	158.4	151.0	90.4	60.6
His	12.9	49.1	62.0	35.0	27.0
Ile	2.2	122.3	124.5	76.4	48.1
Leu	-0.5	195.4	194.9	97.6	97.3
Lys	21.7	142.6	164.4	106.6	57.8
Met	15.0	43.0	58.0	34.8	23.2
Phe	43.0	79.2	122.3	67.1	55.2
Thr	37.8	79.3	117.2	72.6	44.5
Trp	6.4	28.1	34.5	21.2	13.3
Val	-0.3	138.7	138.4	80.1	58.3

the present experiment. Based on the predictions of the CNCPS, the addition of these two AA would have maintained a Lys: Met ratio of 2.8: 1 and balanced Lys at 7.2% of EAA.

Milk Sampling and Analysis

Milk yield was measured daily via electronic parlor measurements or direct weighing throughout the duration of the experiment, and samples were collected at each milking on the last 2 d of each treatment period. Samples were analyzed individually for true protein, fat, lactose, total solids, MUN, and SCC at the Northeast DHIA Inc. using an infrared milk analyzer (Milkoscan 6000; Foss Electric, Hillerød, Denmark). Milk samples taken on the last 2 d of each period were composited by day and an aliquot for each composite was frozen at -15^{o}C until analysis for NPN. Macro-Kjeldahl techniques were utilized to determine the NPN fraction of composited milk samples (Barbano and Lynch, 1991).

Another aliquot of each daily composite was de-fatted and used to determine milk AA composition. A modified procedure of Gehrke et al. (1985) was used to acid hydrolyze milk samples with 6 N hydrochloric acid. Milk samples containing approximately 2 mg N were freeze dried in screw top hydrolysis tubes, and 50 μL of 125 mM norleucine and 5 ml of 6 N HCl were added. Mixtures were flushed with N gas to remove O_2, and were placed in boiling water for 10 minutes before being heated in a block heater at 110^{o}C for 21 h. After filtration through a Whatman 541 filter, all HCl was removed from a 1 ml aliquot by evaporation with N_2 gas. Approximately 0.6 ml of sample was filtered through a 0.45 μm nylon filter into a vial and was frozen at -15^{o}C until analysis for AA composition. A modified preoxidation technique using performic acid (Spindler et al., 1984) was employed to convert the sulfur AA cysteine and methionine to cysteic acid and methionine sulfone, respectively prior to acid

hydrolysis with hydrochloric acid. Milk samples were analyzed by ion exchange High Pressure Liquid Chromatography (HPLC System Gold with 32 Karat software, Beckman-Coulter, Inc., Fullerton, CA) for AA composition. Lithium based buffers (Li292, Li365, Li375) were used to elute AA, and AA concentrations were determined by absorbance at 560 nm following postcolumn derivitazation with a ninhydrin reagent.

Measurements of Arteriovenous Difference

Indwelling intercostal artery catheters were installed at least six days prior to the start of the experiment. The last day of each experimental period was used for collection of blood from an artery and vein of each animal. Arteriovenous differences across the mammary gland were measured according to the procedure of Mackle et al. (2000). Prior to the commencement of the experiment, an indwelling catheter (0.065 in o.d. × 0.030 in i.d.; Micro-Renathane, Braintree Scientific Inc.) was installed in an intercostal artery of each cow to obtain an arterial blood sample, and patency was maintained for the duration of the experiment. To collect a venous blood sample, a catheter (0.080 in o.d. × 0.040 in i.d.; Micro-Renathane, Braintree Scientific Inc.) was inserted in one of the caudal superficial epigastric veins of each animal at least 12 h prior to sampling and was removed immediately following collection of the last blood sample.

Cows were standing for at least 20 minutes prior to blood sampling and remained standing throughout collections. Arterial and venous blood samples were obtained simultaneously into heparinized tubes at 1-h intervals for 12 h. Tubes were placed in ice, and plasma was separated by centrifugation and collected immediately after each sampling. Deproteinization of plasma was achieved by adding 0.07 ml of 50% sulfosalicylic acid containing the internal standard norleucine (2.5 μM) to 0.63 ml of

plasma. Deproteinized plasma samples were vortexed extensively, centrifuged, and the supernatant fractions were composited at 2-h intervals resulting in six pairs of arterial and venous samples per cow on the last day of each period. Samples were filtered through a 0.45 μm filter and frozen at -15°C. An automated ion-exchange HPLC system (HPLC System Gold with 32 Karat software, Beckman-Coulter, Inc., Fullerton, CA) was used to analyze plasma for AA composition. An additional aliquot of plasma was collected at 1200 and 1400 on the last day of each period and frozen until analysis for plasma urea N (PUN). Concentration of PUN was determined by enzymatic colorimetric analysis using commercial kits (Sigma Chemical Co., St. Louis, MO).

Calculations and Statistical Analysis

The Fick Principle assumes that uptake of a particular substrate by the mammary gland is estimated by multiplying the AV difference across the udder with mammary blood flow (MBF). This concept depends on the presence of a marker that is transferred from arterial blood to milk directly, without participating in other intracellular metabolic processes. In this experiment MBF was estimated by utilizing the AA Phe and Tyr, because they are assumed to be secreted in milk with an output to uptake ratio close to 1. The following equation used to calculate MBF was the same as the one reported by Mackle et al. (2000):

$$\text{MBF} = \frac{\text{MP}_{\text{Phe}} + \text{MP}_{\text{Tyr}}}{\text{AV}_{\text{Phe}} + \text{AV}_{\text{Tyr}}} \times \frac{100}{100 - \text{Hct}}$$

MP_{Phe} and MP_{Tyr} represented the concentration of the respective AA in milk, multiplied by the daily milk yield. The AV_{Phe} and AV_{Tyr} were AV differences across the udder for these AA, and Hct signified hematocrit percent, which was determined

immediately after each blood sampling. Extraction efficiency was calculated by dividing the AV difference of the AA by its concentration in arterial plasma and then multiplying by 100. Mammary uptake of AA was calculated by multiplying the AV difference across the mammary gland by the mammary blood flow.

Mean values for each cow were determined for milk yield, milk composition, and DMI from the last two days of each treatment. Effects of treatment were analyzed as a mixed model procedure of JMP (version 8.0, SAS Institute, Cary, NC), using the following model:

$$Y_{ijk} = \mu + C_i + P_j + T_k + \varepsilon_{ijk}$$

where Y_{ijk} is the dependent variable, μ is the overall mean, C_i is the random effect of cow, P_j is the fixed effect of period, T_k is the fixed effect of treatment, and ε_{ijk} is the residual error. Data and residuals were checked for normality, and these distributions provided further justification for using the mixed model. Data are presented as least squares means. Treatment effects were declared significant at $P \leq 0.05$, and trends for treatment effects were declared at $P \leq 0.10$. Since treatments were not expected to be linear, single degree of freedom orthogonal contrasts were also performed to compare individual treatment means.

RESULTS AND DISCUSSION

Diet Composition

Nutrient composition of corn silage, mixed mostly legume silage, and wheat straw were relatively uniform between weekly samples of each forage type. Mixed concentrate ingredients were delivered to the farm in one load, and weekly samples

were similar in chemical composition. In vitro digestibility (24 h) of the corn silage, mixed mostly legume silage, and wheat straw were 79.8%, 77.3%, and 29%.

Chemical composition of the TMR concentrate and forages is presented in Table 5. The formulated CP content of the TMR was 14.4%, however, the mixed mostly legume silage varied from formulation through the experimental period. The actual CP content of the diet and orts were 15.6% and 14.9%, respectively, thus the CP of the diet was higher than planned.

Feed Intake

All cows remained in good health for the duration of the experiment, and DMI was not dramatically suppressed for any extended time during continuous infusions. Dry matter intake, feed efficiency (FE), milk yield, and composition are presented in Table 6. Average DMI was 25.7 ± 1.4 kg/d among treatments. Cows infused with Pro consumed significantly less DM than cows on control (C) and His (H) treatments ($P < 0.05$), and tended to consume less DM than cows on the His and Pro (H+P) treatment ($P < 0.10$). Although dietary AA imbalances have been shown to decrease feed intake in nonruminant species (Harper et al., 1970), AA imbalances in ruminant species are not commonly observed due to the consistency of the AA profile of microbial protein (Allen, 2000). It is unlikely that Pro infusion caused an AA imbalance mainly due to lack of supporting evidence and the assumption that animals are able to cope with NEAA imbalances better than with EAA imbalances (Harper et al., 1970). According to Bruckental et al. (1991), there was no significant difference between DMI of control and Pro infused cows. More studies in which Pro and other NEAA are infused individually might lead to a more conclusive scheme of this effect on feed intake. Vanhatalo et al. (1999) observed slightly higher ($P = 0.05$) total DMI with His infusions as compared with the control. In similar His infusion studies, average DMI

Table 5. Chemical composition of feedstuffs used in this infusion experiment.

Variable	Corn silage	Mixed mostly legume silage	Concentrate
DM, %	40.1	35.9	91.8
CP, % DM	8.5	21.9	20.6
Soluble P, % CP	61.6	68.8	31.7
NDF, % DM	37.3	43.2	23.0
ADF, % DM	22.4	32.6	12.2
Lignin, % DM	2.8	6.9	--
Crude fat, % DM	2.8	4.2	--
Calcium, % DM	0.16	1.51	1.38
Phosphorus, % DM	0.25	0.37	0.50
Magnesium, % DM	0.13	0.33	0.42
Potassium, % DM	1.01	2.56	0.93
Sodium, % DM	0.03	0.06	1.03
24 h IVTD, % DM	79.8	77.3	--
24 h NDFD, % DM	45.8	47.8	--
NE_L, Mcal/kg	1.5	1.4	1.9

Chemical composition was determined by the Dairy One Forage Laboratory, Ithaca, NY.

Table 6. Least squares means for dry matter intake, feed efficiency (FE), milk yield, and milk composition of cows fed a common diet and infused abomasally with water (C), histidine (H), proline (P), or a combination of both AA (H+P).

	Treatment					
Variable	C	H	H+P	P	SE	P^1
DMI, kg/d	26.6[a]	26.3[ab]	25.1[bc]	24.8[c]	0.5	0.04
FE, kg 3.5% FCM/ kg DM	1.95[b]	1.92[b]	1.95[b]	2.11[a]	0.08	0.07
Yield						
Milk, kg/d	50.2	49.6	48.0	48.7	1.7	0.44
3.5% FCM, kg/d	51.8	50.6	49.0	52.4	2.5	0.34
Fat, g/d	1871.7[†‡]	1804.6[†‡]	1736.9[†]	1929.7[‡]	116.1	0.29
Lactose, g/d	2433.9	2427.5	2324.3	2423.9	94.2	0.36
Protein, g/d	1471.8[†]	1473.6[†]	1369.8[‡]	1409.7[†‡]	74.2	0.25
Milk composition, %						
Fat	3.70	3.60	3.63	3.95	0.15	0.29
Lactose	4.85[b]	4.89[b]	4.83[b]	4.97[a]	0.03	0.01
Protein	2.93	2.96	2.85	2.89	0.06	0.33
NPN	0.133[b]	0.135[ab]	0.135[ab]	0.144[a]	0.003	0.11
Urea, mg/dl	8.7	9.7	7.9	10.0	0.9	0.51

[a,b,c]Least squares means within rows were separated by linear contrasts; different superscripts differ ($P \leq 0.05$)
[†,‡] Different superscripts differ ($P \leq 0.10$)
[1]Main effect of treatment.

was relatively the same for control and His treatments (Korhonen et al., 2000; Huhtanen et al., 2002).

Milk Yield and Composition

Despite the lower CP content of the diet and the intensive nature of the experimental procedures, milk yield was maintained at a high level for the duration of the experiment (Table 4). Milk yield and 3.5% fat corrected milk (FCM) averaged 49.1 ± 3.5 and 51.0 ± 5.3 kg/d, respectively, and were not significantly different among treatments. The lack of difference between milk yield of C and P treatments is consistent with results of Bruckental et al. (1991), in which daily milk yield did not differ between Control and Pro infused cows, in both early and mid lactation. According to data from an experiment in which goats were infused into the duodenum with Pro, milk yield was not significantly different between Control and Pro treatments (Alumot et al., 1983).

Abomasal His infusion (6.0 and 6.5 g/d) has been shown to increase milk yield in dairy cows by up to 1.8 kg/d (Vanhatalo et al., 1999; Korhonen et al., 2000; Huhtanen et al., 2002). In an experiment involving infusion of a mixture of AA including and excluding His into the abomasum of lactating goats, milk yield was depressed (P = 0.08) when His was excluded from the AA infusate (Bequette et al., 2000). The authors imposed a single EAA limitation in goats and observed an increase in milk yield when the deficiency was corrected. According to these studies in which His was limiting in both lactating dairy cows and goats, correction of the limitation through postruminal supplementation of His resulted in increased milk yields. Results from the current study imply that postruminal supply of His had no effect on milk production in high producing dairy cows fed corn silage based diets and thus, was not first limiting.

Protein and fat content of milk were not affected by treatments ($P = 0.33$ and 0.29 for protein and fat, respectively). Infusion of Pro alone increased lactose percentage ($P = 0.01$); however lactose yield was not affected by treatments. Results of treatment contrasts showed that fat yield during Pro infusion (1929.7 g/d) tended to be higher ($P \leq 0.10$) than during His plus Pro infusion (1736.9 g/d). There were no other significant differences among other treatments. The protein yield for the cows on the H and H+P treatments tended to be more ($P \leq 0.10$) than those on the H+P treatment, with no other significant differences. There was no overall effect of treatment on NPN and MUN concentration of milk ($P = 0.11$ and 0.51 for NPN and MUN, respectively). However, when linear contrasts were performed between individual treatment means for NPN, the means for treatments H and H+P tended to be lower ($P \leq 0.10$) than for treatment P, and mean for Control was less ($P \leq 0.05$) than for treatment P.

Most research suggests that dietary protein has only minor effects on protein and fat content in milk (Emery, 1978; McCrae et al., 1987; Sutton, 1989), and this is most likely due to the low efficiency of transfer rates (25 to 30%) of dietary protein to milk (Bequette et al., 1998; Jenkins and McGuire, 2006) and that milk protein output is energy driven. However, increasing postruminal supply of AA is of interest, due to the positive responses in milk yield and protein yield observed when casein was infused into the abomasum or duodenum of lactating dairy cows (Clark, 1975; Schwab et al., 1976; Raggio et al., 2006). Productive responses to increased postruminal supply of individual AA are variable depending on the AA, protein and energy content of the ration, level of milk production, and method of supplementation (Cant et al., 2003). Although abomasal infusion of His has been shown to increase protein yield and decrease fat yield in milk of dairy cows (Vanhatalo et al., 1999; Korhonen et al., 2000; Huhtanen et al., 2002), this was not observed in the present study. Based on AA subtraction experiments in which a complete mixture of AA minus His was infused

into either the abomasum or an external iliac artery of lactating dairy cows, a significant increase in fat content of milk was observed with the His lacking infusate as compared with the complete mixture of AA (Cant et al., 2001; Weekes et al., 2006).

Milk composition responses to Pro infusion were unexpected, especially the observed increase ($P = 0.01$) in lactose content. Reviews outlining the effect of diet on milk composition emphasize that lactose content was not significantly changed by dietary manipulations, aside from extreme or unusual feeding situations (Sutton, 1989; Jenkins and McGuire, 2006). Palmiter (1969) suggested that lactose content is affected by concentrations of other osmoregulators, including Na^+ and Cl^- ions, in milk. It has been shown that somatic cell count (SCC) is negatively associated with lactose content in milk across a variety of ruminant species (Vanlandingham et al., 1941; Leitner et al., 2004a; Leitner et al., 2004b). However, there were no significant differences in SCC among treatments of the current study. Intracellular synthesis of Pro in the mammary gland requires α-ketoglutarate and NADPH, both of which are involved in the Krebs cycle (Basch et al., 1997). It is possible that Pro infusion might have altered intracellular metabolism in the mammary gland to spare Krebs cycle intermediates, providing for more galactose for lactose synthesis. Although lactose yield was similar ($P = 0.56$) across treatments, longer experimental periods might have resulted in a shift in mammary metabolism that could have led to greater milk and lactose yields.

Fat yield of cows infused with Pro tended to be higher than that of cows infused with His and Pro simultaneously (643.24 vs. 578.97 for P and H+P, respectively; $P \leq 0.10$). Elevated milk fat percentage, and not fat yield, was observed with duodenal infusion of Pro in lactating goats and cows (Alumot et al., 1983; Bruckental et al., 1991). The mechanism behind the increase in fat content remains unclear, although the authors have suggested that this response is indicative of improved utilization of

available energy for production. It has been suggested that a shift in intracellular mammary metabolism in response to a change in postruminal supply of AA might provide more milk fat precursors or NADPH to facilitate higher rates of lipogenesis (Weekes et al., 2006). It appears that the mammary gland reacted differently to infusion of His plus Pro and that inclusion of His in the infusate mitigated any potential lipogenic effect of infusion of Pro alone. Overall, milk and component yields were numerically lowest for the H+P treatment, which might suggest that an interaction of His and Pro metabolism in the mammary gland or in peripheral tissues does not provide for efficient use of nutrients for milk synthesis. Both His and Pro can be transported across the mammary cell membrane via the sodium-dependent system A; however, His is considered to be transported primarily by system N (Baumrucker et al., 1985). If competition between AA for a shared transport system occurs, then it is possible that this antagonism might affect AA supply for milk production (Bequette et al., 2003); however this proposed mechanism has yet to be substantiated.

Feed Efficiency

There was a tendency (P = 0.07) for increased feed efficiency (FE) during Pro infusion (Table 4). Feed efficiency, estimated as kg 3.5% FCM per kg DMI, is a basic measurement of the efficiency at which dietary energy is converted to milk. According to an experiment performed by Britt et al. (2003), FE was highly and positively correlated to total milk yield and negatively correlated with DIM and DMI. The cows used in the present study were in early lactation and converted DM to milk with efficiencies ranging from 1.92 to 2.11 kg 3.5% FCM/kg feed. The slightly higher FE observed for animals infused with Pro was most likely due to their reduced DMI because no significant change in 3.5% FCM was observed. Further work with more

animals would substantiate the observation that increased absorptive supply of Pro might improve 3.5% FCM feed efficiency by decreasing DMI. Milk N:feed N ratios were 0.364, 0.362, 0.358, and 0.375 for treatments C, H, H+P, and P, respectively.

Mammary Blood Flow

Mammary blood flow (MBF) was estimated using the Fick Principle, which is based on the plasma AV difference and output of Phe and Tyr in milk protein. Therefore, calculated values of MBF are not independent of measured AV differences and milk protein output of Phe and Tyr. There was no treatment effect on absolute MBF, expressed as L blood/min. Although MBF, expressed as L blood/L milk, was not significantly different across treatments, there was a 14% increase (694.8 vs. 606.8 L blood/L milk for P and C, respectively) in MBF with P treatment as compared with the Control ($P = 0.37$). There are limited studies in which Pro was infused into the duodenum of lactating cows and goats, and in those studies MBF was not significantly affected by Pro infusion (Alumot et al., 1983; Bruckental et al., 1991). Although MBF was not significantly affected by treatment, the slightly higher MBF during Pro infusion might be biologically significant. Bequette et al. (2000) showed that abomasal infusion of a mixture of AA lacking His in lactating goats elevated MBF by approximately 33%, as compared with MBF during infusion of a complete mixture of AA including His. The authors speculated that MBF was up-regulated in order to maintain AA supply to the mammary gland when His was limiting or deficient. The slight elevation in MBF observed during Pro infusion might have been a result of a change in the absorptive AA profile. This change might have altered the pattern of AA supplied to the mammary gland, which in turn could have caused the mammary gland to up-regulate blood flow to ensure adequate supply of other AA for intracellular anabolic processes.

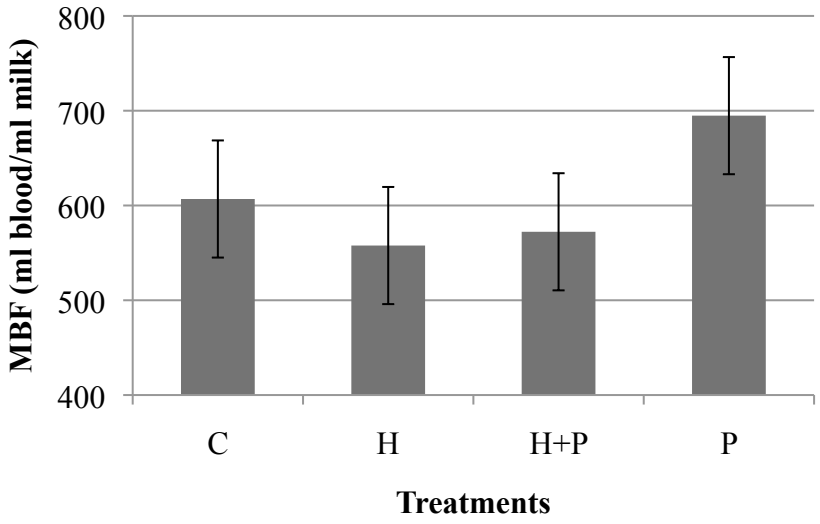

Figure 3. Mammary blood flow for each of the four treatments. Blood flow, which was estimated using the Fick Principle and Phe and Tyr as markers, is expressed as ml of blood per ml of milk, and values represent the LS mean ± SE. Experimental treatments were abomasal infusion of water (C), histidine (H), proline (P), or a combination of the two AA (H+P).

Production of local vasoactive agents within the mammary gland influences the rate of MBF (Davis and Collier, 1985; Prosser et al., 1996). Given this observation, then it is possible that rate of milk synthesis and intracellular mammary metabolism, which might affect the generation of these vasoactive substances, plays an important role in regulating nutrient flow to the mammary gland. The vasorelaxant nitric oxide (NO), which is synthesized from Arg and the activity of NO synthase enzymes, is one potential regulator of MBF that has been shown to increase MBF in lactating goats (Lacasse et al., 1996). Nitric oxide synthesis and intracellular Pro synthesis both utilize Arg as a precursor, and the enzymes that facilitate these processes have been identified in the parenchyma of mammary tissue (Verbeke et al., 1968; Clark et al., 1975; Lacasse et al., 1996). It is possible that if the Arg requirement for Pro synthesis in the mammary gland was reduced, more Arg might be available for synthesis of NO, polyamines, and other compounds, if the required enzymes were present and active. However, there was no evidence to support that postruminal supplementation of Pro decreased the Arg requirement for Pro synthesis or that mammary intracellular Arg metabolism is subject to major changes.

Mammary Amino Acid Utilization

Indwelling intercostal artery catheters remained patent for the duration of the experiment, and arterial plasma concentrations of AA are presented in Table 7. The arterial plasma concentration of His tended to be higher for His infusion than for both Control and Pro infusions ($P \leq 0.10$) and was not significantly different between H and H+P treatments. There was no significant effect of treatment on arterial concentrations of other EAA, with the exception of Cys ($P = 0.01$), which was lower during Pro infusion than during both Control and His infusions. The arterial concentration of Lys, Thr, and Orn, arterial plasma concentration tended to be lower

Table 7. Least squares means for arterial plasma amino acids from cows fed the same total mixed ration and infused abomasally with water (C), histidine (H), proline (P), or a combination of both AA (H+P).

	Treatment					
Variable	C	H	H+P	P	SEM	P^1
			(μM)			
EAA						
Arg	57.5	54.5	52.9	54.8	5.9	0.70
Cys	14.7^{b}	16.9^{a}	15.3^{ab}	12.2^{c}	1.2	0.01
His	24.3†	36.8‡	33.7†‡	24.6†	4.0	0.12
Ile	81.2	76.3	75.4	77.2	4.4	0.71
Leu	97.4	81.5	80.6	87.5	8.0	0.38
Lys	76.6†	72.5†‡	66.8‡	71.9†‡	5.7	0.37
Met	26.9	25.3	25.3	23.5	2.3	0.82
Phe	31.0	28.5	30.7	31.9	3.0	0.78
Thr	77.1†	70.0†‡	61.5‡	66.8†‡	5.1	0.30
Trp	30.2	27.8	26.3	31.9	3.5	0.63
Val	180.3^{a}	161.9^{ab}	152.1^{b}	167.5^{ab}	8.7	0.17
NEAA						
Ala	223.7	212.9	205.0	213.5	15.5	0.62
Asn	39.3	36.2	36.5	37.4	3.4	0.77
Asp	7.4	8.2	9.0	9.4	1.0	0.40
Gln	232.2	221.2	218.3	224.0	16.0	0.54
Glu	57.2	58.9	59.2	57.8	4.0	0.78
Gly	393.0	350.6	372.1	399.4	33.4	0.68
Pro	107.7	103.7	94.6	93.9	15.3	0.80
Ser	92.9	82.6	85.4	89.0	5.0	0.58
Tyr	36.6	34.0	33.0	33.4	2.6	0.60
Cit	65.7	55.7	58.0	69.4	5.2	0.32
Orn	36.0†	29.8†‡	28.8‡	32.4†‡	3.4	0.24

^{a,b,c}Least squares means within rows were separated by linear contrasts; different superscripts differ ($P \leq 0.05$)

†,‡ Different superscripts differ ($P \leq 0.10$)

^{1}Main effect of treatment.

($P \leq 0.10$) during His + Pro infusion than during Control infusion. Also, the mean for arterial concentration of Val was lower ($P \leq 0.05$) during His + Pro infusion than during Control infusion. The arterial plasma concentrations of NEAA were not significantly different between treatments with the exception that Orn concentration tended to be lower ($P \leq 0.10$) for treatment H+P as compared with the Control.

An increase in the arterial plasma concentration of AA is commonly observed when the postruminal supply of those AA is increased (Spires et al., 1975; Guinard and Rulquin, 1994; Mackle et al., 1999). Although arterial plasma concentration of His has been shown to increase with greater absorptive supply of His (Kim et al., 2000; Korhonen et al., 2000), this response has not been well documented when Pro alone was infused. Pro concentration in arterial plasma has been shown to either remain the same or increase with duodenal infusion of casein (Seymour et al., 1990; Guinard and Rulquin, 1994; Mackle et al., 2000), of which Pro comprises approximately 10% (Swaisgood, 1995). The lack of an observed response in plasma Pro concentration to increased postruminal supply in the present experiment might reflect postabsorptive disposal of Pro. If this were the case, it might have occurred because the Pro requirement for milk protein synthesis had been exceeded (Hanigan et al., 2001). According to Hanigan et al., it appears that absorptive supply of AA affects systemic concentrations and might be one of many factors that influence net removal of AA by the udder. Further work in this area would provide more information to determine the extent at which altered postruminal supply of one or more AA affects systemic AA concentrations and AA utilization by other tissues.

There was no overall treatment effect on AV differences or mammary uptake of AA, with the exception that uptake of Cys tended to be lower ($P \leq 0.10$) during His + Pro infusion than during Control infusion (Tables 8 and 9). The AV difference of Cys was lower ($P \leq 0.05$) and AV difference of Cit tended to be lower ($P \leq 0.10$) for

Table 8. Least squares means for mammary arteriovenous difference of plasma amino acids from cows fed the same total mixed ration and infused abomasally with water (C), histidine (H), proline (P), or a combination of both AA (H+P).

	Treatment					
Variable	C	H	H+P	P	SEM	P^1
			(μM)			
EAA						
Arg	29.4	30.9	29.8	27.1	2.8	0.75
Cys	1.8^{a,†}	1.2^{ab,†‡}	1.6^{ab,†}	0.6^{b,‡}	0.3	0.15
His	9.8	10.6	10.0	9.7	1.2	0.89
Ile	33.0	31.3	32.5	26.5	4.1	0.64
Leu	50.0	49.2	47.3	44.2	5.6	0.83
Lys	41.1	42.8	38.5	36.6	4.5	0.60
Met	10.5	10.3	11.0	8.9	1.2	0.57
Phe	15.8	17.6	15.9	14.9	1.9	0.63
Thr	21.7	21.0	22.2	18.7	2.5	0.76
Trp	2.6	2.3	2.8	1.3	1.2	0.83
Val	41.8	40.5	41.9	31.2	4.8	0.39
NEAA						
Ala	46.4	46.0	45.7	31.9	9.4	0.69
Asn	8.3	6.1	9.1	7.9	1.8	0.66
Asp	1.7†‡	1.7†‡	2.2†	1.1‡	0.3	0.34
Gln	52.2	48.5	51.0	35.6	7.7	0.51
Glu	36.5†‡	41.5†	40.6†‡	34.2‡	2.6	0.19
Gly	23.3	-8.3	4.5	-15.7	12.8	0.34
Pro	13.4	23.3	15.7	6.9	8.9	0.68
Ser	22.8	21.2	22.6	19.4	4.5	0.78
Tyr	15.6	17.2	15.6	13.9	1.8	0.83
Cit	2.6†	0.7†‡	1.8†‡	-2.0‡	1.4	0.26
Orn	16.6	14.9	14.8	15.1	2.2	0.83

[a,b,c]Least squares means within rows were separated by linear contrasts; different superscripts differ ($P \leq 0.05$)

[†,‡] Different superscripts differ ($P \leq 0.10$)

[1]Main effect of treatment.

Table 9. Least squares means for mammary amino acid uptake from cows fed the same total mixed ration and infused abomasally with water (C), histidine (H), proline (P), or a combination of both AA (H+P).

	Treatment					
Variable	C	H	H+P	P	SEM	P^1
	(g/kg of milk)					
EAA						
Arg	4.07	3.91	3.94	4.11	0.24	0.86
Cys	$0.34^{a,\dagger}$	$0.22^{ab,\dagger\ddagger}$	$0.28^{ab,\dagger}$	$0.10^{b,\ddagger}$	0.05	0.09
His	1.21	1.17	1.19	1.30	0.09	0.32
Ile	3.45	3.16	3.27	2.99	0.22	0.44
Leu	5.19	4.67	4.77	5.02	0.34	0.42
Lys	4.76	4.54	4.38	4.68	0.35	0.76
Met	1.25	1.11	1.25	1.14	0.09	0.22
Phe	2.08	2.09	2.02	2.58	0.21	0.95
Thr	$2.08^{\dagger}$	$1.79^{\ddagger}$	$2.02^{\dagger\ddagger}$	$1.90^{\dagger\ddagger}$	0.15	0.30
Trp	0.43	0.37	0.46	0.07	0.23	0.63
Val	$3.92^{\dagger}$	$3.43^{\dagger\ddagger}$	$3.75^{\dagger\ddagger}$	$3.04^{\ddagger}$	0.33	0.18
NEAA						
Ala	3.26	2.95	3.16	2.01	0.65	0.52
Asn	1.13	0.79	1.14	1.15	0.20	0.45
Asp	0.18	0.16	0.21	0.12	0.03	0.21
Gln	$6.08^{\dagger}$	$5.07^{\dagger\ddagger}$	$5.63^{\dagger\ddagger}$	$4.18^{\ddagger}$	0.72	0.31
Glu	4.33	4.41	4.49	4.60	0.28	0.89
Gly	$0.51^{\dagger}$	$-0.48^{\dagger\ddagger}$	$0.23^{\dagger\ddagger}$	$-1.47^{\ddagger}$	0.67	0.29
Pro	1.27	2.09	1.38	0.54	0.70	0.54
Ser	1.88	1.57	1.89	1.69	0.31	0.25
Tyr	2.25	2.22	2.18	2.70	0.28	0.64
Cit	$0.42^{\dagger}$	$0.14^{\dagger\ddagger}$	$0.23^{\dagger\ddagger}$	$-0.37^{\ddagger}$	0.21	0.19
Orn	1.72	1.44	1.52	1.73	0.17	0.27

[a,b,c]Least squares means within rows were separated by linear contrasts; different superscripts differ ($P \leq 0.05$)
[†,‡] Different superscripts differ ($P \leq 0.10$)
[1]Main effect of treatment.

treatment P than for treatment C. When AV differences were compared between H+P and P, the AV differences of both Cys and Asp during His + Pro infusion tended to be higher ($P \leq 0.10$) than during Pro infusion. The AV difference of Glu tended to be higher ($P \leq 0.10$) for treatment H (41.5 μMoles/L) than for treatment P (34.2 μMoles/L). When individual treatment means for mammary uptake of AA were evaluated, the most interesting differences were observed between treatments C and P. Uptake of Cit and Cys were lower ($P \leq 0.05$) during Pro infusion, and uptake of Gly and Val tended to be lower ($P \leq 0.10$) during Pro infusion, as compared with Control infusion. Cys uptake by the mammary gland for treatment P tended to be lower than for treatment H+P, and Thr uptake for treatment H tended to be less than for treatment C.

Raggio et al. (2006) observed increased AV differences of all AA ($P \leq 0.05$), except Asp and Glu, with duodenal infusion of casein in early lactation dairy cows. In another experiment in which casein (500 g/d) plus BCAA (88 g/d) were infused into the abomasum of cows in late lactation, AA infusion did not result in significant changes in AV differences of any AA, with the exception of Val (Mackle et al., 2000). These authors observed that arterial concentrations and AV differences of EAA tended to be the lowest for the treatments that produced the highest milk protein yields, which does not support using these measurements to predict AA supply and to identify limiting AA for milk protein production (Mackle et al., 2000).

The results of the present experiment show that AV difference and mammary uptake of Arg were not affected ($P = 0.75$ and 0.86, respectively) by abomasal infusion of 20 g of Pro. These observations are not consistent with data from other Pro infusion experiments performed using small groups of lactating cows (Bruckental et al., 1991) and goats (Alumot et al., 1983). Bruckental et al. reported a 50% decrease in Arg uptake with infusion of 80 g of Pro in early and mid lactation dairy

cows, compared with the control. These authors observed significantly lower AV difference of Arg during Pro infusion, and they reported no change in MBF between treatments. Therefore, the significantly reduced Arg uptake associated with Pro infusion was mainly attributed to the lower AV difference. In the present experiment, no differences in AV difference of Arg or MBF between C and P treatments were observed and could be due to the difference in the amount of Pro infused between the two experiments. We infused a quarter of the amount infused by Bruckental et al. (1991). Thus, we might not have provided enough to allow for the same effect on Arg uptake or the milk yield differences between studies were great enough to not impact Arg uptake.

It appears that abomasal infusion of Pro might have affected uptake of other AA, especially Cit, Cys, Gly, and Val. These AA are extracted and utilized by the mammary gland for different purposes (Mepham, 1982), which makes it difficult to determine the mechanism by which Pro might be altering mammary uptake of these substrates. Cit is an AA taken up by the mammary gland but not secreted in milk (Clark, 1975) and has been shown to be utilized to synthesize Pro and be oxidized to CO_2 in perfused sheep and goat mammary epithelial cells (Roets et al., 1974). The BCAA have been the subject of multiple experiments, because they are the primary AA in casein, are extracted by the mammary gland in excess of their output in milk and donate carbon to form TCA cycle intermediates, CO_2, and NEAA in the mammary gland (Wohlt et al., 1977; Mepham, 1982; Bequette and Backwell, 1997; Mackle et al., 1999). It is possible that increased absorptive supply of Pro might have altered certain metabolic pathways occurring in mammary cells, which caused a reduction in mammary uptake of other AA. Fluctuations in intracellular and extracellular AA concentrations modulate AA transport (Bequette et al., 2003), however this does not

necessarily imply that changes in AA transport will result in higher milk protein synthesis.

Amino acid extraction rates, calculated by dividing the AV difference of an AA by its concentration in arterial plasma, were determined for all AA and presented in Table 10. There was no main effect of treatment for extraction efficiencies of AA ($P > 0.05$), however there was a tendency for treatment differences ($P \leq 0.10$) for extraction efficiency of His, Orn, Phe, and Val. Considering the linear contrasts that were carried out among individual treatment means, His extraction was lower ($P \leq 0.05$) during His infusion and His + Pro infusion than during Control infusion. The constant AV difference was most likely responsible for the lower His extraction rate that was observed as absorptive supply of His was increased. This effect is consistent with other AA infusion experiments in which postruminal infusion of an individual AA resulted in a decrease in extraction efficiency of that AA and a similar AV difference for that AA as compared with the control treatment (Varvikko et al., 1999; Vanhatalo et al., 1999; Korhonen et al., 2000). Additionally, Bequette et al. (2000) reported a drastic increase in efficiency of removal of His by the mammary gland when His supply was limiting.

There were differences in individual treatment means of extraction efficiencies for two of the BCAA. For Leu, extraction efficiency for treatment H tended to be higher ($P \leq 0.10$) than for treatment C and P, separately. Val extraction tended to be lower ($P \leq 0.10$) during Pro infusion (18.3%) as compared with during Control infusion (23.4%) and was lower ($P \leq 0.05$) during Pro infusion than during His (24.9%) or His+Pro infusion (27.6%). Although there were no significant differences among treatment means for Ile extraction, the rate of Ile extraction was numerically less during Pro infusion as compared with the other three treatments. The BCAA represent approximately 22% of milk protein (Swaisgood, 1995) and are required by the

Table 10. Least squares means for extraction rates of plasma amino acids from cows fed the same total mixed ration and infused abomsally with water (C), histidine (H), proline (P), or a combination of both AA (H+P).

	Treatment					
Variable	C	H	H+P	P	SEM	P^1
			(%)			
EAA						
Arg	52.3	57.2	56.9	50.1	4.6	0.27
Cys	12.2	7.4	10.6	6.3	2.3	0.30
His	45.8^{a}	29.0^{b}	29.5^{b}	40.5^{ab}	5.6	0.10
Ile	40.8	40.2	43.2	34.3	4.1	0.38
Leu	51.3†	60.2‡	58.7†‡	51.0†	4.8	0.14
Lys	53.9	58.9	57.3	51.3	4.5	0.43
Met	39.7	41.2	43.8	37.9	4.7	0.73
Phe	51.0^{ab,†}	61.4^{a,‡}	52.2^{ab,†}	46.9^{b,†}	4.1	0.07
Thr	28.4†	30.2†‡	36.5‡	27.5†	2.9	0.21
Trp	9.0	8.4	10.8	0.5	4.7	0.47
Val	23.4^{ab,†}	24.9^{a,†}	27.6^{a,†}	18.3^{b,‡}	2.2	0.06
NEAA						
Ala	21.0	21.4	22.1	15.6	3.8	0.61
Asn	21.2	16.0	25.6	21.0	3.6	0.43
Asp	23.4^{ab,†}	20.8^{ab,†‡}	24.6^{a,†}	11.5^{b,‡}	3.7	0.13
Gln	22.8	21.9	23.3	16.6	3.3	0.56
Glu	63.9†‡	70.3†	68.6†	60.2‡	2.9	0.16
Gly	6.6	-2.7	1.2	-4.7	3.8	0.35
Pro	12.0	20.2	17.3	9.6	7.2	0.69
Ser	25.0	24.5	26.2	21.9	4.9	0.79
Tyr	43.1	50.1	47.3	41.5	3.3	0.31
Cit	4.5†	1.0†‡	2.9†‡	-3.2‡	2.2	0.23
Orn	46.5^{a,‡}	49.9^{ab,†}	51.3^{b,†}	46.1^{a,‡}	3.4	0.07

^{a,b,c}Least squares means within rows were separated by linear contrasts; different superscripts differ ($P \leq 0.05$)
†,‡ Different superscripts differ ($P \leq 0.10$)
^{1}Main effect of treatment.

mammary gland in amounts exceeding what is secreted in milk protein due to the high level of oxidation and utilization in secretory cells (Wohlt et al., 1977; Mepham, 1982). They are catabolized in mammary cells to yield organic acids, carbon skeletons for NEAA synthesis, and CO_2, (Mepham, 1982; Bequette et al., 1998). Pro synthesis in mammary epithelial cells involves several important enzymes, reducing equivalents, and α-ketoglutarate (Basch et al., 1995; Basch et al., 1996), which is a TCA cycle intermediate that can be produced by carbon skeletons donated by BCAA. Decreasing intracellular Pro synthesis might spare α-ketoglutarate, which could continue in its role in the TCA cycle, which could produce glucose or galactose to contribute to lactose synthesis. It appears there might be an interaction between Pro synthesis and production of TCA cycle intermediates, although the mechanism by which increased absorptive supply of individual AA might affect intracellular metabolism and extraction efficiency of other AA is unclear.

Contrasts between individual treatment means showed that extraction efficiency of some of the NEAA were different between certain treatments. The extraction efficiency of Asp tended to be lower for P than for treatment C and was significantly lower for treatment H+P. Also, there was a tendency for Glu extraction to be less during Pro infusion than during either His or His+Pro infusion. Orn extraction tended to be higher ($P \leq 0.10$) for treatment H+P as compared with treatment C and tended to be lower ($P \leq 0.10$) for treatment P as compared with treatment H.

In the case of Cit, the extraction rate during Pro infusion was negative and tended to be lower than during abomasal infusion of water. The negative extraction rate associated with Pro infusion was due to the negative AV difference of this AA. Unlike Arg, Cit does not occur in milk; therefore, if extracted by the mammary gland, it is either broken down or converted into other AA within cells (Roets et al., 1974). It was observed that Cit uptake was reduced during Pro infusion and appears to be

produced since a negative extraction rate implies production, and it is possible that the lower extraction rate is a parallel effect. The negative AV difference of Cit during Pro infusion might indicate that with increased absorptive supply of Pro, Cit was being produced within mammary cells, most likely via activity of NO synthase enzymes that convert Arg to Cit while producing NO gas (Wu and Morris, 1998). In this case, intracellular concentration of Cit might have affected uptake or transport of this AA into the cell (Bequette et al., 2003). There is evidence to support that Arg deprivation can reduce expression of an inducible isoform of NO synthase (Morris et al., 2006). The mechanism by which these processes might be occurring due to a change in absorptive supply of Pro seems complex and might relate to the proposed notion that increasing Pro supply to the mammary gland reduces the Arg requirement for endogenous Pro synthesis (Bruckental et al., 1991).

The current experiment indicates that there is some effect of altering absorptive supply of His and Pro on uptake and utilization of other AA by mammary epithelial cells. Analysis and interpretation of the mammary AA utilization data was difficult because although there were differences in individual treatment means of certain parameters, the sample size of cows was small, and drawing mechanistic conclusions requires further work.

CHAPTER 3: CONCLUSION

The objectives of this research were to determine the effects of abomasal infusion of the AA His and Pro on milk composition and mammary AA utilization in high producing lactating dairy cows fed corn silage based rations. The four animals that were used for the experiment maintained a high level of milk production. The results of this study suggest several key pieces of information. First, although His has been shown to be a limiting AA for milk and milk protein production in dairy cows being fed grass silage based diets, it does not appear that a His limitation occurs in high producing dairy cows being fed corn silage based commercial rations common to the Northeast US dairy industry. When 10 g of His was infused into the abomasum of high producing lactating dairy cows during this experiment, milk yield and milk protein yield were not different than values for the Control treatment. Although His infusion increased the plasma concentration of His, extraction efficiency of His was modestly reduced, which might suggest that the mammary gland was able to regulate uptake of this AA and that under current experimental conditions was not a limiting AA for milk yield or milk protein synthesis. In the small group of cows that were used for the experiment, there appeared to be no benefit of increasing postruminal His supply, in terms of improving milk production, milk protein synthesis, or mammary AA utilization.

The feed intake and efficiency responses that were observed during abomasal infusion of Pro were unexpected. Abomasal infusion of Pro resulted in reduced DMI, which after taking into account the similar milk production among treatments, caused an increase in conversion of feed to milk. Percentage of lactose in milk was higher during Pro infusion as compared with the Control infusion. Although the difference in MBF between the Pro and Control treatments was not significant, there was a 14%

increase when Pro was infused. However, this apparent difference in MBF should be considered carefully because of the small sample size of cows used in the experiment and also because of the MBF estimation technique, which is partially based on the AV difference of Phe and Tyr across the mammary gland. With respect to mammary AA utilization, abomasal infusion of Pro did not decrease Arg uptake, which is inconsistent with previously published work, however it modestly altered mammary uptake and extraction rate of Cit. Pro infusion also altered uptake of Cys, Val, and Gly. Under the conditions of this experiment, abomasal infusion of Pro did not increase milk protein content or yield, nor did it alter Arg uptake by the mammary gland. More research on increased absorptive supply of Pro, using more animals, is needed in order to validate feed intake and mammary AA utilization responses that were observed in this experiment.

Abomasal infusion of His and Pro simultaneously did not improve milk production or milk protein synthesis. DMI was slightly lower during His + Pro infusion, however feed efficiency was similar to the value for the Control. For high producing lactating dairy cows fed corn silage based rations, there seems to be no benefit of increasing the postruminal supply of these two AA together. Supplementing Pro alone resulted in a greater improvement in feed efficiency. Infusion of His + Pro resulted in a lower extraction rate of His, as compared with the control, although generally, it did not alter AV difference across the mammary gland, extraction efficiency, or mammary uptake of AA.

This study emphasizes the effectiveness of feeding rations that are formulated to provide adequate metabolizable energy and protein to support a high level of milk production without overfeeding N. The actual CP content of the experimental ration was 15.6%, and MP supply was balanced not to exceed predicted requirements. Milk production was maintained at a high level under these dietary conditions. Milk N:feed

N ratios were 0.364, 0.362, 0.358, and 0.375 for treatments C, H, H+P, and P, respectively. The results of this experiment provide a framework to investigate the potential benefits of increasing absorptive supply of Pro. There might be significant practical implications in the dairy industry if supplementation of Pro could consistently improve feed efficiency by reducing DMI, although the mechanism by which this might be occurring is unknown.

REFERENCES

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

Alumot, E., I. Bruckental, A. Tadmor, C. Kennit, and P. Holstein. 1983. Effect of proline on arginine uptake and nitrogen metabolism of lactating goats. J. Dairy Sci. 66:1243-1247.

Barbano, D. M., J. M. Lynch, and J. R. Fleming. 1991. Direct and indirect determination of true protein content of milk by Kjeldahl analysis: collaborative study. J. Assoc. Off. Anal. Chem. 74:281-288.

Basch, J. J., E. D. Wickham, H.M. Farrell, Jr., and J.E. Keys. 1995. Ornithine-δ-aminotransferase in lactating bovine mammary glands. J. Dairy Sci. 78:825-831.

Basch, J. J., E. D. Wickham, and H. M. Farrell, Jr. 1996. Pyrroline-5-carboxylate reductase in lactating bovine mammary glands. J. Dairy Sci. 79:1361-1368.

Basch, J. J., E. D. Wickham, and H. M. Farrell, Jr. 1997. Arginase in lactating bovine mammary glands: implications in proline synthesis. J. Dairy Sci. 80:3241-3248.

Baumrucker, C. R. 1984. Cationic amino acid transport by bovine mammary tissue. J. Dairy Sci. 67:2500-2506.

Baumrucker, C. R. 1985. Amino acid transport systems in bovine mammary tissue. J. Dairy Sci. 68:2436-2451.

Bequette, B. J. and F. R. C. Backwell. 1997. Amino acid supply and metabolism by the ruminant mammary gland. P. Nutr. Soc. 56:593-605.

Bequette, B. J., F. R. C. Backwell, and L. A. Crompton. 1998. Current concepts of amino acid and protein metabolism in the mammary gland of the lactating ruminant. J. Dairy Sci. 81:2540-2559.

Bequette, B. J., M. D. Hanigan, A. G. Calder, C. K. Reynolds, G. E. Lobley, and J. C. MacRae. 2000. Amino acid exchange by the mammary gland of lactating goats when histidine limits milk production. J. Dairy Sci. 83:765-775.

Bequette, B. J., M. D. Hanigan, and H. Lapierre. 2003. Mammary uptake and metabolism of amino acids by lactating ruminants. In Amino Acids in Animal Nutrition. J. P. F. D'Mello, ed. Wallingford: CABI Publishing, 347-365.

Bequette, B. J., S. L. Owens, S. W. El-Kadi, N. E. Sunny, and A. Shamay. Use of ^{13}C-mass isotope distribution analysis (MIDA) to define precursors for lactose and amino acid synthesis by bovine mammary explants. J. Dairy Sci. 88(Suppl. 1):289.

Bequette, B. J., and K. Nelson. 2006. The roles of amino acids in milk yield and components. Pages 26-39 in Proc. 17th Florida Ruminant Nutrition Symposium, Gainesville, FL. Univ. of Florida, Gainesville, FL.

Bordin, G., F. C. Raposo, B. de la Calle, A. R. Rodriguez. 2001. Identification and quantification of major bovine milk proteins by liquid chromatography. J. Chromatogr. A. 928:63-76.

Britt, J. S., R. C. Thomas, N. C. Speer, and M. B. Hall. 2003. Efficiency of converting nutrient dry matter to milk in Holstein herds. J. Dairy Sci. 86:3796-3801.

Bruckental, I., I. Ascarelli, B. Yosif, and E. Alumot. 1991. Effect of duodenal proline infusion on milk production and composition in dairy cows. Anim. Prod. 53:299-303.

Chavance, J. 1946. Histamine theory and treatment of laminitis. Vet. Med. 41:199-201.

Cant, J. P., F. Qiao, and C. A. Toerien. 1999. Regulation of mammary metabolism. Pages 203-219 in Proc. of the 8th International Symposium on Protein Metabolism and Nutrition, Aberdeen, United Kingdom.

Cant, J. P., D. R. Trout, F. Qiao, and B. W. McBride. 2001. Milk composition responses to unilateral arterial infusion of complete and histidine-lacking amino acid mixtures to the mammary glands of cows. J. Dairy Sci. 84:1192-1200.

Cant, J. P., R. Berthiaume, H. Lapierre, P. H. Luimes, B. W. McBride, and D. Pacheco. 2003. Responses of the bovine mammary glands to absorptive supple of single amino acids. Can. J. Anim. Sci. 83:341-355.

Clark, J. H. 1975. Lactational responses to postruminal administration of proteins and amino acids. J. Dairy Sci. 58:1178-1197.

Clark, J. H., R. G. Derrig, C. L. Davis, and H. R. Spires. 1975. Metabolism of arginine and ornithine in the cow and rabbit mammary tissue. J. Dairy Sci. 58:1808-1813.

Clark, J. H., H. R. Spires, R. G. Derrig, and M. R. Bennink. 1977. Milk production, nitrogen utilization, and glucose synthesis in lactating cows infused postruminally with sodium caseinate and glucose. J. Nutr. 107:631-644.

Clark, J. H., T. H. Klusmeyer, and M. R. Cameron. 1992. Microbial protein synthesis and flows of nitrogen fractions to the duodenum of dairy cows. J. Dairy Sci. 75:2304-2323

Colin-Schoellen, O., F. Laurent, B. Vignon, J. C. Robert, and B. Sloan. 1995. Interactions of ruminally protected methionine and lysine with protein source or energy level in the diets of cows. J. Dairy Sci. 78:2807-2818.

Colmenero, J. J. O., and G. A. Broderick. 2006. Effect of dietary crude protein concentration on milk production and nitrogen utilization in lactating dairy cows. J. Dairy Sci. 89:1704-1712.

Davis, S. R., R. J. Collier, J. P. McNamara, H. H. Head, and W. Sussman. 1988. Effects of thyroxine and growth hormone treatment of dairy cows on milk yield, cardiac output, and mammary blood flow. J. Anim. Sci. 66:70-79.

Davis, S. R., and R. J. Collier. 1985. Mammary blood flow and regulation of substrate supply for milk synthesis. J. Dairy Sci. 68:1041-1058.

DePeters, E. J., and J. P. Cant. 1992. Nutritional factors influencing the nitrogen composition of bovine milk. J. Dairy Sci. 75:2043-2070.

Derrig, R. G., J. H. Clark, and C. L. Davis. 1974. Effect of abomasal infusion of sodium caseinate on milk yield, nitrogen utilization, and amino acid nutrition of the dairy cow. J. Nutr. 104:151-159.

De Wit, J. N. 1998. Nutritional and functional characteristics of whey proteins in food products. J. Dairy Sci. 81:597-608.

Doepel, L., D. Pacheco, J. J. Kennelly, M. D. Hanigan, I. F. Lopez, and H. Lapierre. 2004. Milk protein synthesis as a function of amino acid supply. J. Dairy Sci. 87:1279-1297.

Emery, R. S. 1978. Feeding for increased milk protein. J. Dairy Sci. 61:825-828

Fox, D. G., L. O. Tedeschi, T. P. Tylutki, J. B. Russell, M. E. Van Amburgh, L. E. Chase, A. N. Pell, and T. R. Overton. 2004. The Cornell Net Carbohydrate and Protein System model for evaluating herd nutrition and nutrient excretion. Anim. Feed Sci. Technol. 112:29-78.

Garner, M. R., J. F. Flint, and J. B. Russell. 2002. *Allisonella histaminiformans* gen. nov., sp. nov.: a novel bacterium that produces histamine, utilizes histidine as its sole energy source, and could play a role in bovine and equine laminitis. System. Appl. Microbiol. 25:498-506.

Garner, M. R., M. R. Gronquist, and J. B. Russell. 2004. Nutritional requirements of *Allisonella histaminiformans*, a ruminal bacterium that decarboxylates histidine and produces histamine. Curr. Microbiol. 49:295-299.

Gehrke, C. W., L. L. Wall, Sr., J. S. Absheer, F. E. Kaiser, and R. W. Zumwalt. 1985. Sample preparation for chromatography of amino acids. Acid hydrolysis of proteins. J. AOAC Int. 68:811-821.

Glass, R. D., and W. E. Knox. 1973. Arginase isozymes of rat mammary gland, liver, and other tissues. J. Biol. Chem. 248:5785-5789.

Guinard, J., and H. Rulquin. 1994. Effect of graded levels of duodenal infusions of casein on mammary uptake in lactating cows. 2. Individual amino acids. J. Dairy Sci. 77:3304-3315.

Hanigan, M. D., J. P. Cant, D. C. Weakley, and J. L. Beckett. 1998. An evaluation of postabsorptive protein and amino acid metabolism in the lactating dairy cow. J. Dairy Sci. 81:3385-3401.

Hanigan, M. D., B. J. Bequette, L. A. Crompton, and J. France. 2001. Modeling mammary amino acid metabolism. Livest. Prod. Sci. 70:63-78.

Harper, A. E., N. J. Benevenga, and R. M. Wohlhueter. 1970. Effects of ingestion of disproportionate amounts of amino acids. Physiol. Rev. 50:428-558.

Huhtanen, P., A. Vanhatalo, and T. Varvikko. 2002. Effects of abomasal infusions of histidine, glucose, and leucine on milk production and plasma metabolites of dairy cows fed grass silage diets. J. Dairy Sci. 85:204-216.

Igarashi, K., and K. Kashiwagi. 2000. Polyamines: mysterious modulators of cellular functions. Biochem. Biophys. Res. Commun. 271:559-564.

Jenkins, T. C., and M. A. McGuire. 2006. Major advances in nutrition: impact on milk composition. J. Dairy Sci. 89:1302-1310.

Jonker, J. S., R. A. Kohn, and J. High. 2002. Dairy herd management practices that impact nitrogen utilization efficiency. J. Dairy Sci. 85:1218-1226.

Kim, C. H., J. J. Choung, and D. G. Chamberlain. 2000. The effects of intravenous administration of amino acids and glucose on the milk production of dairy cows consuming diets based on grass silage. Grass Forage Sci. 55:173-180.

Kim, C. H., J. J. Choung, and D. G. Chamberlain. 2001. Estimates of the efficiency of transfer of L-histidine from blood to milk when it is the first-limiting amino acid for secretion of milk protein in the dairy cow. J. Sci. Food Agric. 81:1150-1155.

Kim, S. W., and G. Wu. 2009. Regulatory role for amino acids in mammary gland growth and milk synthesis. Amino Acids. 37:89-95.

Korhonen, M., A. Vanhatalo, T. Varvikko, and P. Huhtanen. 2000. Responses to graded postruminal doses of histidine in dairy cows fed grass silage diets. J. Dairy Sci. 83:2596-2608.

Lacasse, P., V. C. Farr, S. R. Davis, and C. G. Prosser. 1996. Local secretion of nitric oxide and the control of mammary blood flow. J. Dairy Sci. 79:1369-1374.

Lacasse, P., and C. G. Prosser. 2003. Mammary blood flow does not limit milk yield in lactating goats. J. Dairy Sci. 86:2094-2097.

Lapierre, H., R. Berthiaume, G. Raggio, M. C. Thivierge, L. Doepel, D. Pacheco, P. Dubreuil, and G. E. Lobley. 2005. The route of absorbed nitrogen into milk protein. Anim. Sci. 80:11-22.

Lapierre, H., D. Pacheco, R. Berthiaume, D. R. Ouellet, C. G. Schwab, P. Dubreuil, G. Holtrop, and G. E. Lobley. 2006. What is the true supply of amino acids for a dairy cow?. J. Dairy Sci. 89(E. Suppl.):E1-E14.

Lapierre, H., Lobley, G. E., D. R. Ouellet, L. Doepel, and D. Pacheco. 2007. Amino acid requirements for lactating dairy cows: reconciling predictive models and biology. Pages 39-59 in Proc. Cornell Nutr. Conf., Syracuse, NY., Cornell Univ., Ithaca, NY.

Leitner, G., M. Chaffer, A. Shamay, F. Shapiro, U. Merin, E. Ezra, A. Saran, and N. Silanikove. 2004a. Changes in milk composition as affected by subclinical mastitis in sheep. J. Dairy Sci. 87:46-52.

Leitner, G., U. Merin, and N. Silanikove. 2004b. Changes in milk composition as affected by subclinical mastitis in goats. J. Dairy Sci. 87:1719-1726.

Leng, R. A., and J. V. Nolan. 1984. Nitrogen metabolism in the rumen. J. Dairy Sci. 67:1072-1089.

Maas, J. A., J. France, and B. W. McBride. 1977. Model of milk protein synthesis. A mechanistic model of milk protein synthesis in the lactating bovine mammary gland. J. Theor. Biol. 187:363-378.

Mackle, T. R., D. A. Dwyer, and D. E. Bauman. 1999. Effects of branched-chain amino acids and sodium caseinate on milk protein concentration and yield from dairy cows. J. Dairy Sci. 82:161-171.

Mackle, T. R., D. A. Dwyer, K. L. Ingvartsen, P. Y. Chouinard, D. A. Ross, and D. E. Bauman. 2000. Effects of insulin and postruminal supply of protein on use of amino acids by the mammary gland for milk protein synthesis. J. Dairy Sci. 83:93-105.

Marini, J. C., D. G. Fox, and M. R. Murphy. 2008. Nitrogen transactions along the gastrointestinal tract of cattle: a meta-analytical approach. J. Anim. Sci. 86:660-679.

McCrae, I., F. Harding, and D. D. Muir. 1987. Milk compositional quality-opportunities for manipulation. J. Soc. Dairy Technol. 40:57-58.

Mepham, T. B. 1982. Amino acid utilization by lactating mammary gland. J. Dairy Sci. 65:287-298.

Mepham, T. B., and J. L. Linzell. 1966. A quantitative assessment of the contribution of individual plasma amino acids to the synthesis of milk proteins by the goat mammary gland. Biochem. J. 101:76-83.

Mepham, T. B., P. Gaye, P. Martin, and J. C. Mercier. 1998. Biosynthesis of milk protein. In Dairy Chemistry and Biochemistry. P. F. Fox and P. L. H. McSweeney, ed. London, UK: Blackie Academic and Professional, 491-543.

Metcalf, J. A., R. J. Mansbridge, J. S. Blake, J. D. Oldham, and J. R. Newbold. 2008. The efficiency of conversion of metabolisable protein into milk true protein over a range of metabolisable protein intakes. Animal. 2:1193-1202.

Misciattelli, L., V. F. Kristensen, M. Vestergaard, M. R. Weisbjerg, K. Sejrsen, and T. Hvelplund. 2003. Milk production, nutrient utilization, and endocrine responses to increased postruminal lysine and methionine supply in dairy cows. J. Dairy Sci. 86:275-286.

Morris, S. M. 2006. Arginine: beyond protein. Am. J. Clin. Nutr. 83(suppl.):508S-512S.

Nocek, J. E. 1997. Bovine acidosis: Implications on laminitis. J. DairySci. 80:1005-1028.

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

Palmiter, R. D. 1969. What regulates lactose content in milk?. Nature. 221:912-914.

Pisulewski, P. M., H. Rulquin, J. L. Peyraud, and R. Verite. 1996. Lactational and systemic responses of dairy cows to postruminal infusions of increasing amounts of methionine. J. Dairy Sci. 79:1781-1791.

Prosser, C. G., S. R. Davis, V. C. Farr, and P. Lacasse. 1996. Regulation of blood flow in the mammary microvasculature. J. Dairy Sci. 79:1184-1197.

Purdie, N. G., C. E. A. Borsy, C. Chui, J. Imada, P. Stahel, C. Longo, A. K. Shoveller, V. R. Osborne, and J. P. Cant. 2009. J. Dairy Sci. 92(E. Suppl):51.

Raggio, G., S. Lemosquet, G. E. Lobley, H. Rulquin, and H. Lapierre. 2006. Effect of casein and propionate supply on mammary protein metabolism in lactating dairy cows. J. Dairy Sci. 89:4340-4351.

Roets, E., R. Verbeke, A. Massart-Leën, and G. Peeters. 1974. Metabolism of [^{14}C]Citrulline in the perfused sheep and goat udder. Biochem. J. 144:435-446.

Rogers, J. A., U. Krishnamoorthy, and C. J. Sniffen. 1987. Plasma amino acids and milk protein production by cows fed rumen-protected methionine and lysine. J. Dairy Sci. 70:789-798.

Schwab, C. G., L. D. Satter, and A. B. Clay. 1976. Response of lactating dairy cows to abomasal infusion of amino acids. J. Dairy Sci. 59:1254-1270.

Schwab, C. G., C. K. Bozak, N. L. Whitehouse, and M. M. A. Mesbah. 1992a. Amino acid limitation and flow to the duodenum at four stages of lactation. 1. Sequence of lysine and methionine limitation. J. Dairy Sci. 75:3486-3502.

Schwab, C. G., C. K. Bozak, N. L. Whitehouse, and V. M. Olson. 1992b. Amino acid limitation and flow to the duodenum at four stages of lactation. 2. Extent of lysine limitation. J. Dairy Sci. 75:3503-3518.

Seymour, W. M., C. E. Polan, and J. H. Herbein. 1990. Effects of dietary protein degradability and casein or amino acid infusions on production and plasma amino acids in dairy cows. J. Dairy Sci. 73:735-748.

Shennan, D. B., I. D. Millar, and D. T. Calvert. 1997. Mammary-tissue amino acid transport systems. Proc. Nutr. Soc. 56:177-191.

Spindler, M., R. Stadler, and H. Tanner. 1984. Amino acid analysis of feedstuffs: determination of methionine and cystine after oxidation with performic acid and hydrolysis. J. Agric. Food Chem. 32:1366-1371.

Spires, H. R., J. H. Clark, R. G. Derrig, and C. L. Davis. 1975. Milk production and nitrogen utilization in response to postruminal infusion of sodium caseinate in lactating cows. J. Nutr. 105:1111-1121.

Sutton, J. D. 1989. Altering milk composition by feeding. J. Dairy Sci. 72:2801-2814.

Swaisgood, H. E. 1995. Protein and amino acid composition of bovine milk. In Handbook of Milk Composition. R. G. Jensen, ed. San Diego, CA: Academic Press, 464-468.

Tamminga, S. and J. D. Oldham. 1980. Amino acid utilisation by dairy cows. II. Concept of amino acid requirements. Livest. Prod. Sci. 7:453-463.

Tylutki, T. P., D. G. Fox, V. M. Durbal, L. O. Tedeschi, J. B. Russell, M. E. Van Amburgh, T. R. Overton, L. E. Chase, and A. N. Pell. 2008. Cornell Net Carbohydrate and Protein System: A model for precision feeding of dairy cattle. Anim. Feed Sci. Technol. 143:174-202.

Vanhatalo, A., P. Huhtanen, V. Toivonen, and T. Varvikko. 1999. Response of dairy cows fed grass silage diets to abomasal infusions of histidine alone or in combinations with methionine and lysine. J. Dairy Sci. 82:2674-2685.

Vanlandingham, A. H., C. E. Weakley, Jr., E. N. Moore, and H. O. Henderson. 1941. Mastitis. I. The relationship of the development of mastitis to changes in the chlorine, lactose, and casein number in milk. J. Dairy Sci. 24:383-398.

Varvikko, T., A. Vanhatalo, T. Jalava, and P. Huhtanen. 1999. Lactation and metabolic responses to graded abomasal doses of methionine and lysine in cows fed grass silage diets. J. Dairy Sci. 82:2659-2673.

Verbeke, R., G. Peeters, A. Massart-Leën, and G. Cocquyt. 1968. Incorporation of DL-[2-14C]Ornithine and DL-[5-14C]Arginine in milk constituents by the isolated lactating sheep udder. Biochem. J. 106:719-724.

Verbeke, R. E., E. Roets, A. M. Massart-Leën, and G. Peeters. 1972. Metabolism of [U-^{14}C]-L-threonine and [U-^{14}C]-L-phenylalanine by isolated perfused udder. J. Dairy Res. 39:239-250.

Wallace, R. J. 1996. Ruminal microbial metabolism of peptides and amino acids. J. Nutr. 126:1326S-1334S.

Weekes, T. L., P. H. Luimes, and J. P. Cant. 2006. Responses to amino acid imbalances and deficiencies in lactating dairy cows. J. Dairy Sci. 89:2177-2187.

Wohlt, J. E., J. H. Clark, R. G. Derrig, and C. L. Davis. 1977. Valine, leucine, and isoleucine metabolism by lactating bovine mammary tissue. J. Dairy Sci. 60:1875-1882.

Wu, G., and S. M. Morris. 1998. Arginine metabolism: nitric oxide and beyond. Biochem. J. 336:1-17.

Yip, M. C. M., and W. E. Knox. 1972. Function of arginase in lactating mammary gland. Biochem. J. 127:893-899.