# INFLUENCE OF DIETARY FAT AND PROTEIN ON NUTRIENT SUPPLY AND UTILIZATION BY THE LACTATING BOVINE MAMMARY GLAND

BY

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DOCTOR OF PHILOSOPHY

in

Animal Science (Dairy)

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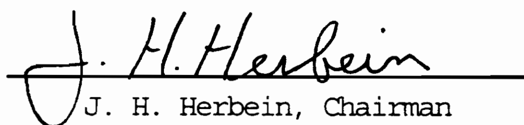

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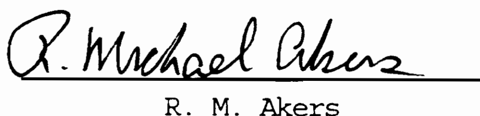

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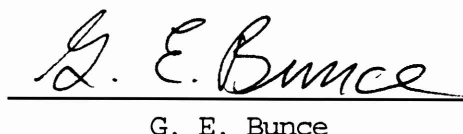

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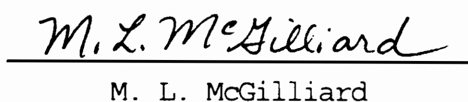

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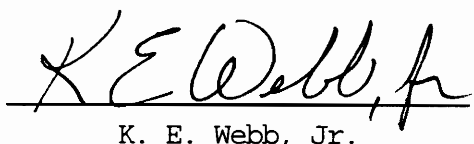

K. E. Webb, Jr.

December, 1993
Blacksburg, VA

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Brian John Wonsil

Committee Chairman: J. H. Herbein
Animal Science

**(ABSTRACT)**

The objective of this study was to determine whether dietary fat supplementation and level of undegradable intake protein (UIP) could affect daily milk output and composition by influencing nutrient supply to the mammary gland. Three lactating Holstein cows (60, 68, and 74 d postpartum) were used in an incomplete 4 x 4 Latin square design (2 x 2 factorial) and fed diets (15.9% CP and 19.5% ADF) with 0% or 2.5% partially hydrogenated tallow and 33% or 41% UIP. A 5:2.5:1 mixture of dried brewer's grains, corn gluten meal, and blood meal was substituted for soybean meal to raise dietary UIP from 33% to 41% UIP. Despite similar DM intake across treatments, cows produced 9% more milk per day when fed 2.5% supplemental fat, 41% UIP, or the combination of 2.5% fat and 41% UIP when compared to the control diet. Fat supplementation depressed milk protein percentage but not daily milk protein output. Mammary blood flow was estimated using the Fick principle at 6-hr intervals for 24 h. Concentration of individual nutrients in arterial (carotid) and venous (abdominal vein) blood and corresponding blood flows were used to calculate nutrient uptakes by the mammary gland. Calculated carbon uptake was 95 to 101% of output when using estimated carbon content of nutrients, and 100 to 106% when using an elemental analyzer to determine actual carbon output in milk. Uptake of glucose, β-hydroxybutyrate, lactate, pyruvate, acetate, and $O_2$ were not affected by dietary treatment. Triacylglycerol concentration in arterial blood and uptake of long-chain fatty acids were elevated by fat supplementation, resulting in milk fat with a higher percentage of 18-

carbon fatty acids and a lower ratio of saturated to unsaturated fatty acids. Arterial essential and total amino acid (AA) concentrations in plasma and whole blood were elevated when cows were fed 41% versus 33% UIP. However, mammary arteriovenous differences, extraction percentages, and uptakes of most AA were not significantly affected by dietary treatments. Across treatments, peptide AA accounted for ~10% of AA in arterial whole blood but no net uptake of peptide AA by the lactating gland was detected. Results indicated that dietary fat supplementation at two levels of UIP can increase milk production by altering mammary lipid metabolism, thereby improving the efficiency of milk synthesis. However, depression of milk protein percentage in response to dietary fat supplementation was not alleviated by elevating arterial essential and total AA through higher dietary UIP.

## DEDICATION

I dedicate this dissertation to my wife, Kelly Wonsil.

**ACKNOWLEDGEMENTS**

As with most endeavors in life, completion of this degree would not have been possible without the help of many individuals.

I would like to begin by thanking the members of my graduate committee including: Dr. J. H. Herbein, Dr. R. M. Akers, Dr. G. E. Bunce, Dr. M.L. McGilliard, and K.E. Webb, Jr.. As a group, they have helped me become a better scientist but more importantly a better person. Their support, guidance, and friendship will never be forgotten. A special thanks to my major professor Dr. Herbein for taking a chance on a farm boy from Wisconsin.

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Special thanks to the John Lee Pratt Animal Nutrition Program for providing financial support throughout my graduate program.

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# CHAPTER 1

# INTRODUCTION

In general, the rate of milk synthesis by the lactating mammary gland is a function of nutrient concentrations in blood, rate of extraction of each nutrient, and rate of blood flow through the gland. Modifying nutrient supply to the gland or nutrient uptake may influence milk and milk component production. For example, a current trend in formulation of diets for high producing dairy cows is to increase the percentage of fat from approximately 4% to 7% of dietary DM, thereby providing additional energy for milk production. Although supplemental fat frequently increases milk production, the concentration of protein in milk often decreases, suggesting an alteration in nutrient utilization within the gland. Specifically, as milk production increases, the requirement for precursors of milk components (i.e. glucose, fatty acids and AA) increases and may become rate-limiting if precursor availability to the mammary gland remains constant or decreases.

In theory, raising undegradable intake protein (UIP), without adversely affecting microbial protein production, should increase the level of AA ultimately reaching the mammary gland and alleviate milk protein depression caused by fat supplementation. However, numerous production trials have shown that high producing cows respond inconsistently to supplemental fat and increased dietary UIP. It is apparent that advances in diet formulation, genetics, and management practices have superseded our ability to address the changing metabolic requirements of the lactating mammary gland.

Therefore, the overall objective of this experiment was to evaluate how supplemental fat and level of dietary UIP alter daily milk output and composition by monitoring: 1) concentration of nutrients in arterial blood, 2) daily flow of nutrients to the mammary gland (nutrient concentration times blood flow), and 3) uptake of nutrients by the mammary gland (arteriovenous difference times blood flow). Specific objectives included:

1. Corroborate earlier findings that supplemental fat increases efficiency of energy utilization within the gland leading to greater milk synthesis.

2. Determine if there is any relationship between blood flow to the mammary gland and milk protein depression in cows fed fat-supplemented diets.

3. Determine whether a blend of dietary proteins more resistant to rumen microbial fermentation than soybean meal can increase circulating levels of AA available to the gland, thereby alleviating milk protein depression.

4. Compare uptakes of free AA determined from blood plasma versus whole blood as the mammary gland's source of AA.

5. Determine the level of circulating peptide AA (<10,000 Da) in arterial blood of lactating dairy cows.

6. Determine if there is net uptake of peptide AA by the bovine mammary gland.

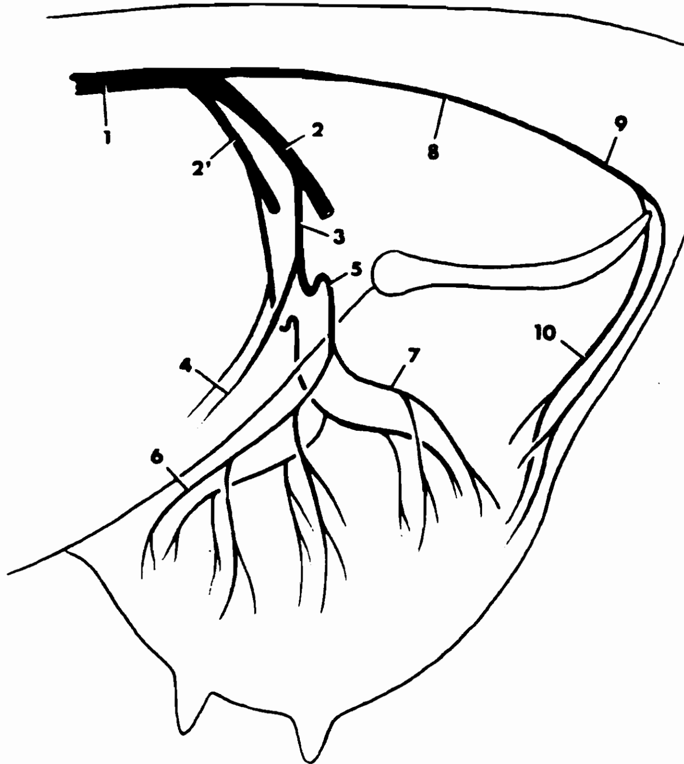
# CHAPTER 2

# REVIEW OF LITERATURE

## Vasculature of the mammary gland

As previously stated by Davis and Collier (1985), the mammary gland is dependent upon its blood supply to provide substrates at the appropriate rates to sustain a given level of milk production. The arterial blood supply of the mammary gland is illustrated in Figure 2.1. After leaving the heart via the ascending aorta, arterial blood enters either the anterior (to head) or posterior dorsal aorta. The dorsal aorta continues until it bifurcates into the left and right internal and external iliac arteries. Blood destined for the udder flows through the pudendoepigastric trunk via the inguinal canal, before reaching the external pudenal. Upon entering the mammary gland, the external pudenal bifurcates into the cranial and caudal mammary arteries.

Whereas one source of paired vessels supply arterial blood to the mammary gland, blood leaves the udder either through the external pudenal veins or cranial superficial epigastric veins (see Figure 2.2). Blood leaving via the external pudic veins eventually reaches the heart via the caudal vena cava. On the other hand, blood from the epigastric veins returns to the heart via the jugular vein and anterior vena cava (not shown in Figure 2.2).




1. Posterior Aorta
2. External iliac
   2'. Right external iliac
3. Pudendoepigastric trunk
4. Caudal epigastric
5. External pudendal
6. Cranial mammary
7. Caudal mammary
8. Internal iliac
9. Internal pudenal
10. Ventral perineal

Figure 2.1. Arterial blood supply to the mammary gland.

A. Caudal vena cava
B. External iliac
C. B'. External iliac
D. Pudendoepigastric trunk
E. External pudenal
F. Caudal mammary
G. Cranial mammary
H. Cranial superficial epigastric
I. Milk well
J. Internal thoracic
K. Internal iliac
L. Internal pudendal
M. Ventral perineal

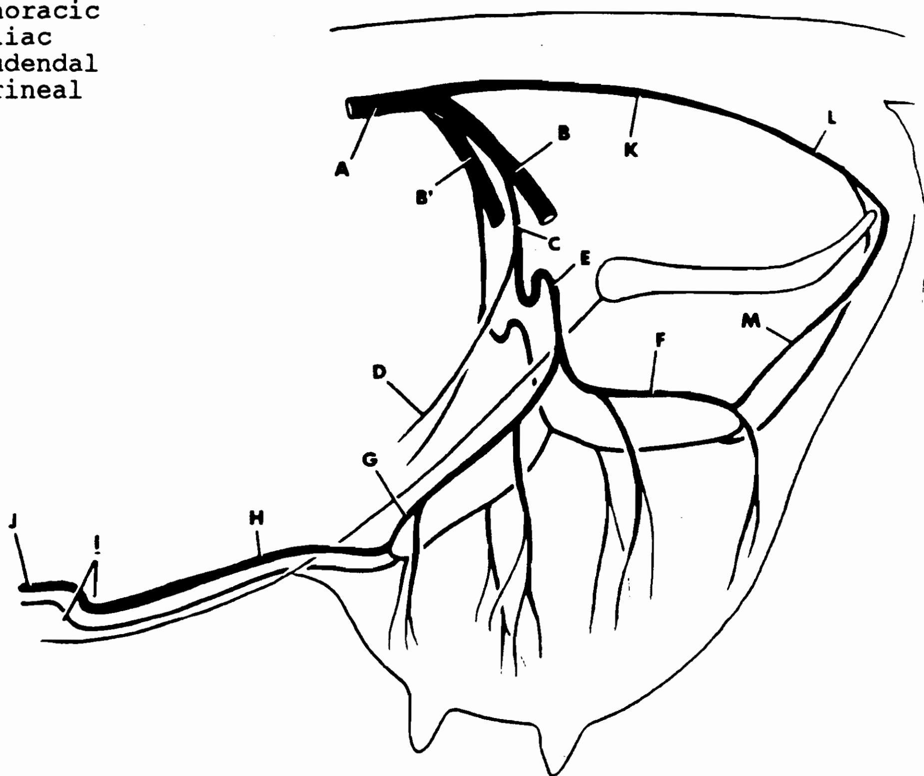



Figure 2.2. Venous drainage from the mammary gland.

**Lymphatic system in the mammary gland**

Although the lymphatic system is best known for its role as a transporter of chylomicra from the gastrointestinal tract of mammalian species, another important function is that it carries proteins and other blood components away from tissue spaces not removed via the blood capillary system. Until a recent report by Gorewit et al. (1993a), no one had characterized afferent lymphatic flow (and composition) from the supramammary lymph node in the lactating dairy cow. Gorewit et al. (1993a) observed total lymphatic flow ranged from 13 to 45 mL/h, resulting from 62 to 67 pulsatile episodes per h. Mean concentrations of selected nutrients in lymph and plasma (mg/dL), respectively, were: protein (800, 5800); glucose (80.7, 80.1); and triglycerides (8.0, 19.0). If total lymph flow was as high as ~100 mL/h, this would translate to only ~2.4 L/d and would represent only .009% of average daily blood flow (27,000 L/d) observed by Cant et al. (1993b). Therefore, the contribution of lymph to flux of major nutrients across the mammary gland is quantifiable, but normally not measured (Bickerstaffe et al. 1974, Cant et al. 1993b, Peeters et al. 1979).

**Regulation of mammary blood flow**

Although physiological regulation of mammary blood flow is critical, it is still not known whether mammary metabolism influences blood flow or visa versa. There are however, a number of well documented examples of controlling mammary blood flow. For example, 10, 20, and 60 mU of oxytocin injected into the right pudenal artery of lactating cows increased half-udder blood flow by 20, 28, and 33%

(Gorewit et al. 1989). Previously, Reynolds et al. (1968) reported that massaging the udder of goats increased mammary blood flow 30 to 50% above control levels. Conversely, epinephrine has been shown to inhibit milk ejection by limiting the availability of oxytocin to the mammary gland (Gorewit et al. 1989). Gorewit and Aromando (1985), Gorewit et al. (1989), and our lab (data not shown) found that epinephrine acted peripherally on the gland and significantly reduced mammary blood flow (nearly 100%). Recently, Gorewit et al. (1993b) observed that angiotensin I, II, and III are vasoconstrictive to isolated mammary arteries. These examples represent relatively short-term regulation of mammary blood flow. Sustained regulation of blood flow, as suggested by Collier (1985) and Kronfeld et al. (1968), is apparently via mechanisms that regulate milk production, because milk yield and mammary blood flow are positively correlated.

Additional support for the relationship between blood flow and milk yield was provided by Mepham (1982), who found that milk production stimulated by exogenous growth hormone resulted in increased mammary blood flow. Similarly, Davis et al. (1988a) observed a 21% increase in milk production and 33% (3.3 to 4.4 L/min) increase in blood flow when cows were given growth hormone injections. Increased circulating concentrations of IGF-1 after bST administration coupled with the fact that type I and type II IGF receptors are present in mammary tissue during lactogenesis (Dehoff et al., 1988) has led researchers to investigate the relationship between IGF-1 and mammary blood flow. Recently, Prosser et al. (1990) observed that infusion of free IGF-1 into the pudenal artery of dairy goats enhanced milk production (half-udder) 25% and blood flow 22%.

Alternatively, Cant et al. (1993a) have reported that dietary fat decreased mammary blood flow 7% ($P < .07$) when cows were fed 4% yellow

grease. They theorized that a reduction in concentration of a vasodilator, such as adenosine, may reduce mammary blood flow via closure of precapillary sphincters. Temperature has also been shown to affect mammary blood flow. McBride and Christopherson (1984) reported that lactating ewes exposed to acute cold exposure (0°C) for 8 wk had a ratio of mammary blood flow to milk yield of 360 ± 110 compared to 664 ± 179 for controls. An interesting observation was that mammary blood flow was redistributed within the gland such that milk secretion was not significantly reduced.

Posture (standing or lying) appears to affect mammary blood flow. (McBride and Christopherson 1984, Metcalf et al. 1992, Rulquin and Caudal 1993). Rulquin and Caudal (1993) observed that lying induced a 24% increase in mammary blood flow which led to a 13% underestimation of average daily mammary blood flow if only "standing" values were used. Metcalf et al. (1992) observed a 22 to 28% increase in mammary blood flow when lactating cows laid down, depending on whether flow was determined by para-amino hippuric acid estimations or direct measurement with ultrasonic flow probes. A similar trend was observed in ewes (McBride and Christopherson 1984). These findings led Metcalf et al. (1992) to suggest that dairyman should utilize husbandry methods that encouraged cows to spend a greater proportion of their time lying down.

**Mammary blood flow measurement**

Since all milk precursors originate from blood, it is not surprising that accurate estimations of blood flow become critical when evaluating nutrient uptake by the mammary gland. Methods of estimating mammary blood flow include direct measurement by ultrasonic blood flow probes (Gorewit et al. 1989, Gorewit et al. 1993b, Rulquin and Caudal 1993, Rulquin and Vérité 1993), electromagnetic flow probes (Davis et al. 1988a, Davis et al. 1988b, McBride and Christopherson 1984, Peeters et al. 1979) and thermodilution (Bickerstaffe et al. 1974, Linzell 1957), versus indirect measurements by the Fick principle (Bickerstaffe et al. 1974, Cant et al. 1993a, Cant et al. 1993b, Davis et al. 1978, Linzell 1960a), antipyrine absorption (Rasmussen 1965), nitrous oxide diffusion (Reynolds et. al 1968), sodium bromsulphalein dilution (Thompson, et al. 1991), and para-amino hippuric acid (DiRienzo 1990, Metcalf et al. 1992).

Direct measurements of blood flow using either ultrasonic probes, thermodilution technique, or electromagnetic probes are advantageous because they can measure blood flow in short intervals over long sampling periods. However, the electromagnetic probes and the thermodilution technique are not without limitations. For example, as previously stated by Gorewit et al. (1989), the electromagnetic probe size must be chosen to provide ~10% constrictive fit so that the probe is tight enough to provide proper alignment, but not cause an aneurism. Additionally, unless the electromagnetic probes are calibrated in vivo, they are subject to potentially large zero offset (Peeters et al. 1979, Sellers et al. 1964).

Although accurate, the thermodilution technique as described by Linzell (1966) is not very applicable to determining blood flow in the

lactating dairy cow. Briefly, the technique involves injecting sterile, pyrogen-free saline at a known infusion rate and lower temperature into the milk vein, and then monitoring the temperature drop in blood downstream from the infusion point to calculate blood flow. Because there are two sources of venous drainage from the bovine mammary gland (see Figure 2.2), it is anatomically impossible to "clamp" the external pudenal veins (as done in the goat by Linzell 1976) to obtain total mammary flow through the epigastric vein only.

Ultrasonic probes are considered a promising alternative to the electromagnetic probes because they give direct measurement of net volume, independent of the flow profile. Recently, Gorewit et al. (1989) and Rémond et al. (1993) measured the zero offset of Transonic® ultrasonic flow probes and observed that readings from implants on the pudic artery and rumen artery were only -2.5 and 1.29 mL/min, respectively. Additionally, the ultrasonic probes require only acoustic contact with the vessel, thereby reducing risk of aneurysms (Gorewit et al. 1989). However, Hunington et al. (1990) and Metcalf et al. (1992) reported that ultrasonic blood flow probe readings correlated poorly with para-amino hippuric acid dilution estimations, and over time tended to underestimate flow.

Indirect measurement of blood flow requires using a nonendogenous indicator (i.e. nitrous oxide diffusion (Reynolds et al. 1968), antipyrine absorption (Rasmussen 1965), sodium bromsulphalein dilution (Thompson, et al. 1991), or para-amino hippuric acid (DiRienzo 1990, Metcalf et al. 1992)) or endogenous compounds that are stoichiometrically transferred from blood to milk such that mammary gland uptake equals output in milk. Generally, methionine (Davis and Bickerstaffe 1978), and tyrosine plus phenylalanine (Cant et al. 1993a and Cant et al. 1993b) have been popular choices as indicators for

determining mammary blood flow. The principal advantage of using endogenous indicators is that they are evenly distributed in blood; whereas, nitrous oxide, antipyrine, and para-amino hippuric acid methodologies assume that the compounds are thoroughly mixed with blood at the sampling sites.

## Arteriovenous sampling methodology

Collection of blood from catheters placed in the aorta (or coccygeal artery/vein) and epigastric vein (milk vein) allow the determination of arteriovenous difference of nutrients across the mammary gland. Other sources of arterial blood include the external iliac artery (Clark et al. 1974) or coccygeal artery/vein (also known as the tail artery/vein). The use of tail vein blood as representative of arterial blood has been previously validated by Emery et al. (1965) and subsequently was used to study uptake across the mammary gland (Cant et al. 1993a, Cant et al. 1993b, Hanigan et al. 1991, Illg et al. 1987, Miller et al. 1991, Seymour et al. 1990).

All studies mentioned above used the epigastric vein as the source of venous blood. As previously reported by Linzell (1960b), this source of blood would not be acceptable in virgin female goats or cows prior to lactation, because the arrangement of valves within the vein allows blood to flow towards the udder (abdominal venous blood). However, Linzell (1960b) went on to say that during lactation, the valves become incompetent and blood from the mammary gland flows through the milk vein under positive pressure. Ideally, it would be advantageous to occlude the pudenal veins while sampling the epigastric vein as described by Linzell (1960b) or Peeters et al. (1979), but this normally is not done

because the level of non-mammary blood potentially contaminating epigastric samples from bovine is considered minimal (Cant et al. 1993a).

## Glucose utilization by the lactating mammary gland

The glucose requirement of the lactating ruminant's mammary gland is due primarily to glucose utilization for lactose synthesis. Hardwick et al. (1961) used isolated perfused mammary glands to show that during glucose deprivation, mammary lactose and water secretion ceased. Glucose metabolism in ruminants is unique because, unlike monogastrics, much of the dietary nonfiber carbohydrate is fermented to VFA in the rumen (Bugaut 1987). Therefore, the ruminant must continually synthesize glucose via gluconeogenesis to meet requirements for maintenance and milk synthesis (Young 1976). Circulating glucose concentrations in ruminants commonly range between 2.2 and 4.7 mM (Cant et al. 1993b, Miller et al. 1991) which is approximately 50 to 80% of levels observed in humans. Therefore, ruminants have developed strategies to spare glucose for lactose synthesis in the mammary gland. Specifically, ruminants use acetate and β-hydroxybutyrate to synthesize fatty acids, whereas nonruminants primarily use acetyl CoA generated by oxidative decarboxylation of pyruvate (Bauman and Davis 1974).

Dietary fat generally does not affect glucose concentrations (Palmquist and Conrad 1978, Schauff et al. 1992), although increases (Elliot et al. 1993, Jenkins and Jenny 1989, Khorasani et al. 1992, Kronfeld et al. 1980) and decreases (Cant et al. 1993b, Palmquist and Moser 1981) have been reported. Decreases in arterial glucose concentrations have been attributed to reduced starch flow to the small

intestine or altered OM fermentation in the rumen, resulting in decreased propionate production (Cant et al. 1993b). Cant et al. (1993b) and Miller et al. (1991) reported that mammary arteriovenous (A-V) difference for glucose was not significantly affected by dietary fat, which supports earlier findings of Rulquin (1981) that glucose A-V difference is independent of arterial glucose concentration. Glucose extraction ratios of 17.5%, 18.4%, and 26.3% have been reported by Cant et al. (1993b), Peeters et al. (1979), and Bickerstaffe et al. (1974), respectively. Level of dietary UIP generally does not affect arterial glucose concentrations (Palmquist et al. 1993, Sahlu et al. 1993).

After Threadgold and Kuhn (1984) theorized that glucose transport across the mammary plasma membrane was the rate-limiting step in milk synthesis, researchers have used molecular biological techniques to characterize glucose transporters in the mammary gland. Burnol et al. (1990) reported that, unlike muscle and adipose tissue, the lactating rat mammary gland contains only insulin-independent glucose transporters. Similarly, Prosser (1988) reported primarily insulin-independent receptors on the plasma membrane of the mammary epithelial cell. Tesseraud et al. (1992) recently confirmed these findings by showing that insulin infusions in vivo did not affect uptake of glucose by the mammary gland.

Since glucose is the precursor molecule for lactose, and lactose is the osmotic regulator of milk volume, the only way to increase milk production is to increase lactose synthesis. It has been previously estimated that lactose accounts for 70% of the glucose extracted by the mammary gland (Bickerstaffe et al. 1974) thereby representing 1.42 kg of glucose uptake per 1 kg lactose output. A similar glucose uptake to lactose output ratio was reported by Peeters et al. (1979). Therefore, it is not surprising that Kronfeld et al. (1968) noted a linear

relationship between glucose uptake by the mammary gland and milk production.

## Lactate utilization by the lactating mammary gland

Lactate, a precursor for glucose synthesis, is absorbed from the digestive tract and is produced endogenously within portal-drained viscera, liver, muscle, and adipose (Brockman 1993). Weekes and Webster (1975) estimated that ~15% of circulating lactate is derived from propionate. The concentration of circulating lactate in lactating dairy cows ranges from .3 and 2.4 mM (Miller et al. 1991), and lactate is extracted by the lactating mammary glands of cows (10%), goats (28%), and pigs (15%) (Collier 1985). Additionally, Oddy et al. (1985) found that net uptake of lactate by the lactating ovine mammary gland was about 20% of glucose uptake on a molar basis. Although lactate is generally used as a glucose precursor in the liver (Cori cycle), Forsberg et al. (1985) demonstrated in vitro that the mammary gland primarily oxidizes lactate or uses it as a substrate for fatty acid synthesis.

## Acetate utilization by the lactating mammary gland

Unlike other VFA, Bergman and Wolff (1971) showed little metabolism of acetate in the rumen wall or the liver, which accounts for the higher circulating concentration of acetate compared to other VFA. Another source of endogenous acetate is metabolism of LCFA in the liver (Baird et al. 1974). Arterial acetate concentrations ranging from 1.0

to 3.0 mM in lactating cows have been reported (Bickerstaffe et al. 1974, Bjorkman and Forslund 1986, Cant et al. 1993b, Miller et al. 1991). Additionally, extraction of arterial acetate by the mammary gland of ruminants ranges from 40 to 80% (Bickerstaffe et al. 1974, Cant et al. 1993b, Collier 1985, McClymont 1951, Miller et al. 1991). The wide range of extractions under physiological conditions supports the theory that arterial acetate extraction appears to be a simple linear function of blood concentration (Cant et al. 1993b, Miller et al. 1991). Uptake of acetate by the mammary gland does not appear to be influenced by insulin (Laarveld et al. 1985).

McClymont (1951) was one of the first to report that the mammary gland utilizes acetate. Acetate serves as the major energy source and precursor of fatty acids in the ruminant mammary gland. Balmain et al. (1954) used sheep mammary slices to show that 97% of fatty acids synthesized in vitro were derived from acetate but only 3% from glucose. Later, Bickerstaffe et al. (1974) estimated that in cows and goats, 29 to 47% of acetate taken up by the mammary gland is oxidized. In the lactating ewe, 17 to 29% of milk fatty acids were synthesized from acetate (King et al. 1985).

**Propionate utilization by the lactating mammary gland**

Although propionate represents a major end product of microbial fermentation, 85 to 90% is removed from portal vein blood in a single pass through the liver (Brockman 1993) and converted to glucose. Therefore, quantitative uptake of propionate by extrahepatic tissues has been considered minimal and usually ignored (Bickerstaffe et al. 1974, Cant et al. 1993b).

**Butyrate utilization by the lactating mammary gland**

The primary function of butyrate is its role as a precursor for production of ketone bodies in the rumen epithelium (Bugaut 1987, Emmanual 1980). Production of ketone bodies from butyrate was first demonstrated in vitro by Pennington (1952) and in vivo by Annison et al. (1957). In a review of the literature, Bugaut (1987) reported that as much as 70 to 90% of absorbed butyrate is metabolized to ketone bodies in the rumen wall. Butyrate concentration in arterial plasma of dairy cattle is normally below .1 mM (Bjorkman and Forslund 1986). Therefore, the low level of butyrate in blood precluded its quantification in recent mammary A-V difference and uptake studies (Cant et al. 1993b, Miller et al. 1991).

**Ketone utilization by the lactating mammary gland**

Circulating β-hydroxybutyrate and acetoacetate originate primarily from ketogenesis of NEFA in the liver and in the case of β-hydroxybutyrate, as discussed above, oxidation of butyrate as it is absorbed across the rumen wall (Leng and West 1969). Rumen-derived acetate and propionate contribute relatively small or negligible quantities to ketone bodies formation (Pennington 1954). Leng and Annison (1964) reported that β-hydroxybutyrate accounted for ~85% of circulating ketone bodies in sheep. Recently, Miller et al. (1991) found arterial β-hydroxybutyrate concentrations ranging from .7 to 4.7 mM for cows consuming similar diets but blocked by previous milk producing potential (5,900 to 13,600 kg milk in a previous lactation). Mammary gland extractions of β-hydroxybutyrate were 40%, 57%, and 15% for the cow, goat, and pig, respectively (Collier 1985). Miller et al. (1991) and Cant et al. (1993b) suggested that arterial concentration of

β-hydroxybutyrate is the major determinant of mammary gland A-V difference and extraction (i.e. as arterial concentrations go up, A-V differences and extractions increase linearly). In the mammary gland, β-hydroxybutyrate is primarily used as a primer molecule for fatty acid synthesis but cannot furnish significant quantities of carbon for elongation or de novo fatty acid synthesis because the ruminant mammary gland lacks a functional citrate-cleavage pathway (Bauman and Davis 1974, Palmquist et al. 1969).

## Oxygen and carbon dioxide flux across the lactating mammary gland

Like other nutrients previously mentioned, the amount of $O_2$ available for tissue utilization is a function of its concentration in blood and blood flow. Oxygen uptake is closely linked to fat, protein, and lactose synthesis due to the requirement for ATP (via oxidative phosphorylation). Therefore rate of $O_2$ utilization is controlled by the rate of energy expenditure within the cells (Guyton 1991). As previously stated by Davis et al. (1988b), one possible mechanism by which mammary tissue could increase milk synthesis without altering $O_2$ consumption would be to increase mammary utilization of preformed long-chain fatty acids, thereby decreasing mammary ATP requirements for fatty acid synthesis.

The primary product of oxidative metabolism by cells is $CO_2$. Only a small portion of $CO_2$ is transferred in the dissolved state (7%), compared to 70% as bicarbonate ($HCO_3^-$) and 23% as carbaminohemoglobin ($CO_2HHB$) (Guyton 1991). Like $O_2$ uptake, $CO_2$ output from the mammary gland can be used as an indicator of mammary gland metabolism. For example, Cant et al. (1993b) observed an increased mammary A-V

difference for $CO_2$ when cows consumed fat-supplemented diets. They attributed their findings to increased LCFA uptake and reduced de novo fatty acid synthesis from acetate. The acetate was oxidized, thereby increasing $CO_2$ output.

## Triacylglycerol and NEFA utilization by the lactating mammary gland

Triacylglycerol (TG) fatty acids and NEFA represent ~5.5% of the total fatty acids in bovine plasma (Moore et al. 1969). Generally, supplemental fat increases TG (Cant et al. 1993b, Chow et al. 1990) and NEFA (Schauff et al. 1992, Chow et al. 1990, Palmquist and Conrad 1978). To our knowledge, increasing dietary UIP does not influence arterial concentrations of TG or NEFA. Uptake of TG is dependent on lipoprotein lipase found on capillaries of the mammary gland. Therefore, elevated arterial TG represent increased dietary fat absorption from the intestine relative to utilization by mammary tissue or adipose tissue (Cant et al. 1993b). It is very common to observe higher extractions of TG and negligible or negative extractions of NEFA when cows consume supplemental fat (Cant et al. 1993b, Davis and Collier 1985). The higher negative extraction of NEFA represents increased capillary lipoprotein lipase activity with incomplete uptake of released fatty acids (West et al. 1972). True LCFA uptake can be calculated by subtracting the esterified fatty acids in TG and NEFA in venous samples from the esterified fatty acids in TG and NEFA in arterial samples.

**Plasma and milk urea N**

Plasma urea N is often used as an indicator of dietary CP percentage, level of dietary CP degradability, and postruminal protein supply (Roseler et al. 1993). Dietary fat has been previously shown to increase (Cant et al. 1993a), decrease (Palmquist and Conrad 1978) or not affect (Schauff et al. 1992) plasma urea N. Generally, casein N, whey protein N, and NPN constitute approximately 78, 17, and 5%, respectively, of the total N in milk. DePeters and Cant (1992) have reported that nutrition, breed, parity, stage of lactation, environment, and disease could markedly affect the distribution of N-containing compounds in milk. As seen previously for fat-induced milk protein depression, when expressed as a percentage of total N, casein N and whey N generally decrease and urea N increases (DePeters and Cant 1992, DePeters et al. 1989, Dunkley et al. 1977, Jenkins and Jenny 1992). Milk urea N arises primarily from the passive diffusion of urea from plasma to milk (Clark et al. 1978). Given the high solubility of urea and its potential to diffuse freely across mammary epithelial cells, a positive correlation between plasma urea N and milk urea N has been observed (Cant et al. 1993a, DePeters et al. 1989, Roseler et al. 1993). However, the mammary gland also synthesizes some urea N (Clark et al. 1974, Mepham and Linzell 1974b).

**Amino acid utilization by the lactating mammary gland**

As previously described by Mepham (1982), AA uptake is dependent on arterial concentration and rate of blood flow, which together determine the quantity of AA available to the gland per unit time and the transfer efficiency of amino acids across the basal membranes of the secretory cells. Once inside the cell, these AA could either be used for milk protein synthesis, retained in the cells in the form of structural proteins or enzymes, metabolized to $CO_2$, urea, and NEAA, or pass unchanged in to milk, blood, or lymph.

Dietary UIP (Seymour et al., 1990), sodium caseinate infusions into the duodenum (Cant et al. 1993a, Clark et al. 1977, Seymour et al. 1990), and dietary rumen-protected Met and Lys (Schwab et al. 1992, Seymour et al., 1990) can effectively influence circulating concentrations of individual AA. Supplemental fat has been shown to decrease plasma NEAA and total AA concentrations (Cant et al. 1993a, Casper and Schingoethe 1989, Mohamed et al. 1988).

Although plasma AA have traditionally been used to characterize AA supply to the mammary gland, it is now generally accepted that whole blood AA provide a better indication of arterial AA concentrations because erythrocyte AA have been shown to contribute to amino acid transport in lactating cows (Hanigan et al.,1991) and growing calves (Koeln et al. 1993, McCormick and Webb 1982). Another relatively new concept is that the plasma peptide AA pool in nonlactating ruminants may account for 50 to 80% of the available AA pool (DiRienzo 1990, Koeln et al. 1993, Seal and Parker 1991). Webb et al. (1992 and 1993) have suggested that although free AA in plasma derived from undegraded dietary protein, microbial protein, and endogenous proteins have been considered the primary sources of AA for peripheral tissues, studies

that do not account for peptide AA may potentially underestimate total AA uptake by the lactating gland. Recent findings by Pan et al. (1993) and Wang et al. (1993) provided in vitro verification that muscle and mammary cells in culture can utilize peptide AA. Regardless of source, circulating AA concentrations reflect not only the rate of entry of AA into circulation but also their removal by the mammary gland and other peripheral tissues. Circulating AA concentrations, therefore, reflect the net result of AA metabolism throughout the body. For example, Champredon et al. (1990) observed that when Met requirements for milk production become limiting in the lactating goat, an adaptive mechanism occurs such that AA utilization by extramammary tissues decreases.

Mepham and Linzell (1974) observed that despite daily arterial AA fluctuations in lactating goats, A-V differences for the EAA varied only slightly. In contrast, Hanigan et al. (1992) found that as arterial EAA concentrations increased, A-V differences and extraction ratios increased linearly. Specifically, Casper and Schingoethe (1989) observed that dietary fat decreased A-V differences for several EAA and total AA, and proposed that dietary fat decreased AA extractions by the mammary gland due to low concentrations of bST in blood. However, Cant et al.(1993a), could not substantiate this hypothesis because supplemental fat either increased or did not affect A-V differences and the percent extraction of AA in plasma and whole blood.

Mepham (1982) previously categorized free AA into three groups based on their uptake and utilization in the mammary gland. Group I contained AA that are stoichiometrically transferred from blood to milk, and include Met, Phe, Trp, and Try. Group II contained EAA taken up in excess of milk output, and include Arg, His, Ile, Leu, Lys, Thr, and Val. Group III contained the remaining NEAA that are not taken from blood in sufficient quantities to account for their output in milk (i.e.

Pro). Of particular interest is the high mammary gland uptake of arginine relative to its output in milk across ruminant species (Clark et al. 1974, Davis et al. 1978, Mepham and Linzell 1967). Mepham and Linzell (1967) showed that excess arginine taken up by goat mammary tissue is converted to proline by pathways involving the formation of ornithine and urea. These findings were later verified in the lactating dairy cow (Clark et al. 1974).

**Effect of dietary fat and level of UIP on milk production**

About 50 years ago, Maynard et al. (1941) and Loosli et al. (1944) found that concentrates containing 3 to 4% supplemental fat increased milk production by 2 to 10%. More recently, Mattias et al. (1982) fed concentrates containing 5% tallow and found that second-lactation cows produced more milk. Wonsil (1990) reported that 3% partially hydrogenated tallow or 3% tallow coated with casein and corn syrup solids increased milk production. However, there have also been reports that supplemental fat did not increase milk production. Yellow grease (Cant et al. 1993b), tallow (Palmquist and Conrad 1980), protected tallow (Dunkley et al. 1977), or prilled fat (Grummer 1988, Shauff and Clark 1989) supplying up to 5% of diet DM did not improve milk production. Lack of response to dietary fat supplementation may be due to stage of lactation (early versus mid to late lactation) or use of cows with lower genetic potential for milk production.

Abomasal infusions of casein (~400 g/d) increased milk production by 4 to 10% above controls consuming 18% CP diets containing soybean meal as the primary protein supplement (Clark et al. 1977, Cohick et al. 1986, Derrig, et al. 1974). This has led to the practice of using

highly undegradable protein sources to potentially increase the level of AA reaching the small intestine and reduce the level of N wasted via excessive microbial proteolysis in the rumen. However, when dietary UIP is elevated at the expense of microbial protein production, the quality and quantity of metabolizable AA ultimately reaching the mammary gland may not change or could decrease (Clark et al. 1992). Therefore, it is not uncommon that diets containing UIP with an inappropriate balance of EAA do not improve milk production (Holter et al. 1993, Schwab 1989, Winsryg et al. 1991).

## Effect of dietary fat and level of UIP on milk fat production

Palmquist et al. (1978) used [1-$^{14}$C]-linoleic acid to estimate that approximately 50% of milk fatty acids are synthesized de novo in the mammary gland from acetate and β-hydroxybutyrate, 40-45% from dietary fatty acids, and less than 10% from adipose. Storry et al. (1973) reported that fatty acid uptake by the mammary gland inhibited de novo synthesis of short chain fatty acids. It was proposed that acetyl-CoA carboxylase was inhibited by increased concentrations of long chain acyl-CoA. However, fat supplementation, especially during early lactation, can increase milk fat concentration and fat-corrected milk production. Protected lipid supplements (i.e. Ca-salts of long chain fatty acids, formaldehyde treated oil, and hydrogenated fats) increased milk fat percent (Palmquist 1976). Palmquist (1976) theorized that uptake of long chain fatty acids by mammary gland exceeded the compensatory reduction in short chain acid synthesis resulting in higher milk fat secretion. Alternatively, supplemental fat did not improve milk fat percent when tallow (Palmquist and Conrad 1978), Ca-salts of

long chain fatty acids (Grummer 1988, Shauff and Clark 1989) or prilled fat (Grummer 1988, Shauff and Clark 1989) were fed. In spite of no change or a decrease in milk fat percent, milk fat yield may increase if milk volume increases. It is apparent that stage of lactation, energy status of the animal, DM intake, and "health" of rumen environment could influence milk fat production (Wonsil 1990).

**Effect of dietary fat and level of UIP on milk fatty acid composition**

Dietary fats also can alter fatty acid composition of milk fat. For example, a given fatty acid may be ingested, absorbed, and transported without alteration to the mammary gland where it can be esterified and incorporated into milk fat in a higher proportion than is found in control animals (Wonsil 1990). Alternatively, unsaturated (i.e. 18:2) fatty acids may undergo biohydrogenation to 18:0 in the rumen prior to being esterified in the mammary gland for incorporation into milk fat. However, mammary tissue also has the capacity to desaturate 18:0 to 18:1 before incorporation into milk fat (Kinsella 1972). This may be a compensatory response of the mammary gland to maintain milk fat globule fluidity when the gland receives large amounts of saturated fatty acids.

In general, increased uptake and secretion of dietary fatty acids appears to decrease de novo production of 4:0 through 14:0 fatty acids by the mammary gland. Cant et al. (1993b) found that dietary supplemention with 4% yellow grease resulted in milk fat with a higher concentration of dietary LCFA and a lower concentration of short and medium chain fatty acids derived from de novo synthesis. Similar responses to dietary fat were reported previously (Chow et al. 1990,

Khorasani et al. 1991, Schauff et al. 1992, Storry et al. 1973). Canale et al. (1990) found cows fed a blend of animal-vegetable fat or Ca-salts of long chain fatty acids produced milk fat with a higher percentage and yield of 16:0 through 18:2 and lower percentages of 4:0 through 14:0. Steele (1984) reported that cows fed tallow and ground nut oil had higher yields of 18:0 through 18:2 and reduced yields of 4:0 through 14:0 compared to cows fed an unsupplemented diet. Similarly, Schneider et al. (1990) also observed reduced 4:0 through 14:0 milk fatty acids when Ca-salts of long chain fatty acids were fed or if bST was administered.

**Effect of dietary fat and level of UIP on milk protein production**

Banks et al. (1984), Cant et al. (1993b), and Murphy and Morgan (1983) reported that milk protein yield was not influenced by supplemental fat because higher milk production compensated for a lower milk protein percentage, which is a common response to supplemental dietary fat. Recently, Grummer et al. (1993) observed that a small but linear decrease in milk protein percentage as tallow supplementation was increased from 0 to 3% of the diet DM. The mechanism for supplemental fat-induced milk protein depression has been theorized to be a function of inadequate AA absorbed from the small intestine (Clark 1975), altered glucose metabolism (Smith et al. 1978), insulin resistance (Palmquist and Moser 1981), decreased somatotropin release from the anterior pituitary (Casper and Schingoethe 1989), or, most recently, reduced mammary blood flow (Cant et al. 1993a).

## Chapter 3

### AVAILABILITY AND UTILIZATION OF CARBON SOURCES FOR MILK SYNTHESIS BY DAIRY COWS FED SUPPLEMENTAL FAT AT TWO LEVELS OF UNDEGRADABLE INTAKE PROTEIN

### (ABSTRACT)

The objective of this study was to determine whether dietary fat supplementation and level of undegradable intake protein (UIP) could influence daily milk output and composition by regulating nutrient supply to the mammary gland. Three lactating Holstein cows (60, 68, and 74 d postpartum) were used in an incomplete 4 x 4 Latin square design (2 x 2 factorial) and fed diets (15.9% CP and 19.5% ADF) with 0% or 2.5% partially hydrogenated tallow and 33% or 41% UIP. A 5:2.5:1 mixture of dried brewer's grains, corn gluten meal, and blood meal was substituted for soybean meal to raise dietary UIP from 33% to 41% UIP. Despite similar DM intake across treatments, cows produced 9% more milk per day when fed 2.5% supplemental fat, 41% UIP, or the combination of 2.5% fat and 41% UIP when compared to the control diet. Mammary blood flow was estimated using the Fick principle at 6-hr intervals for 24 h. Concentration of individual nutrients in arterial (carotid) and venous (abdominal vein) blood and corresponding blood flows were used to calculate nutrient uptakes by mammary glands. Fat supplementation depressed milk protein percentage but not daily milk protein output. Calculated carbon uptake was 95 to 101% of output when using estimated carbon content of nutrients, and 100 to 106% when using an elemental analyzer to determine actual carbon output in milk. Uptake of glucose, β-hydroxybutyrate, lactate, pyruvate, acetate, and $O_2$ were not affected by dietary treatment. Triacylglycerol concentration in arterial blood and uptake of long-chain fatty acids increased 16% and 33% with fat supplementation, respectively, resulting in milk fat with a higher

percentage of 18-carbon fatty acids and lower percentages of 6:0 through 16:0. Results indicated that dietary fat supplementation at two levels of UIP increased milk production by altering mammary lipid metabolism and improving efficiency of milk synthesis.

## INTRODUCTION

A current trend when formulating diets for high producing dairy cows is to increase the level of dietary fat and the proportion of undegradable intake protein (UIP), theoretically providing additional energy and AA for milk production. However, it is not uncommon that adoption of these practices does not improve milk production or reduce fat-induced milk protein production (Holter et al. 1993, Schwab 1989, Winsryg et al. 1991). Recently, Cant et al. (1993a and 1993b) used 4% dietary yellow grease and ruminal and abomasal sodium caseinate infusions (5.04 kg/d) to show that dietary fat increased the efficiency of milk production (i.e. less blood volume required to meet the energy demands to synthesize a given volume of milk), but inhibited milk protein percentage by reducing mammary blood flow and altering AA utilization.

To our knowledge, no one has specifically addressed potential changes in the metabolic requirements of the lactating mammary gland when cows are consuming diets that contain supplemental fat and different levels of UIP. Therefore, the overall objective of this experiment was to evaluate whether supplemental fat and level of undegradable intake protein (UIP) alters daily milk output and composition by monitoring concentration of nutrients in arterial blood, daily flow of nutrients to the mammary gland, and balance of nutrients across the mammary gland. Specifically, we wanted to test the hypothesis of Cant et al. (1993a and 1993b) that supplemental fat increases the efficiency of energy utilization within the gland, leading to greater milk synthesis. A companion paper (see Chapter 4), will addressed whether supplemental fat-induced milk protein depression can be attributed to reduced mammary blood flow, thereby inhibiting AA utilization.

## Materials and Methods

### Surgical Procedures

All procedures for this study were approved by the Virginia Polytechnic Institute and State University Institutional Animal Care and Use Committee. Approximately three weeks following parturition, four cows were prepared for surgical installation of a blood flow probe at the Virgnia-Maryland College of Veterinary Medicine as described by Gorewit et al. (1989). After induction and maintenance of anesthesia, a 15 to 18 cm incision was made over the inguinal region between the medial thigh and lateral surface of the mammary gland. The right external pudic artery was isolated by blunt dissection immediately ventral to the inguinal canal. After cleaning fatty deposits from around the vessel, a 16-mm ultrasonic blood flow probe (Transonic Systems, Ithaca, NY) and sheath were placed around then sutured to the artery. This ensured proper alignment of the probe around the vessel and theoretically diminished future fat inclusion in the vicinity of the acoustic reflector of the probe. The flow probe wires were routed subcutaneously and externalized ~15 cm above the rear udder. Additionally, the right carotid artery of each cow was surgically elevated to a subcutaneous position to facilitate arterial blood sampling. Following surgery, cows were returned to a tie stall facility at the Dairy Nutrition Research Barn at the Virginia Tech Dairy Center and given 5 mL Banamine® (50 mg/mL) and 30 mL Penicillin G Procaine (300,000 units/mL) every 12 h for 3 d and 7 d, respectively, to reduce post-operative stress and infection. One of the original four cows was removed from the study due to chronic mastitis during the first period of an experiment intended to be a 4 x 4 Latin square.

**Experimental Design and Diets**

Three lactating Holstein cows (60, 68, and 74 d postpartum) were used in an incomplete 4 x 4 Latin square design (2 x 2 factorial) because one cow was removed from the study due to chronic mastitis. Cows were fed diets containing 0% added fat and 33% UIP **(CNTL)**, 2.5% added fat (Alifet®; Alifet USA Inc., Cincinnati, OH) and 33% UIP **(FAT)**, 0% added fat and 41% UIP **(UIP)**, or 2.5% added fat and 41% UIP **(BOTH)**. Dietary ingredients and chemical composition are given in Table 3.1 (see page 48). Diets were fed as a total mixed diet at 6 h intervals (Figure 3.1 see page 65) and contained 15.9% CP and 19.5% ADF. A 5:1 ratio of supplemental fat and corn gluten meal replaced corn grain in FAT and BOTH to raise dietary crude fat from 3.5% to 6.0% without changing the CP content. A 5:2.5:1 mixture of dried brewer's grains, corn gluten meal, and blood meal was substituted for soybean meal in UIP and BOTH to raise dietary UIP from 33% to 41% UIP. This combination of protein sources was chosen to provide a reduced rate of protein degradation in the rumen and an essential AA **(EAA)** chemical score relative to milk protein that would compliment the chemical score of rumen microorganisms (Table 3.2).

**Measurements and Sampling**

Forages and concentrates were sampled on alternate weeks throughout the experiment. Dry matter intakes and milk production were measured daily but only d 17 through d 21 of each 21-d period were used to ascertain effects of dietary treatments on DM intake and milk production, respectively.

On d 16 of each period, a progesterone releasing intravaginal device **(PRID)** (PRID®; Sanofi, Paris, France) was inserted into the

vagina of each cow. These devices were used as synchronizing agents to prevent estrus and the unwanted physiological effects that estrus could have on DM intake and milk production during the 24-h sampling period.

Catheters were implanted on d 17 of each period in the right carotid artery and right superficial epigastric vein to determine nutrient exchange across the mammary gland. Preceding catheterizations, cows were given 500 *u*l of Butorphanol (10 mg/mL) as an analgesic. The carotid artery catheter was Tygon® tubing (.02 mm i.d., .06 mm o.d., Fisher Scientific Co., Pittsburgh, PA) inserted through a Centrasil® guide catheter (Baxter, Deerfield, IL) temporarily placed in the carotid artery. The milk vein catheter used was Tygon® tubing (.02 mm i.d., .06 mm o.d., Fisher Scientific Co., Pittsburgh, PA) inserted into a 15 cm Teflon catheter (B-D I.V.™, Becton-Dickinson, Rutherford, NJ). Cows were given 20 mL of Penicillin G Potassium (500,000 units/mL) and moved to elevated stalls in a ventilated metabolism room for the remainder of the period. Catheters were flushed at 4-h intervals with 10 mL of .9% saline followed with 5 mL of heparinized saline (100 units/mL).

The last 24 h of each period were used for milk and blood collection to calculate arterial concentrations, arteriovenous **(A-V)** differences, extractions, and net uptakes of nutrients by the mammary gland. After the first milking at 0530 h, arterial and venous samples were collected simultaneously at 3-h intervals for the next 24 h (Figure 3.1). Blood samples were immediately placed on ice, packed cell volume determined, and plasma harvested by centrifugation at 2,500 x *g* for 15 min prior to storage at -20°C. Following the last collection of milk (0530 h) and blood (0600 h), all catheters and PRIDS were removed, and cows were returned to tie stalls in the research barn.

**Analyses**

Dry matter content of forages and concentrates was determined by freeze-drying to a constant weight. Lyophilized samples were ground through a 1 mm screen in a Wiley mill (Arthur J. Thomas, Philadelphia,, PA) and analyzed for acid detergent fiber (Goering and Van Soest 1970), nitrogen (AOAC 1980), and crude fat (AOAC 1980). Milk samples collected during the 24-h period were analyzed for fat by acid hydrolysis, di-ethyl ether, and petroleum ether extraction (AOAC 1980), nitrogen by Kjeldahl analysis (AOAC 1980), and lactose (Kurz and Wallenfels 1974). Additionally, milk fat, casein, and whey fractions were separated using ultracentrifugation as described by Donovan and Lonnerdal (1989). Carbon and nitrogen content of milk fractions were determined by elemental carbon, hydrogen, and nitrogen (CHN) analyses (CHNS/O Analyzer, Perkin Elmer, Norwalk, CT).

Triplicate analyses were conducted for nutrients in whole blood or plasma samples for all sampling times, except VFA, lactate, and pyruvate, which were analyzed only in samples collected at 0900 h, 1500 h, 2200 h, and 0300 h. Glucose (Procedure No. 16-UV, Sigma Diagnostics®, St. Louis, MO), β-hydroxybutyrate (Williamson and Mellanby 1974b), acetoacetate (Williamson and Mellanby, 1974a), lactate (Procedure No. 826-UV, Sigma Diagnostics®, St. Louis, MO), pyruvate (Procedure No. 726-UV, Sigma Diagnostics®, St. Louis, MO), triglyceride (Procedure No. 337, Sigma Diagnostics®, St. Louis, MO), glycerol (Procedure No. 337, Sigma Diagnostics®, St. Louis, MO), and NEFA (NEFA C, Wako Chemicals USA, Inc., Richmond, VA) were analyzed spectrophotometrically. Whole blood was analyzed for $O_2$, $CO_2$, $HCO_3^-$, $Na^+$, and $K^+$, using a blood gas analyzer (Stat Profile 2 Blood Gas Analyzer, Nova Biomedical, Waltham, MA).

Volatile fatty acids were extracted from plasma using the alcohol

extraction procedure of Remesy and Demingne (1974) and determined by gas-liquid chromatography using isocaproic acid as an internal standard. Samples were injected by auto-sampler into a Hewlett Packard 5890A gas chromatograph equipped with a flame ionization detector (Hewlett Packard Co., Sunvale, CA). Samples were split 5:1 onto a 30 m DB-FFAP glass capillary column with .25 mm i.d. and .25 um film thickness (J & W Scientific, Folsom, CA). Injector and detector temperatures were set at 125°C and 225°C, respectively. A temperature program initiated runs at 60°C, held for 2 min, warmed to 144°C at a rate of 10°C/min, held for 3 min, and then warmed to 190°C at a rate of 70°C/min and held for 8 min. Calibration mixtures containing 1 mM, 2mM, and 3mM acetate, propionate, isobutyrate, butyrate, isovalerate, and valerate were used to identify peaks and construct a multi-level calibration table via a HP 3396A integrator (Hewlett Packard Co., Avondale, PA). Response and recovery factors were used to determine weight percentages of individual VFA in the analyzed samples.

Fatty acids in plasma and milk fat were extracted and methylated by direct transesterification (Outen et al. 1976) using heptadecenoic and undecenoic acid (NU-Check-Prep Inc., Elysian, MN) as internal standards, respectively. Samples were injected by auto-sampler into a Hewlett Packard 5890A gas chromatograph equipped with a flame ionization detector (Hewlett Packard Co., Sunvale, CA). Samples were split 100:1 onto a 30 m DB-225 glass capillary column with .25 mm i.d. and .15 um film thickness (J & W Scientific, Folsom, CA). Injector and detector temperatures were set at 225°C. For fatty acids in plasma, a temperature program initiated runs at 190°C, held for 1 min, warmed to 205°C at a rate of 5°C/min, held for 12 min, and then warmed to 220°C at a rate of 5°C/min and held for 17 min. For fatty acids in milk fat, a temperature program initiated runs at 60°C, warmed to 205°C at a rate of 5°C/min, held for 12 min, and then warmed to 220°C at a rate of 5°C/min

and held for 7 min. Identification of peaks was based on relative retention times of commercial standards (NU-Check-Prep Inc., Elysian, MN). Calibration mixtures containing fatty acid methyl esters were used to construct a multi-level calibration table via a HP 3396A integrator (Hewlett Packard Co., Avondale, PA). Response and recovery factors were used to determine weight percentages of individual fatty acids in the analyzed samples.

Carbon balances across the mammary gland were ascertained two ways. The first method utilized nutrient uptake and output determined by chemical analyses and multiplied by carbon percentage of each nutrient. The second method utilized nutrient uptake, calculated as indicated above, and nutrient output determined by CHN analyses.

**Blood Flow Measurements**

A T101D Transonic Flowmeter (Transonic System Inc., Ithaca, NY) and TM04 Ultrsonic Flowprobe Scanner (Transonic System Inc., Ithaca, NY) were used in conjunction with LabTech Notebook Version 4.12 (Laboratory Technologies Co., Wilmington, MA) computer software for data aquistion. Individual blood flow measurements (3 readings at 10 sec intervals/30 sec per cow) were compiled to generate average blood flow measurements at 30-min intervals across treatments to observe potential effects of feeding, milking, and position (lying and standing) on mammary blood flow (Figure 3.2). Additionally, four 6-h averages of blood flow were determined for comparison with blood flow calculated using the Fick equation (Appendix Table A1 see page 122).

The Fick equation was used to deterime blood for the current study because mammary blood flow measured by the ultrasonic flow probes was precise but not accurate. The logic and justification for using the Fick equation have been addressed previously (Cant et al. 1993a).

Briefly, compounds that are stoichiometrically transferred from blood to milk such that mammary gland uptake equals milk output can be used to estimate blood flow. As suggested by Cant et al. (1993a and 1993b), Phe and Tyr were chosen to calculate blood flow. Additionally, plasma proteins were assumed to constitute 3.5% of the total milk protein. Thus, blood flow through the mammary gland (L/min) was calculated as follows:

$$\text{Blood flow} = \frac{(Y_B \times .965) + Y_F}{Y_{A-V}}$$

$Y_B$ = Phe + Tyr output in milk protein (mol/min)

$Y_F$ = free Phe + Tyr output in milk (mol/min)

$Y_{A-V}$ = Phe + Tyr A-V difference (mol/L)

Values for Phe and Tyr output as part of casein and in the free form were obtained from reversed phase HPLC determinations (See Materials and Methods Chapter 4). Concentrations of Phe and Tyr in whey were assumed to be 224 mg/g of whey N and 214 mg/g of whey N, respectively (Hambraeus 1982). Mammary blood flow was not corrected for afferent mammary lymphatic flow, previously characterized by Gorewit et al. (1993a) at 13 to 45 mL/h.

**Statistical Analyses**

Data were analyzed by ANOVA for a randomized incomplete block design using the general linear models procedure of PC-SAS (1990) including effects of treatments (df = 3), periods (df = 3), and cows (df = 2) with repeated measurements on time (df = 7, 3, 1, or 0). Effects of dietary treatments on arterial nutrient concentrations, A-V differences, and extractions were based on 8 estimations (3 h intervals), blood flow and uptakes were based on 4 estimations (6 h intervals), milk composition was based on 2 estimations (12 h intervals), and DM intake and milk production were based on a single estimation. All results are shown as least-squares means and pooled SEM. Orthogonal contrasts for treatment means included effects due to 0% versus 2.5% FAT, 33% versus 41% UIP, and the interaction between FAT and UIP.

**Results**

Overall, 2.5% supplemental fat and 41% UIP did not influence DM intake, due to a significant interaction between the dietary ingredients (Table 3.3). Intake of FAT and UIP was increased 4% compared to CNTL, but these effects were not additive when BOTH was fed. Cows produced 8%, 7%, and 11% more milk when fed FAT, UIP, and BOTH, respectively. Daily output of milk components was not significanly influenced by diet, despite a lower milk protein percentage due to supplemental fat.

Mammary blood flow determined by the Fick equation and expressed as L/min and L/kg of milk was not significantly affected by treatment (Table 3.3). Compared to blood flows estimated by the Fick equation, ultrasonic blood flow probes underestimated blood flow from 47% to 57% (Appendix Table A1). Therefore, flow determined by the Fick equation was used to calculate net uptake of nutrients.

Arterial glucose, β-hydroxybutyrate, acetoacetate, and lactate concentrations were not affected by dietary treatment (Table 3.4), but lactate concentrations tended ($P < .08$) to be lower when cows were fed 41% UIP. The significant interaction for pyruvate was due to higher concentrations when FAT and UIP were fed versus a lack of a similar response when BOTH was fed. Dietary treatments did not effect A-V differences and extractions of glucose, β-hydroxybutyrate, acetoacetate, lactate, or pyruvate. Similarily, net uptakes of glucose, β-hydroxybutyrate, acetoacetate, lactate, and pyruvate expressed as mol/d and g/d were not affected by dietary treatments (Table 3.5). Additionally, uptake of acetoacetate was not significantly different from zero ($P < .05$). Molecular weights used to convert data from mol/d to g/d are presented in Appendix Table A2. Across treatments, the ratio of glucose uptake to lactose output averaged 1.38 to 1.

Arterial concentrations, A-V differences, extractions, and uptakes

of acetate, butyrate, and isovalerate (Tables 3.6 and 3.7) were not affected by dietary treatments. A significant interaction for propionate concentration and uptake was due to the type of response noted earlier for DM intake and arterial lactate. In general, VFA were not affected by dietary treatment and uptake appeared to be a function of arterial blood concentration.

Arterial concentrations, A-V differences, extractions, and uptakes of $O_2$ and $Na^+$ (Tables 3.8 and 3.9) were not affected by dietary treatments. When cows were fed the 41% UIP, arterial $K^+$ concentration decreased, but A-V differences, extractions, and uptakes of $K^+$ were unaffected. Although arterial concentration of $CO_2$ and $HCO_3^-$ were not affected by dietary treatment, A-V differences, extractions, and outputs of $CO_2$ and $HCO_3^-$ were lower when cows consumed the fat-supplemented diets. Across treatments, 95% of $CO_2$ was transported as $HCO_3^-$. Overall, the mammary gland was a net importer of $O_2$ and $K^+$, and a net exporter of $Na^+$, $CO_2$, and $HCO_3^-$.

Although supplemental fat did not significantly influence arterial concentrations of TG, NEFA, LCFA, or glycerol (Table 3.10), A-V differences and extractions of TG and NEFA increased. The higher negative extraction of NEFA represents increased capillary lipoprotein lipase activity with incomplete uptake of released fatty acids. When fatty acids from TG and NEFA were combined to estimate total LCFA in plasma, uptake of LCFA (g/d) increased when cows received supplemental fat (Table 3.11). The 41% UIP treatments did not effect arterial concentrations, extractions, or uptakes of TG, NEFA, LCFA, and glycerol. Additionally, glycerol A-V differences were negligible, so glycerol uptake was not included in Table 3.11.

Our attempts to quantify the plasma LCFA via gas-liquid chromatograpy yielded inconclusive results. Dietary treatments did not significantly influence individual or total fatty acid concentrations

(mg/L) in plasma (Table 3.12), but suppplemental fat increased 16:0 and 18:1, and tended to increase 18:3(n-3) concentrations when expressed as a percent of total fatty acids. Arteriovenous differences and net uptakes were not significantly different from zero for individual or total fatty acids (Table 3.13), contradicting data from TG and NEFA analysis which indicated net uptake of LCFA.

Additional evidence indicating net uptake of dietary LCFA is presented in Table 3.14. In general, as uptake and secretion of dietary LCFA into milk fat increased, there was an apparent decrease in de novo synthesis of short and medium chain fatty acids (6:0 through 16:0), increased 4:0, 18:0 and 18:1, decreased saturated fatty acids, and increased 18:0 through 18:3(n-3). As a result, output (g/d) of 4:0, 18:0, 18:1, unsaturated fatty acids, and 18:0 through 18:3(n-3) was higher when cows were fed supplemental fat (Table 3.15). When cows consumed 41% UIP, however, there was a trend towards increased de novo synthesis of short and medium chain fatty acids (6:0 through 16:0), but generally, no change in fatty acid output (g/d).

Nutrient balances across the mammary gland (including amino acid data from Chapter 4) are given in Table 3.16. The overall trend was that nutrient uptake matched nutrient output when cows were fed FAT and BOTH but were ~7% below expected output when cows consumed either CNTL or UIP. Carbon balance is presented in Table 3.17. Carbon uptake estimates were determined from chemical analyses of nutrients and carbon percentage composition are listed (see Appendix Table A3 for carbon uptakes by mammary gland based on plasma CHN analyses). Carbon output, however, was determined not only by chemical analyses of output and carbon percentage composition, but also by CHN analyses. Carbon uptake matched calculated carbon output when cows were fed FAT and BOTH, but were ~5% below expected when cows consumed either CNTL or UIP. A similar pattern was evident when carbon output was based on CHN analyses, but all means were 100% or greater.

**Discussion**

Providing 2.5% supplemental fat, 41% UIP, or combining 2.5% supplemental fat with 41% UIP had a positive influence on milk production. Compared to CNTL, cows produced 8%, 7%, and 11% more milk when fed FAT, UIP, and BOTH, respectively. Dry matter intake tended to increase when cows were fed 2.5% supplemental fat (1 kg/d) or 41% UIP (.7 kg/d) compared to CNTL or BOTH. However, the efficiency of milk production increased from 1.28 kg milk/kg feed for CNTL to 1.43 kg milk/kg feed for BOTH. Supplemental fat and higher UIP previously have been shown to increase (Jenkins and Jenny 1992, Mattias et al. 1982) or not affect (Mantysaari et al. 1989, Schwab 1989, Winsryg et al. 1991) milk production.

In the current study and others (Banks et al. 1984, Cant et al. 1993b, Murphy and Morgan 1983), milk protein yield was not influenced by supplemental fat because the higher milk production compensated for lower milk protein percentage. The mechanism for supplemental fat-induced milk protein depression was theorized to be a function of inadequate AA absorbed from the small intestine (Clark et al. 1975), altered glucose metabolism (Smith et al. 1978), insulin resistance (Palmquist and Moser 1981), decreased somatotropin release from the anterior pituitary (Casper and Schingoethe 1989), or most recently, reduced mammary blood flow (Cant et al. 1993a).

Milk yield and mammary blood flow are generally thought to be positively correlated (Collier 1985, Kronfeld et al. 1968). As evidence, cows injected with bST had increased milk yield with corresponding increases in mammary blood flow (Mepham et al. 1984, Rulqin and Vérité 1993). In the current study, mammary blood flow determined by the Fick equation and expressed as L/min and L/kg of milk was not significantly affected by treatment (Table 3.3). However, blood

flow tended to be highest when cows consumed UIP (17.7 L/min), lowest for CNTL (14.8 L/min), and intermediate with fat-supplementation (16.8 L/min). This was in contrast to blood flow data measured by the ultrasonic blood flow probes (Appendix Table A1) which showed no effect of dietary treatments on flow (8.25 L/min).

Ultrasonic blood flow data in Figure 3.2 demonstrates that blood flow is not static, but fluctuates during the day. This illustrates an advantage of using blood flow probes because they can theoretically provide a better estimation of average blood flow during a sampling interval (i.e. only a limited number of simultaneously collected samples can be analyzed for AA content to estimate blood flow using the Fick principle). However, we discovered that compared to Fick estimations, ultrasonic probes underestimated blood flow from 47 to 57% and averaged only ~340 L/kg of milk, compared to ~681 L/kg of milk for Fick estimations. Glucose and AA uptake fell short of output in milk when calculated using blood flow obtained from ultrasonic flow probe measurements (data not shown). Cows used to determine blood flow by ultrasonic flow probes in previous reports (Gorewit et al. 1989, Rulqin and Vérité 1993) produced less than 66% of the milk observed in the current study. Our data agrees with recent findings of Metcalf et al. (1992) which suggest ultrasonic probes underestimate blood flow during chronic implantation. Therefore, the Fick equation was used to calculate net nutrient uptake and balance across the mammary gland.

Overall, 2.5% supplemental fat and 41% UIP did not influence arterial glucose concentrations. However, the tendency (*P* < .07) for a significant interaction between FAT and UIP may reflect lower DM intake and subsequent lower propionate production in the rumen when cows consumed BOTH. Dietary fat also did not affect plasma glucose concentration in other studies (Palmquist and Conrad 1978, Schauff et al. 1992), although increases (Elliot et al. 1993, Jenkins and Jenny 1989, Khorasani et al. 1992, Kronfeld et al. 1980) and decreases (Cant et al. 1993b, Palmquist and Moser 1981) have been reported.

Arteriovenous glucose differences were not significantly affected by dietary treatment, which supports earlier findings of Cant et al. (1993b) and Miller et al. (1991) that glucose A-V difference is independent of arterial glucose concentration. Across treatments, glucose extraction was 23.5%, slightly higher than the 17.5% observed by Cant et al. (1993b) but lower than the 26.3% reported by Bickerstaffe et al. (1974). Since glucose is the precursor molecule for lactose, and lactose is the osmotic regulator of milk volume, the only way to increase milk production is to increase lactose synthesis. It has been previously estimated that lactose accounts for ~70% of the glucose extracted by the mammary gland (Bickerstaffe et al. 1974), which is similar to the 1.38:1 ratio of glucose uptake to lactose output across dietary treatments in the current study. Therefore, it is not surprising that Kronfeld et al. (1968) noted a linear relationship between glucose uptake by the mammary gland and milk production. Yet in our study, glucose uptake was not significantly affected by dietary treatment even though milk production was increased by supplemental fat and 41% UIP, which suggested a change in glucose utilization in the gland.

Arterial concentrations of β-hydroxybutyrate, acetoacetate, and lactate, were not significantly affected by dietary treatment which

indicates rumen fermentation patterns and liver metabolism were not significantly altered by either 2.5% supplemental fat or 41% UIP. Circulating β-hydroxybutyrate and acetoacetate originate primarily from ketogenesis of NEFA in the liver and in the case of β-hydroxybutyrate, oxidation of butyrate as it is absorbed across the rumen wall (Leng and West 1969). Across treatments, β-hydroxybutyrate accounted for 84.5% of circulating ketone bodies which is similar to the 85% reported for sheep (Leng and Annison 1964). Dietary treatments did not significantly affect A-V differences, extractions, or uptakes of β-hydroxybutyrate, acetoacetate, lactate, or pyruvate. In the mammary gland, β-hydroxybutyrate is primarily used as a primer molecule for fatty acid synthesis. Across treatments, lactate uptake was only about 10.6% of glucose uptake on a molar basis, compared to 20% observed in lactating sheep (Oddy et al. 1985). Forsberg et al. (1985) used an in vitro study to show that the mammary gland has a high capacity for lactate oxidation and conversion to fatty acids. A similar fate for pyruvate would be expected, but could not be documented. Net uptake of acetoacetate was negligible in the current study, and to our knowledge uptake has never been determined using the A-V difference technique in other studies.

Across treatments, acetate concentrations were 1.65 mM which is slightly lower than the 2.17 mM observed by Cant et al. (1993b) but within the physiological range of 1.3 to 3.0 mM given by Miller et al. (1991). Propionate and butyrate accounted for only 9.4% and 6.9% of circulating VFA, respectively, which is evidence of propionate metabolism in the liver and butyrate metabolism in the rumen wall (Annison 1983). Across treatments, uptake of propionate and butyrate was only 2.7% and 1.8% of circulating VFA, respectively, and therefore contribute only indirectly to mammary metabolism.

Dietary treatments did not affect arterial concentrations, A-V differences, extractions, and uptakes of $O_2$ and $Na^+$. Decreased arterial $K^+$ concentrations observed when cows consumed 41% UIP most likely reflects the 18% lower $K^+$ concentration of protein by-products versus soybean meal. However, A-V differences, extractions, and uptakes of $K^+$ were unaffected by treatment. Oxygen uptake is closely linked to fat, protein, and lactose synthesis due to the requirement for ATP (via oxidative phosphorylation). Even though cows produced ~10% more total milk solids (lactose, fat, and protein) when fed the supplemented diets versus CNTL (4.44 versus 3.99 kg/d)(see Table 3.3), mammary $O_2$ consumption was 31.3, 32.4, 35.9, and 29.8 g/kg of milk for CNTL, FAT, UIP, and BOTH, respectively. As previously stated by Davis et al. (1988b), one possible mechanism by which mammary tissue could increase milk synthesis without altering $O_2$ consumption would be to increase mammary utilization of preformed long-chain fatty acids, thereby decreasing mammary ATP requirements for fatty acid synthesis.

Arteriovenous differences, extractions, and outputs of $CO_2$ were lower when cows consumed the fat-supplemented diets. Conversely, Cant et al. (1993b) observed similar $CO_2$ output from the mammary gland when cows consumed fat-supplemented diets because higher $CO_2$ output into venous blood was offset by lower blood flow. They attributed their findings to increased LCFA uptake and reduced de novo fatty acid synthesis, but suggested that increased acetate oxidation resulted in similar $CO_2$ output compared to the control.

Previously, Wonsil (1990) observed that 2.5% supplemental fat (same product) increased the amount of fatty acids (g/d) apparently absorbed from the small intestine. In the current study, lack of a significant increase in arterial concentration of TG when cows were fed supplemental fat (Table 3.10) suggests that absorption of fat from the gut was not greater than mammary gland and adipose lipoprotein lipase

activity. Higher A-V differences and extractions of TG and NEFA when cows consumed 2.5% supplemental fat indicates increased mammary gland uptake. The higher negative extraction of NEFA apparently represents increased capillary lipoprotein lipase activity with incomplete uptake of released fatty acids (West et al. 1972). True LCFA uptake was calculated by subtracting the esterified fatty acids in TG and NEFA in venous samples from the esterified fatty acids in TG and NEFA in arterial samples. After multiplying A-V differences by their respective blood flows, it was observed that cows fed the fat-supplemented diets had 33% higher LCFA uptake (539 versus 361 g/d). As uptake of LCFA increased, de novo synthesis of short chain and medium chain fatty acids decreased (see Table 3.14). Similar responses were reported previously (Cant et al. 1993b, Khorasani et al. 1991, Schauff et al. 1992, Steele 1984, Storry et al. 1973).

Because TG and NEFA fatty acids represent only ~5.5% of the total fatty acids in bovine plasma (Moore et al. 1969), our efforts to assess uptake of individual LCFA by the A-V technique were not successful. Total concentration of fatty acids in plasma was high relative to A-V difference and extraction (1.3%). Isolation of TG prior to fatty acid analysis by gas-liquid chromatography may have increased our ability to quantify individual LCFA uptake using the A-V difference technique. There was relatively close agreement between total TG (mg/L) in arterial plasma as determined by gas-liquid chromatography (when estimated as 5.5% of total LCFA in plasma) and the results from analyses with TG kits for CNTL (83.3, 75.7), FAT (87.12, 85.5), UIP (72.5, 73.8), and BOTH (82.3,85.6). Additionally, the tendency for fat-supplemented diets to increase 18:0 and 18:1 concentrations in plasma (mg/L) (Table 3.12) was reflected in the 18:0 and 18:1 concentrations in milk fat (Table 3.14).

Nutrient balances across the mammary gland (including amino acid data from Chapter 4) are given in Tables 3.16. The overall trend was

for nutrient uptake to equal nutrient output when cows were fed FAT and BOTH, but uptake was ~7% below output when cows consumed either CNTL or UIP. Considering potential errors in determining blood flow and nutrient concentrations in blood, uptake data observed for the current study and others (Bickerstaffe et al. 1974, Cant et al. 1993b) come remarkably close to accounting for nutrient output from the mammary gland. As shown previously (Cant et al. 1993b), dietary fat-supplemention resulted in milk fat with a higher concentration of dietary LCFA and a lower concentration of short and medium chain fatty acids derived from de novo synthesis, which spared glucose for lactose synthesis without significantly increasing $O_2$ demands and thereby increased the efficiency of milk production.

Similar to the nutrient balance noted above, carbon uptake equaled carbon output when cows were fed FAT and BOTH but was ~5% below expected when cows consumed either CNTL or UIP (Table 3.17). However, when carbon output was based on CHN analyses, uptake for all dietary groups was 100% of output or greater. This indicates that carbon output determined by chemical analyses and carbon percentages was overestimated to a small extent. Uptakes of carbon by mammary gland were not based on plasma CHN analyses, because data indicated that uptake was negligible or highly variable (Appendix Table A3). To our knowledge, we are the first to use CHN analyses in combination with the A-V difference technique to assess carbon (or nitrogen) uptake by the lactating mammary gland. However, the carbon concentration in arterial and venous plasma was very high relative to its A-V difference and extraction; thus, the procedure was not sensitive enough to determine carbon uptake.

## Conclusions

The observation that calculated carbon uptake was 95 to 101% of output when using estimated carbon content of nutrients, and 100 to 106% when using an elemental analyzer to determine actual carbon output in milk suggests that our experiment adequately accounted for nutrient balance across the mammary gland. Although uptake of glucose, β-hydroxybutyrate, lactate, pyruvate, acetate, and $O_2$ were not affected by dietary treatment, uptake of long-chain fatty acids increased 33%. The greater rate of dietary LCFA uptake decreased de novo fatty acid synthesis and resulted in milk fat with a higher percentage of long-chain fatty acids derived from the diet. Overall, results indicated that dietary fat supplementation at two levels of UIP can increase milk production by altering mammary lipid metabolism and improving efficiency of milk synthesis. However, the fat-induced milk protein depression observed in this study could not be explained by reduced mammary blood flow, non-nitrogenous nutrient uptake, or overall balance (including AA) of nutrients across the gland. Therefore, the following chapter will address AA utilization in the mammary gland and its role in milk protein production.

**TABLE 3.1.** Dietary ingredients and chemical composition.

| | Treatments[1] | | | |
|---|---|---|---|---|
| Item | CNTL | FAT | UIP | BOTH |
| **Dietary ingredients** | | % of DM | | |
| Alfalfa silage | 27.0 | 27.0 | 27.0 | 27.0 |
| Corn silage | 26.0 | 26.0 | 26.0 | 26.0 |
| Corn grain | 35.5 | 32.5 | 36.0 | 33.0 |
| Soybean meal | 9.5 | 9.5 | 1.5 | 1.5 |
| Dried brewer's grains | -- | -- | 5.0 | 5.0 |
| Corn gluten meal | -- | .5 | 2.5 | 3.0 |
| Blood meal | -- | -- | 1.0 | 1.0 |
| Soy hulls | 1.0 | 1.0 | -- | -- |
| Fat | -- | 2.5 | -- | 2.5 |
| Mineral-vitamin mix[2] | .4 | .4 | .4 | .4 |
| Monocalcium phosphate | .2 | .2 | .2 | .2 |
| Magnesium oxide | .1 | .1 | .1 | .1 |
| Sodium bicarbonate | .2 | .2 | .2 | .2 |
| Trace mineralized salt | .1 | .1 | .1 | .1 |
| **Chemical composition** | | | | |
| DM, % | 59.5 | 59.5 | 59.5 | 59.5 |
| CP, % of DM | 15.8 | 15.8 | 15.9 | 15.9 |
| UIP, % of CP[3] | 33.0 | 33.0 | 41.0 | 41.0 |
| ADF, % of DM | 19.5 | 19.5 | 19.5 | 19.5 |
| Crude fat, % of DM | 3.5 | 6.0 | 3.5 | 6.0 |
| $NE_L$,[3] Mcal/kg | 1.65 | 1.74 | 1.65 | 1.74 |

[1]CNTL = 0% added fat and 33% UIP; FAT = 2.5% added fat and 33% UIP; UIP = 0% added fat and 41% UIP; BOTH = 2.5% added fat and 41% UIP.

[2]Contained: 21.5% Ca; 10.0% P; 12% NaCl; 4% Mg; .3% K; .2% S; .001% Co; .02% Cu; .006% I; .75% Fe; .1% Mn; .003% Se; .13% Zn; .1% F, and 162,000 IU of vitamin A/kg, 54,000 IU of vitamin D/kg, and 54 IU of vitamin E/kg.

[3]Undegradable intake protein (UIP) and $NE_L$ were calculated from NRC (1989).

**TABLE 3.2.** Chemical score of protein sources relative to milk protein.[1,2]

| Protein source | Arg | His | Ile | Leu | Lys | Met | Phe | Thr | Tyr | Val |
|---|---|---|---|---|---|---|---|---|---|---|
| Soybean meal | 89 | 89 | 55 | 56 | 70 | 56 | 100 | 74 | 75 | 60 |
| Brewer's grains | 53 | 56 | 74 | 83 | 34 | 78 | 100 | 65 | 87 | 65 |
| Corn gluten meal | 36 | 67 | 40 | 100 | 18 | 100 | 100 | 60 | 30 | 48 |
| Blood meal | 33 | 100 | 10 | 93 | 91 | 45 | 100 | 86 | 76 | 70 |
| Blend[3] | 52 | 68 | 56 | 84 | 41 | 77 | 100 | 67 | 70 | 61 |
| Rumen microbes | 79 | 90 | 61 | 54 | 100 | 97 | 97 | 100 | 99 | 66 |

[1]Adapted from Chandler (1989).
[2]Individual essential AA of milk protein were used as the reference (100).
[3]Blend score of protein sources in diets UIP and BOTH.

**TABLE 3.3.** Daily dry matter intake, milk composition and output, and blood flow.

| | Treatments | | | | | Effect ( $P$ = ) [2] | | |
|---|---|---|---|---|---|---|---|---|
| | CNTL[1] | FAT | UIP | BOTH | SEM | FAT | UIP | INTER |
| | ----------- kg/d ----------- | | | | | | | |
| DM intake | 25.5 | 26.6 | 26.2 | 25.6 | .3 | .91 | .81 | .05 |
| Milk production | 32.8 | 35.5 | 35.2 | 36.6 | .4 | .03 | .04 | .28 |
| Fat | 1.37 | 1.55 | 1.46 | 1.54 | .06 | .10 | .59 | .44 |
| Protein | 1.07 | 1.14 | 1.16 | 1.17 | .03 | .21 | .14 | .25 |
| Lactose | 1.55 | 1.76 | 1.81 | 1.73 | .07 | .41 | .19 | .14 |
| (Total) | 3.99 | 4.45 | 4.43 | 4.44 | -- | -- | -- | -- |
| | -------- g/100 g milk -------- | | | | | | | |
| Fat | 4.22 | 4.39 | 4.14 | 4.20 | .17 | .57 | .50 | .78 |
| Protein | 3.29 | 3.25 | 3.30 | 3.19 | .02 | .02 | .24 | .13 |
| Lactose | 4.73 | 4.96 | 5.15 | 4.75 | .14 | .58 | .53 | .12 |
| Blood flow | | | | | | | | |
| L/min | 14.82 | 16.98 | 17.70 | 16.61 | .80 | .49 | .17 | .10 |
| L/kg of milk | 658 | 687 | 731 | 650 | 32 | .49 | .62 | .20 |

[1]CNTL= 0% added fat and 33% UIP; FAT= 2.5% added fat and 33% UIP; UIP= 0% added fat and 41% UIP; BOTH= 2.5% added fat and 41% UIP.

[2]Probability of effects due to 0% versus 2.5% FAT, 33% versus 41% UIP, and the interaction (INTER) between FAT and UIP.

**TABLE 3.4.** Arterial concentrations, arteriovenous differences (A-V), and mammary gland extractions of glucose, β-hydroxybutyrate, acetoacetate, lactate, and pyruvate.[1]

| | Treatments | | | | | Effect ( *P* = ) | | |
|---|---|---|---|---|---|---|---|---|
| Item | CNTL | FAT | UIP | BOTH | SEM | FAT | UIP | INTER |
| **Arterial** | ------------ mM ------------ | | | | | | | |
| Glucose | 3.18 | 3.28 | 3.24 | 3.10 | .03 | .65 | .23 | .07 |
| β-OH Butyrate | 1.09 | .99 | 1.17 | 1.08 | .07 | .72 | .72 | .99 |
| Acetoacetate | .17 | .16 | .17 | .17 | .01 | .41 | .77 | .42 |
| Lactate | .51 | .51 | .47 | .45 | .02 | .53 | .08 | .57 |
| Pyruvate | .11 | .12 | .13 | .11 | .01 | .15 | .55 | .01 |
| **A-V** | ------------ mM ------------ | | | | | | | |
| Glucose | .718 | .764 | .766 | .754 | .032 | .85 | .83 | .74 |
| β-OH Butyrate | .467 | .409 | .413 | .404 | .030 | .75 | .78 | .81 |
| Acetoacetate† | .004 | .002 | .006 | .001 | .005 | .62 | .95 | .78 |
| Lactate | .074 | .050 | .051 | .045 | .013 | .58 | .59 | .73 |
| Pyruvate | .016 | .019 | .024 | .012 | .004 | .47 | .96 | .30 |
| **Extraction** | ------------ % ------------ | | | | | | | |
| Glucose | 22.5 | 23.1 | 23.5 | 24.5 | .9 | .76 | .66 | .95 |
| β-OH Butyrate | 44.4 | 39.1 | 34.8 | 37.5 | 1.3 | .55 | .06 | .14 |
| Acetoacetate† | 2.2 | .5 | 3.3 | -1.6 | 3.4 | .60 | .94 | .80 |
| Lactate | 14.5 | 7.2 | 7.8 | 7.9 | 2.9 | .59 | .65 | .58 |
| Pyruvate | 14.9 | 13.2 | 15.7 | 12.1 | 3.3 | .66 | .99 | .88 |

[1]Dietary treatments and effects of treatments are defined in Table 3.3.
†Least squares means in a row did not differ from zero ($P > .05$).

**TABLE 3.5.** Mammary gland uptakes of glucose, β-hydroxybutyrate, acetoacetate, lactate, and pyruvate.[1]

| | Treatments | | | | | Effect ( $P=$ ) | | |
|---|---|---|---|---|---|---|---|---|
| Item | CNTL | FAT | UIP | BOTH | SEM | FAT | UIP | INTER |
| | ----------- mol/d ----------- | | | | | | | |
| Glucose | 10.92 | 13.33 | 13.53 | 13.04 | .60 | .41 | .33 | .24 |
| β-OH Butyrate | 10.15 | 9.64 | 11.09 | 9.11 | .89 | .68 | .95 | .81 |
| Acetoacetate† | .06 | .10 | .08 | -.08 | .13 | .80 | .71 | .66 |
| Lactate | 1.50 | 1.38 | 1.37 | 1.10 | .36 | .77 | .75 | .91 |
| Pyruvate | .31 | .51 | .65 | .26 | .11 | .52 | .78 | .11 |
| | ------------ g/d ------------ | | | | | | | |
| Glucose | 1967 | 2402 | 2438 | 2350 | 97 | .41 | .33 | .24 |
| β-OH Butyrate | 1046 | 993 | 1142 | 938 | 91 | .68 | .95 | .81 |
| Acetoacetate† | 6 | 11 | 8 | -8 | 13 | .80 | .71 | .66 |
| Lactate | 137 | 125 | 124 | 100 | 32 | .77 | .75 | .91 |
| Pyruvate | 28 | 45 | 57 | 23 | 9 | .52 | .78 | .11 |

[1]Dietary treatments and effects of treatments are defined in Table 3.3.
†Least squares means in a row did not differ from zero ($P > .05$).

**TABLE 3.6.** Arterial concentrations, arteriovenous differences (A-V), and mammary gland extractions of volatile fatty acids (VFA).[1]

| | Treatments | | | | | Effect ( $P$ = ) | | |
|---|---|---|---|---|---|---|---|---|
| VFA[2] | CNTL | FAT | UIP | BOTH | SEM | FAT | UIP | INTER |
| **Arterial** | ------------ mM ------------ | | | | | | | |
| Acetate | 1.894 | 1.790 | 1.402 | 1.522 | .088 | .98 | .22 | .68 |
| Propionate | .163 | .181 | .174 | .153 | .004 | .71 | .19 | .03 |
| Butyrate | .132 | .122 | .124 | .125 | .003 | .15 | .42 | .10 |
| Isovalerate | .030 | .022 | .026 | .027 | .002 | .41 | .96 | .31 |
| **A-V** | ------------ mM ------------ | | | | | | | |
| Acetate | 1.244 | 1.145 | .847 | .910 | .083 | .95 | .29 | .77 |
| Propionate | .020 | .028 | .026 | .012 | .003 | .42 | .18 | .13 |
| Butyrate | .019 | .011 | .011 | .011 | .003 | .33 | .41 | .33 |
| Isovalerate† | .002 | .001 | .002 | .003 | .001 | .95 | .74 | .40 |
| **Extraction** | ------------ % ------------ | | | | | | | |
| Acetate | 65.6 | 63.2 | 58.3 | 59.8 | 2.6 | .95 | .50 | .80 |
| Propionate | 12.5 | 15.3 | 14.3 | 8.3 | 1.7 | .49 | .29 | .12 |
| Butyrate | 14.3 | 8.7 | 8.8 | 8.9 | 1.7 | .38 | .40 | .37 |
| Isovalerate† | 6.2 | 4.2 | 5.4 | 8.0 | 3.2 | .94 | .68 | .52 |

[1]Dietary treatments and effects of treatments are defined in Table 3.3.
[2]Valerate was not detected.
†Least squares means in a row did not differ from zero ($P > .05$).

**TABLE 3.7.** Mammary gland uptakes of volatile fatty acids (VFA).[1]

| | Treatments | | | | | Effect ( $P$ = ) | | |
|---|---|---|---|---|---|---|---|---|
| VFA[2] | CNTL | FAT | UIP | BOTH | SEM | FAT | UIP | INTER |
| | ----------- mol/d ----------- | | | | | | | |
| Acetate | 18.85 | 19.85 | 15.60 | 16.32 | 1.71 | .84 | .45 | .98 |
| Propionate | .31 | .51 | .49 | .21 | .05 | .30 | .13 | .01 |
| Butyrate | .29 | .18 | .22 | .20 | .04 | .38 | .72 | .55 |
| Isovalerate† | .03 | .02 | .03 | .04 | .02 | .79 | .69 | .62 |
| | ------------ g/d ------------ | | | | | | | |
| Acetate | 1132 | 1192 | 937 | 980 | 103 | .84 | .45 | .98 |
| Propionate | 23 | 38 | 36 | 16 | 4 | .30 | .13 | .01 |
| Butyrate | 25 | 16 | 19 | 17 | 4 | .38 | .72 | .55 |
| Isovalerate† | 3 | 2 | 3 | 4 | 2 | .79 | .69 | .62 |

[1]Dietary treatments and effects of treatments are defined in Table 3.3.
[2]Valerate was not detected.
†Least squares means in a row did not differ from zero ($P$ > .05).

**TABLE 3.8.** Arterial concentrations, arteriovenous differences (A-V), and mammary gland extractions of oxygen ($O_2$), carbon dioxide ($CO_2$), bicarbonate ($HCO_3^-$), sodium ($Na^+$), and potassium ($K^+$).[1]

| Item[2] | Treatments | | | | | Effect ( $P$ = ) | | |
|---|---|---|---|---|---|---|---|---|
| | CNTL | FAT | UIP | BOTH | SEM | FAT | UIP | INTER |
| **Arterial** | ------------ mM ------------ | | | | | | | |
| $O_2$ | 5.6 | 5.6 | 5.6 | 5.5 | .1 | .43 | .81 | .74 |
| $CO_2$ | 25.9 | 24.8 | 24.7 | 25.0 | .3 | .59 | .43 | .32 |
| $HCO_3^-$ | 24.8 | 23.8 | 23.6 | 24.0 | .3 | .61 | .40 | .29 |
| $Na^+$ | 141.42 | 141.70 | 142.17 | 141.25 | .22 | .29 | .61 | .16 |
| $K^+$ | 4.09 | 4.17 | 4.01 | 4.01 | .04 | .25 | .03 | .24 |
| **A-V** | ------------ mM ------------ | | | | | | | |
| $O_2$ | 1.5 | 1.5 | 1.5 | 1.4 | .1 | .61 | .91 | .69 |
| $CO_2$ | -2.5 | -2.0 | -2.2 | -1.9 | .2 | .05 | .25 | .80 |
| $HCO_3^-$ | -2.3 | -1.9 | -2.2 | -1.8 | .2 | .03 | .24 | .88 |
| $Na^+$ | -.31 | -.31 | -.28 | -.58 | .09 | .50 | .58 | .50 |
| $K^+$ | .06 | .09 | .07 | .06 | .01 | .29 | .28 | .08 |
| **Extraction** | ------------ % ------------ | | | | | | | |
| $O_2$ | 26.6 | 26.9 | 27.1 | 26.2 | .9 | .82 | .97 | .63 |
| $CO_2$ | -9.7 | -8.2 | -9.3 | -7.5 | .8 | .08 | .43 | .90 |
| $HCO_3^-$ | -9.6 | -8.1 | -9.3 | -7.4 | .7 | .06 | .42 | .78 |
| $Na^+$ | -.2 | -.2 | -.2 | -.4 | .1 | .50 | .58 | .50 |
| $K^+$ | 1.5 | 2.3 | 1.8 | 1.5 | .2 | .41 | .42 | .10 |

[1]Dietary treatments and effects of treatments are defined in Table 3.3.
[2]Least squares means differed from zero ($P < .05$).

**TABLE 3.9.** Mammary gland uptakes of oxygen ($O_2$), carbon dioxide ($CO_2$), bicarbonate ($HCO_3^-$), sodium ($Na^+$), and potassium ($K^+$).[1]

| Item[2] | Treatments | | | | | Effect ( *P* = ) | | |
|---|---|---|---|---|---|---|---|---|
| | CNTL | FAT | UIP | BOTH | SEM | FAT | UIP | INTER |
| | ---------- mol/d ---------- | | | | | | | |
| $O_2$ | 32.1 | 35.9 | 39.5 | 34.1 | 1.5 | .81 | .43 | .23 |
| $CO_2$ | -53.4 | -49.2 | -57.0 | -44.5 | 4.8 | .02 | .77 | .10 |
| $HCO_3^-$ | -50.4 | -46.3 | -54.7 | -41.9 | 4.4 | .01 | .99 | .14 |
| $Na^+$ | -7.1 | -7.0 | -6.1 | -13.8 | 2.2 | .44 | .55 | .41 |
| $K^+$ | 1.2 | 2.3 | 1.7 | 1.4 | .2 | .10 | .26 | .02 |
| | ------------ g/d ------------ | | | | | | | |
| $O_2$ | 1027 | 1149 | 1264 | 1090 | 47 | .81 | .43 | .23 |
| $CO_2$ | -2353 | -2166 | -2510 | -1960 | 210 | .02 | .77 | .09 |
| $HCO_3^-$ | -3076 | -2827 | -3340 | -2559 | 270 | .01 | .99 | .14 |
| $Na^+$ | -165 | -161 | -139 | -318 | 51 | .44 | .55 | .41 |
| $K^+$ | 48 | 89 | 67 | 53 | 8 | .10 | .26 | .02 |

[1]Dietary treatments and effects of treatments are defined in Table 3.3.
[2]Least squares means differed from zero ($P < .05$).

**TABLE 3.10.** Arterial concentrations, arteriovenous differences (A-V), and mammary gland extractions of triacylglycerols (TG), nonesterified fatty acids (NEFA), long-chain fatty acids (LCFA), and glycerol.[1]

| | Treatments | | | | | Effect ( $P$ = ) | | |
|---|---|---|---|---|---|---|---|---|
| Item | CNTL | FAT | UIP | BOTH | SEM | FAT | UIP | INTER |
| **Arterial** | ------------ uM ------------ | | | | | | | |
| TG | 66.6 | 78.7 | 63.8 | 77.7 | 3.3 | .35 | .41 | .48 |
| NEFA | 81.0 | 81.7 | 82.1 | 84.8 | 3.6 | .87 | .84 | .92 |
| LCFA | 141.2 | 151.8 | 138.9 | 153.7 | 5.0 | .38 | .66 | .53 |
| Glycerol | 10.8 | 11.1 | 10.2 | 10.2 | .3 | .91 | .44 | .87 |
| **A-V** | ------------ uM ------------ | | | | | | | |
| TG | 33.9 | 47.0 | 29.2 | 46.9 | 2.8 | .02 | .52 | .53 |
| NEFA | -11.9 | -21.9 | -6.3 | -16.7 | 3.3 | .04 | .17 | .96 |
| LCFA | 18.3 | 19.9 | 19.8 | 25.1 | 3.7 | .41 | .41 | .65 |
| Glycerol† | .5 | .3 | -.1 | -.2 | .2 | .69 | .10 | .83 |
| **Extraction** | ------------ % ------------ | | | | | | | |
| TG | 50.9 | 60.5 | 50.5 | 60.5 | 2.7 | .05 | .68 | .69 |
| NEFA | -17.4 | -26.0 | -8.4 | -21.8 | 4.0 | .04 | .13 | .51 |
| LCFA | 12.5 | 13.5 | 14.7 | 16.7 | 2.3 | .45 | .26 | .99 |
| Glycerol† | 2.7 | 2.6 | -2.8 | -2.4 | 2.3 | .97 | .51 | .93 |

[1]Dietary treatments and effects of treatments are defined in Table 3.3.
†Least squares means in a row did not differ from zero ($P$ > .05).

**TABLE 3.11.** Mammary gland uptakes of triacylglycerols (TG), nonesterified fatty acids (NEFA), long-chain fatty acids (LCFA), and glycerol.[1]

| Item | Treatments | | | | SEM | Effect ( *P* = ) | | |
|---|---|---|---|---|---|---|---|---|
| | CNTL | FAT | UIP | BOTH | | FAT | UIP | INTER |
| | ---------- mmol/d ---------- | | | | | | | |
| TG | 515 | 820 | 507 | 806 | 54 | .01 | .84 | .97 |
| NEFA | -179 | -389 | -112 | -334 | 61 | .06 | .44 | .94 |
| LCFA | 279 | 340 | 338 | 383 | 64 | .46 | .47 | .91 |
| | ------------ g/d ------------ | | | | | | | |
| TG | 456 | 726 | 448 | 713 | 48 | .01 | .84 | .97 |
| NEFA | -51 | -110 | -32 | -94 | 17 | .06 | .44 | .94 |
| LCFA | 355 | 536 | 367 | 541 | 40 | .02 | .83 | .93 |

[1]Dietary treatments and effects of treatments are defined in Table 3.3.

**TABLE 3.12.** Arterial concentrations of fatty acids (FA) in plasma.[1]

| FA | Treatments | | | | SEM | Effect ( *P* = ) | | |
|---|---|---|---|---|---|---|---|---|
| | CNTL | FAT | UIP | BOTH | | FAT | UIP | INTER |
| | ---------- mg/L ---------- | | | | | | | |
| 16:0 | 246 | 278 | 274 | 247 | 6 | .92 | .95 | .23 |
| 18:0 | 317 | 347 | 280 | 330 | 7 | .11 | .25 | .65 |
| 18:1 | 104 | 128 | 61 | 126 | 5 | .08 | .28 | .34 |
| 18:2(n-6) | 640 | 615 | 529 | 589 | 27 | .83 | .44 | .62 |
| 18:3(n-3) | 48 | 44 | 24 | 36 | 3 | .69 | .18 | .43 |
| 20:3(n-6) | 92 | 103 | 88 | 97 | 2 | .07 | .29 | .87 |
| 20:4(n-6) | 67 | 69 | 62 | 71 | 2 | .20 | .59 | .37 |
| Saturated FA | 563 | 625 | 554 | 577 | 8 | .08 | .18 | .31 |
| Unsaturated FA | 951 | 959 | 765 | 920 | 35 | .48 | .34 | .53 |
| Total FA | 1514 | 1584 | 1318 | 1496 | 41 | .36 | .31 | .68 |
| | ------ g/100 g fatty acids ----- | | | | | | | |
| 16:0 | 20.3 | 19.5 | 19.8 | 19.2 | .2 | .04 | .18 | .63 |
| 18:0 | 21.6 | 21.8 | 21.4 | 20.8 | .3 | .73 | .32 | .44 |
| 18:1 | 5.2 | 7.5 | 5.2 | 7.1 | .1 | .01 | .38 | .25 |
| 18:2(n-6) | 39.6 | 37.9 | 40.2 | 39.6 | .5 | .27 | .24 | .53 |
| 18:3(n-3) | 2.3 | 2.5 | 2.0 | 2.4 | .1 | .10 | .26 | .50 |
| 20:3(n-6) | 6.2 | 6.4 | 6.7 | 6.3 | .2 | .77 | .70 | .43 |
| 20:4(n-6) | 4.8 | 4.4 | 4.5 | 4.6 | .1 | .22 | .84 | .26 |

[1]Dietary treatments and effects of treatments are defined in Table 3.3.

**TABLE 3.13.** Mammary gland arteriovenous differences (A-V) and uptakes of fatty acids from plasma.[1]

| | Treatments | | | | | Effect ( *P* = ) | | |
|---|---|---|---|---|---|---|---|---|
| FA[2] | CNTL | FAT | UIP | BOTH | SEM | FAT | UIP | INTER |
| **A-V** | | mg/L | | | | | | |
| 16:0 | -2.6 | 1.2 | -.9 | -1.4 | 2.4 | .43 | .82 | .31 |
| 18:0 | -1.3 | -3.1 | 5.2 | -3.7 | 4.5 | .32 | .56 | .48 |
| 18:1 | -.1 | .5 | 1.6 | .6 | 2.8 | .96 | .85 | .87 |
| 18:2(n-6) | -19.4 | 6.8 | -22.7 | -7.5 | 13.6 | .05 | .25 | .44 |
| 18:3(n-3) | -1.8 | 1.2 | -1.1 | -1.8 | 1.4 | .39 | .40 | .20 |
| 20:3(n-6) | -.1 | 2.9 | -2.1 | .2 | 2.5 | .33 | .38 | .89 |
| 20:4(n-6) | .2 | .4 | -.6 | -.9 | 1.7 | .98 | .52 | .88 |
| Total FA | -25.1 | 9.8 | -20.6 | -14.6 | 20.7 | .29 | .58 | .44 |
| **Uptake** | | g/d | | | | | | |
| 16:0 | -36 | 20 | -10 | -38 | 43 | .61 | .54 | .18 |
| 18:0 | 10 | -87 | 134 | -107 | 79 | .16 | .57 | .49 |
| 18:1 | 10 | -15 | 36 | -2 | 51 | .75 | .84 | .95 |
| 18:2(n-6) | -344 | 154 | -465 | -170 | 211 | .05 | .12 | .40 |
| 18:3(n-3) | -30 | 26 | -20 | -33 | 22 | .33 | .28 | .16 |
| 20:3(n-6) | -1 | 44 | -25 | -7 | 43 | .53 | .46 | .80 |
| 20:4(n-6) | 8 | 2 | 4 | -21 | 27 | .58 | .64 | .74 |
| Total FA | -383 | 144 | -346 | -372 | 326 | .39 | .14 | .35 |

[1]Dietary treatments and effects of treatments are defined in Table 3.3.
[2]Least squares means in all rows did not differ from zero ( P > .05 ).

**TABLE 3.14.** Fatty acid (FA) content of milk fat.[1]

| | Treatments | | | | | Effect ( $P$ = ) | | |
|---|---|---|---|---|---|---|---|---|
| FA | CNTL | FAT | UIP | BOTH | SEM | FAT | UIP | INTER |
| | ------ g/100 g fatty acids ----- | | | | | | | |
| 4:0 | 4.74 | 4.86 | 4.71 | 5.14 | .03 | .01 | .03 | .11 |
| 6:0 | 4.44 | 4.36 | 4.62 | 4.46 | .03 | .03 | .02 | .27 |
| 8:0 | 2.66 | 2.60 | 2.86 | 2.65 | .03 | .02 | .03 | .08 |
| 10:0 | 4.99 | 4.59 | 5.45 | 4.67 | .06 | .01 | .03 | .05 |
| 12:0 | 5.11 | 4.40 | 5.43 | 4.59 | .11 | .01 | .11 | .60 |
| 14:0 | 12.53 | 11.92 | 13.05 | 11.82 | .25 | .04 | .46 | .31 |
| 14:1 | 1.29 | 1.16 | 1.29 | 1.25 | .02 | .03 | .13 | .13 |
| 15:0 | 1.25 | 1.21 | 1.29 | .94 | .05 | .03 | .10 | .14 |
| 15:1 | .11 | .17 | .28 | .18 | .02 | .44 | .03 | .05 |
| 16:0 | 32.37 | 29.91 | 31.28 | 28.79 | .54 | .02 | .14 | .98 |
| 16:1(n-7) | 1.34 | 1.24 | 1.19 | 1.21 | .03 | .31 | .08 | .19 |
| 17:0 | .49 | .51 | .46 | .45 | .01 | .47 | .04 | .28 |
| 17:1 | .03 | .07 | .08 | .02 | .01 | .32 | .92 | .12 |
| 18:0 | 10.34 | 11.70 | 9.89 | 11.93 | .12 | .01 | .43 | .07 |
| 18:1 | 16.47 | 19.41 | 15.82 | 19.99 | .64 | .01 | .97 | .42 |
| 18:2(n-6) | 1.62 | 1.67 | 2.06 | 1.74 | .06 | .12 | .03 | .15 |
| 18:3(n-3) | .23 | .22 | .23 | .21 | .01 | .14 | .63 | .42 |
| Sat. FA | 78.91 | 76.05 | 79.03 | 75.42 | .71 | .03 | .75 | .64 |
| Unsat. FA | 21.09 | 23.94 | 20.96 | 24.58 | .71 | .03 | .75 | .64 |
| Sat : Unsat | 3.74 | 3.18 | 3.80 | 3.08 | .13 | .01 | .90 | .61 |
| 18:0 to 18:3 | 28.65 | 33.00 | 28.00 | 33.86 | .68 | .01 | .89 | .35 |

[1]Dietary treatments and effects of treatments are defined in Table 3.3.

**TABLE 3.15.** Daily fatty acid (FA) output in milk.[1]

| | Treatments | | | | | Effect ( $P$ = ) | | |
|---|---|---|---|---|---|---|---|---|
| FA | CNTL | FAT | UIP | BOTH | SEM | FAT | UIP | INTER |
| | ----------- g/d ----------- | | | | | | | |
| 4:0 | 65 | 75 | 68 | 79 | 3 | .05 | .35 | .94 |
| 6:0 | 61 | 68 | 67 | 68 | 2 | .15 | .20 | .29 |
| 8:0 | 37 | 41 | 42 | 41 | 1 | .37 | .16 | .22 |
| 10:0 | 68 | 72 | 79 | 72 | 3 | .50 | .12 | .14 |
| 12:0 | 70 | 69 | 79 | 71 | 3 | .24 | .15 | .34 |
| 14:0 | 171 | 186 | 190 | 182 | 6 | .63 | .31 | .16 |
| 14:1 | 17 | 18 | 19 | 19 | 1 | .68 | .32 | .94 |
| 15:0 | 17 | 19 | 19 | 15 | 1 | .28 | .26 | .15 |
| 15:1 | 2 | 3 | 4 | 3 | 1 | .70 | .05 | .07 |
| 16:0 | 442 | 466 | 455 | 442 | 14 | .71 | .72 | .28 |
| 16:1(n-7) | 18 | 19 | 17 | 19 | 1 | .34 | .51 | .99 |
| 17:0 | 7 | 8 | 7 | 7 | 1 | .12 | .24 | .21 |
| 17:1 | 1 | 1 | 1 | 1 | 1 | .45 | .97 | .55 |
| 18:0 | 142 | 181 | 143 | 184 | 6 | .01 | .77 | .97 |
| 18:1 | 226 | 301 | 231 | 308 | 19 | .03 | .77 | .98 |
| 18:2(n-6) | 22 | 26 | 30 | 27 | 2 | .88 | .11 | .17 |
| 18:3(n-3) | 3 | 2 | 4 | 2 | 2 | .28 | .53 | .24 |
| Total | 1369 | 1555 | 1455 | 1540 | 57 | .09 | .55 | .52 |
| Sat. FA | 1080 | 1183 | 1149 | 1160 | 36 | .22 | .58 | .30 |
| Unsat. FA | 289 | 372 | 306 | 380 | 23 | .05 | .64 | .85 |
| 18:0 to 18:3 | 393 | 512 | 408 | 522 | 27 | .03 | .69 | .93 |

[1]Dietary treatments and effects of treatments are defined in Table 3.3.

**TABLE 3.16**. Nutrient balances across the mammary gland.[1]

| Item[2] | Treatments | | | |
|---|---|---|---|---|
| | CNTL | FAT | UIP | BOTH |
| **Uptake, g/d** | | | | |
| Glucose | 1967 | 2402 | 2438 | 2350 |
| β-Hydroxybutyrate | 1046 | 993 | 1142 | 938 |
| Lactate | 137 | 125 | 124 | 100 |
| Pyruvate | 28 | 45 | 57 | 23 |
| Acetate | 1132 | 1192 | 937 | 980 |
| Propionate | 28 | 45 | 57 | 23 |
| Butyrate | 25 | 16 | 19 | 17 |
| LCFA[3] | 355 | 536 | 367 | 541 |
| AA | 1020 | 1153 | 1103 | 1230 |
| Blood proteins | 38 | 40 | 41 | 41 |
| Total | 5771 | 6540 | 6264 | 6236 |
| **Output, g/d** | | | | |
| Lactose | 1550 | 1760 | 1810 | 1730 |
| FA | 1365 | 1552 | 1450 | 1537 |
| AA | 950 | 1076 | 1024 | 1055 |
| $CO_2$ | 2353 | 2166 | 2510 | 1960 |
| Total | 6218 | 6554 | 6794 | 6282 |
| **Uptake/output X 100, %** | 93 | 100 | 92 | 99 |

[1]Dietary treatments and effects of treatments are defined in Table 3.3.
[2]Determined from chemical analyses of output and carbon percentage composition.
[3]Uptake determined by TG and NEFA analyses (Table 3.11).

**TABLE 3.17.** Carbon balances across the mammary gland.[1]

| Item | Treatments | | | |
|---|---|---|---|---|
| | CNTL | FAT | UIP | BOTH |
| **C Uptake, g/d** | 2581 | 2966 | 2811 | 2852 |
| **C Output,[2] g/d** | | | | |
| Milk C | 2071 | 2354 | 2275 | 2325 |
| $CO_2$ C | 642 | 591 | 685 | 535 |
| Total C | 2713 | 2945 | 2960 | 2860 |
| **Uptake/output X 100, %** | 95 | 101 | 95 | 100 |
| **C Output,[3] g/d** | | | | |
| Milk C | 1942 | 2227 | 2099 | 2160 |
| $CO_2$ C | 642 | 591 | 685 | 535 |
| Total C | 2584 | 2818 | 2784 | 2695 |
| **Uptake/output X 100, %** | 100 | 105 | 101 | 106 |

[1]Dietary treatments and effects of treatments are defined in Table 3.3.
[2]Determined from chemical analyses of output and carbon percentage composition.
[3]Determined from CHN analyses (PE 2400 Series 11 CHNS/O Analyzer, Perkin Elmer, Norwalk, CT).

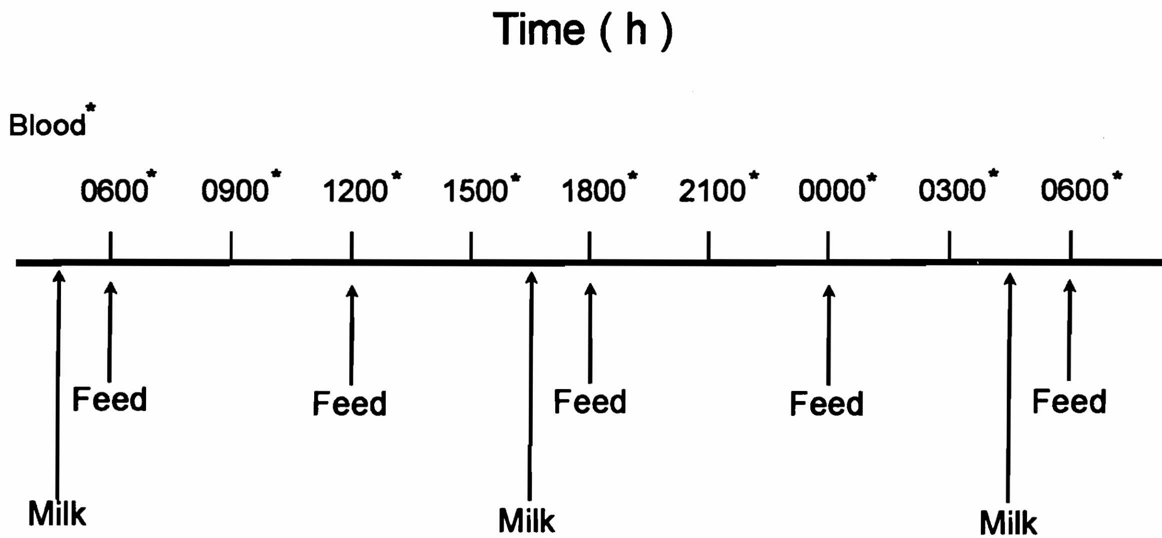



Figure 3.1. Schedule for blood sampling, feeding, and milking during the last 24 h of each 21-d period.

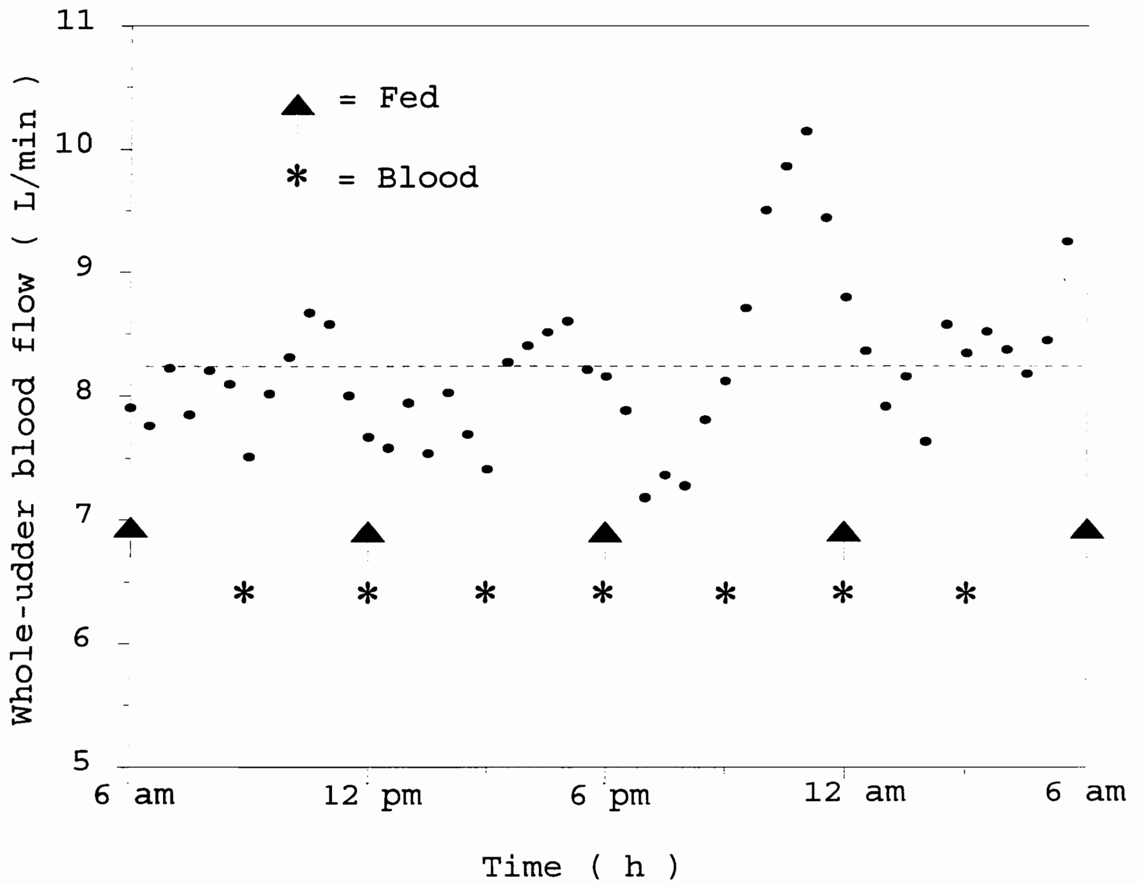



Figure 3.2 Average 30 min whole-udder blood flow for 24-h sampling periods across cows, periods, and treatments as determined by ultrasonic blood flow probes.

# CHAPTER 4

## AMINO ACID AVAILABILITY AND UTILIZATION FOR MILK PROTEIN SYNTHESIS BY DAIRY COWS FED SUPPLEMENTAL FAT AT TWO LEVELS OF UNDEGRADABLE INTAKE PROTEIN


### (ABSTRACT)

The objective of this study was to determine whether dietary level of undegradable intake protein (UIP) could increase efficiency of AA utilization for milk production and alleviate milk protein depression caused by supplemental fat. Three lactating Holstein cows (60, 68, and 74 d postpartum) were used in an incomplete 4 x 4 Latin squre design (2 x 2 factorial) and fed diets (15.9% CP and 19.5% ADF) with 0% or 2.5% partially hydrogenated tallow and 33% or 41% UIP. A 5:2.5:1 mixture of dried brewer's grains, corn gluten meal, and blood meal was substituted for soybean meal to raise dietary UIP from 33% to 41% UIP. Mammary blood flow was estimated using the Fick principle at 6-h intervals for 24 h. Concentration of nutrients in arterial (carotid) and venous (abdominal vein) blood and corresponding blood flow were used to calculate nutrient uptake by mammary glands. Diets containing 41% UIP increased arterial essential and total AA concentrations in plasma and whole blood; whereas, arterial plasma but not whole blood total AA concentrations were decreased by dietary fat supplementation. Mammary arteriovenous differences, extractions, and uptakes of all whole blood or most plasma derived AA were not significantly affected by dietary treatments. Red blood cell contribution to AA uptake (g/d) was negligible. Across treatments, peptide AA accounted for ~10% of AA in arterial whole blood but no net uptake of peptide AA by the lactating gland was detected. Depression of milk protein percentages when cows consumed the fat-supplemented diets was not alleviated by raising the circulating levels of arterial essential and total AA through higher dietary UIP.

## INTRODUCTION

A current practice when formulating diets for high producing dairy cows is to add supplemental fat, increase the proportion of undegradable intake protein (UIP), or a combination of both. When dietary UIP is elevated at the expense of microbial protein production, the quality and quantity of metabolizable AA ultimately reaching the mammary gland may not change (Clark et al. 1992). Therefore, it is not uncommon that adoption of these practices does not improve milk production (Holter et al. 1993, Schwab 1989, Winsryg et al. 1991) but also raises questions about the suitability of increasing dietary UIP to alleviate supplemental fat-induced milk protein depression.

Cant et al. (1993a), Cant et al. (1993b), and DePeters and Cant (1992) have reported that dietary fat-induced milk protein depression may be due to changes in AA utilization within the gland. Specifically, Cant et al. (1993a) used 4% yellow grease and ruminal and abomasal sodium caseinate infusions (5.04 kg/d) to show that dietary fat-induced milk protein depression was caused by increased efficiency of milk production, decreased arterial EAA concentrations, and increased extraction of AA from blood in conjunction with decreased mammary blood flow. Additionally, the plasma peptide AA pool in nonlactating ruminants has been shown to account for 50 to 80% of the available AA pool (DiRienzo 1990, Koeln et al. 1993, Seal and Parker 1991). Webb et al. (1992 and 1993) have suggested that although free AA in plasma derived from undegraded dietary protein, microbial protein, and endogenous proteins have been considered the primary sources of AA, studies that do not account for peptide AA may underestimate total AA uptake by the lactating gland.

Therefore, the objective of this experiment was to quantify uptake of whole blood free AA, plasma free AA, and whole blood peptide AA by lactating mammary glands and AA output in milk to determine if dietary level of UIP could increase the efficiency of AA utilization for milk production and alleviate milk protein depression caused by supplemental fat.

## Materials and Methods

The experimental protocol was described previously (Chapter 3, Page 29). Three lactating Holstein cows (60, 68, and 74 d postpartum) were used in an incomplete 4 x 4 Latin square design (2 x 2 factorial) because one cow was dropped from the study due to chronic mastitis. Cows were fed diets containing 0% added fat and 33% UIP **(CNTL)**, 2.5% added fat (Alifet®; Alifet USA Inc., Cincinnati, OH) and 33% UIP **(FAT)**, 0% added fat and 41% UIP **(UIP)**, or 2.5% added fat and 41% UIP **(BOTH)**. Dietary ingredients and chemical composition are given in Table 3.1 (page 48). Diets were fed as a total mixed diet and contained 15.9% CP and 19.5% ADF. A 5:1 ratio of supplemental fat and corn gluten meal replaced corn grain in FAT and BOTH to raise dietary crude fat from 3.5% to 6.0% while maintaining crude protein content. A 5:2.5:1 mixture of dried brewer's grains, corn gluten meal, and blood meal was substituted for soybean meal in UIP and BOTH to raise dietary UIP from 33% to 41% UIP. This combination of protein sources was chosen to provide a reduced rate of protein degradation in the rumen and an EAA chemical score relative to milk protein (Chapter 3 Table 3.2) that would compliment the chemical score of rumen microorganisms.

Each period was 21 d with the last day used for 24 h of sample collection. Daily intakes and milk production were used to calculate a weekly average but only d 17 through d 21 and d 21 of each period were used to ascertain effects of dietary treatments on DM intake and milk production, respectively.

On d 16 of each period, a progesterone releasing intravaginal device **(PRID)** (PRID®; Sanofi, Paris, France) was inserted into the vagina of each cow. These devices were used as synchronizing agents to prevent estrus and the unwanted physiological effects of estrus on DM intake and milk production during the 24 h sampling period.

Catheters were implanted on d 17 of each period in the right carotid artery and right superficial epigastric vein to determine nutrient exchange across the mammary gland. Preceding catheterizations, cows were given 500 *ul* of Butorphanol (10 mg/mL) as an analgesic. The carotid artery catheter was Tygon® (.02 mm i.d., .06 mm o.d., Fisher Scientific Co., Pittsburgh, PA) inserted through a Centrasil® guide catheter (Baxter, Deerfield, IL) temporarily inserted into in the carotid artery. The epigastric vein catheter was Tygon® (.02 mm i.d., .06 mm o.d., Fisher Scientific Co., Pittsburgh, PA) inserted into a 15 cm Teflon catheter (B-D I.V.™, Becton-Dickinson, Rutherford, NJ). Cows were given 20 mL of Penicillin G Potassium (500,000 units/mL) and moved to elevated stalls in a ventilated room for the remainder of the period. Catheters were flushed at 4-h intervals with 10 mL of .9% saline followed with 5 ml of heparinized saline (100 units/mL).

The last 24 h of each period were used for milk and blood collection to calculate arterial concentrations, arteriovenous **(A-V)** differences, extractions, and net uptakes of AA by the mammary gland. After the first milking at 0530 h, arterial and venous samples were collected simultaneously at 6-h intervals begining at 0900 h for the next 24 h. Blood samples were immediately placed on ice, packed cell volume determined, and plasma harvested by centrifugation at 2500 x *g* prior to storage at -20°C. Following the last collection of milk and blood, catheters and PRID were removed, and cows were returned to tie stalls in the Dairy Nutrition Research barn.

Plasma obtained at 0900, 1500, 2100, and 0300 h was prepared (in triplicate) for urea (Weatherburn 1967). Plasma and whole blood obtained at 0900, 1500, 2100, and 0300 h were prepared (in triplicate) for AA analyses by ultrafiltration. A 1:1 mixture of plasma and internal standard (.2 mM norleucine in .1N HCl) was placed into Ultrafree®-CL Filters (Millipore, Bedford, MA) and filtered at 4,500 x *g*

for 30 min. Similarly, a 1:2 mixture of whole blood and internal standard (.15 mM norleucine in 25% thiodiglycol and .1N HCl) was placed into Ultrafree®-CL Filters and filtered at 4,500 x g for 30 min. The filters excluded protein and peptides with a molecular weight greater than 10,000 da. Filtrates were purged with N prior to storage at -20°C.

Prior to AA analyses, duplicate aliquots of plasma and whole blood filtrates were hydrolyzed as previously described (Cohen et al. 1989). Concentrations of AA in hydrolyzed (total AA) and non-hydrolyzed filtrates (free AA) were derivitized (Bidlingmeyer et al. 1984) and quantified on a PICO-TAG Amino Acid Analysis System (Waters Div. of Millipore, Millipore Corp. Milford, MA.). Peptide AA were calculated as the difference between total AA and free AA. Mammary uptake of free AA and peptide AA was calculated using A-V differences and blood flow calculated with the Fick equation. A description of the Fick equation was presented previously (Chapter 3, Page 35). Casein AA were determined (in duplicate) by weighing 10 mg of casein into 20 mL glass ampules and then adding 15 mL 6N HCl. The ampules were sealed and then autoclaved for 6 h at 270°F. The derivitization procedure was the same as above (Bidlingmeyer et al. 1984) with minor modifications. Nitrogen content of plasma and milk fractions were determined by CHN analyses (PE 2400 Series 11 CHNS/O Analyzer, Perkin Elmer, Norwalk, CT).

Data were analyzed by ANOVA for a randomized incomplete block design using the general linear models procedure of PC-SAS (1990) including effects of treatments (df = 3), periods (df = 3), and cows (df = 2) with repeated measurements on time (df = 3, or 1). Effects of dietary treatments on mammary blood flow, arterial AA concentrations, A-V differences, extractions, and AA uptake were based on 4 estimations (6 h intervals); whereas, milk N fractions and AA output were based on 2

estimations (12 h intervals). All results are shown as least squares means and pooled SEM. Orthogonal contrasts for treatment means included effects due to 0% versus 2.5% FAT, 33% versus 41% UIP, and the interaction between FAT and UIP.

**Results**

Carotid plasma and whole blood free AA and urea concentrations are displayed in Table 4.1. Increasing dietary UIP from 33% to 41% increased concentrations of His, Leu, Phe, Val, Pro, Tyr, EAA, Total AA, plasma Cit, and whole blood Orn, but only tended to increase whole blood Cit, and plasma Orn. However, 41% UIP tended (P<.07) to decrease whole blood and Met and Thr concentrations, and also decreased Thr and tended (P<.07) to decrease Met concentration in plasma. Supplemental fat decreased Trp and Ser in plasma and whole blood. Plasma NEAA and total AA were decreased when cows consumed the fat-supplemented diets, but there was no diet effect when corresponding data for whole blood were compared.

Arteriovenous differences for plasma and whole blood free AA were not affected by dietary treatment (Table 4.2). Additionally, A-V differences for Gly, Hyl, Hyp, Cit, and urea were negligible (not significantly different from zero). Lack of an effect of diet on A-V differences was due to dietary alterations in arterial free AA concentrations (Table 4.1) being offset by changes in mammary gland extraction efficiencies. (Table 4.3). For example, arterial concentration of Leu was highest for UIP and BOTH, but extraction was only 32% to 35% compared to 43% when cows were fed diets with a lower UIP content.

Because A-V differences were not significantly affected by treatment, alterations in free AA uptake could only be accompished through changes in mammary blood flow. Mammary blood flow expressed as L/min and L/kg of milk, however, was not affected by treatment (Table 4.4). Consequently, uptakes expressed as mol/d and g/d of either plasma (Tables 4.5 and 4.6) or whole blood (Tables 4.7 and 4.8) free AA were numerically but not significantly different, with the exception of

greater Leu uptake from plasma when cows were fed 41% UIP. Molecular weights used to convert data from mol/d to g/d are presented in Appendix Table A4. Across dietary treatments, uptake of free AA averaged 3.21 g per 100 g of milk. This suggests milk protein depression observed in this experiment (Chapter 3, Table 3.3) was not due to changes in blood flow or total uptake of free AA (including Cit and Orn), but rather the metabolic fate of AA inside the mammary gland. Additionally, our data indicate the contribution of red blood cells to the transport of free AA into the mammary gland (g/d) was not consistent because, with few exceptions, the difference between whole blood and plasma AA uptake was not significantly different from zero (Table 4.9).

Estimates of peptide AA contribution to the available pool of AA in arterial whole blood are presented in Table 4.10. Negative least-squares means for His, Met, Asp+Asn, Glu+Gln, Ser, and Tyr represent degradation of these AA during hydrolysis of peptide AA. Prior to data summarization and statistical analyses, AA data had been corrected (up or down) to account for degradation or false enrichment of AA standards due to the hydrolysis (Appendix Table 5). Theoretically, the hydrolyzed fraction which contains peptide AA plus free AA cannot be lower than free AA estimations. Therefore, the above mentioned AA must be ignored when interpreting results. Except for Gly, dietary treatments had little effect on peptide AA contribution to the available pool of AA in arterial whole blood (Table 4.10). Across treatments, peptides (mol wt <10,000 Da) accounted for 9.8% of arterial AA with the majority (63.5%) apparently containing a Gly residue (see Figure 4.1, Page 100). However, we observed negligible uptake or release of peptide AA by the mammary gland (Table 4.11).

Supplemental fat decreased total N concentration of milk, increased casein N and urea N output (g/d), but did not affect total N output (Table 4.12). Casein N and whey N as a proportion of total N

were not influenced by diet. Urea N as a proportion of milk (g/100g), urea N output and urea N as a percent of total N output (g/d), were increased by supplemental dietary fat.

Output of EAA and total AA in milk (Table 4.13) were similar across treatments because when cows were fed supplemental fat they had greater milk production but lower milk protein percentage (Chapter 3, Table 3.3). Ratios of AA uptake to output presented in Table 4.14 were not affected by dietary treatment. In general, it appeared that AA uptakes from whole blood across treatments were adequate (ratio greater than 1.0) to account for their output in milk via protein synthesis. The overall efficiency of AA utilization, AA carbon utilization, and AA nitrogen utilization by the mammary gland was 88.3%, 86.5%, and 72.8%, respectively (Table 4.15).

## Discussion

Increasing dietary UIP from 33% to 41% increased arterial concentrations of EAA and total AA. These findings support earlier work indicating that dietary UIP (Seymour et al. 1990), sodium caseinate infusions into the duodenum (Cant et al. 1993a, Clark et al. 1977, Seymour et al. 1990), and dietary rumen-protected Met and Lys (Schwab et al. 1992, Seymour et al. 1990) can effectively influence circulating concentrations of individual AA. In the current study, increased concentrations of His, Leu, Phe, Val, Pro, and Tyr in arterial blood of cows fed UIP or BOTH reflected the limited ruminal degradation of AA in the blend of dried brewer's grains, corn gluten meal, and blood meal compared to soybean meal (Table 3.2). The chemical score of the protein blend, however, did not exceed that of soybean meal for Arg, His, Ile, Lys, Thr. Additionally, microbes supply 35 to 66% of the non-ammonia N passing to the small intestine (Clark et al. 1992), so microbial protein can make a major contribution to the pool of circulating AA. For example, the chemical scores for Met and Thr in the protein blend were 21 points higher and 7 points lower than soybean meal alone, respectively, yet the 41% UIP diets lowered both Met and Thr concentrations in blood compared to the 33% UIP diets.

Supplemental fat decreased plasma NEAA and total AA concentrations as previously reported (Cant et al. 1993a, Casper and Schingoethe 1989, Mohamed et al. 1988). However, whole blood AA concentrations (excluding Trp and Ser) were not decreased by supplemental fat in the current study or Cant et al. (1993a). It is generally accepted that whole blood AA provide the better indication of arterial AA concentrations because erythrocyte AA have been shown to contribute to amino acid transport in lactating cows (Hanigan et al. 1991) and growing calves (Koeln et al. 1993, McCormick and Webb 1982). However, because circulating AA

concentrations are a function of rate of entry of AA into circulation and also their removal by the mammary gland and other peripheral tissues, the concentration of an individual AA in blood may not be a reliable indicator of the amount of the AA absorbed from the intestine. As an example, Champredon et al. (1990) observed that when Met requirements for milk production become limiting in the lactating goat, an adaptative mechanism occurs such that AA utilization by extramammary tissues decreases.

Increased circulating plasma and whole blood Cit and Orn when cows consumed 2.5% supplemental fat and 41% UIP would suggest increased catabolism of AA via the urea cycle in the liver (Vicini et al. 1988). However, we observed only a numerical, but nonsignificant, increase in plasma urea when cows were fed supplemental fat and(or) UIP. The lack of a dietary fat effect was unexpected because supplemental fat induced a higher content of urea N in milk and higher daily urea N output (Table 4.12). Given the high solubility of urea and its potential to diffuse freely across mammary epithelial cells, a positive correlation between plasma urea N and milk urea N would be expected (Cant et al. 1993a, DePeters et al. 1992) unless the higher rate of urea observed in milk collected in the current study reflected urea synthesis within the gland that preceded a change in plasma urea.

Although the 41% UIP diet had a positive effect on arterial concentrations of many AA, A-V differences for plasma and whole blood free AA (Table 4.2) were not affected by dietary treatment. In agreement, Mepham and Linzell (1974) observed that despite daily arterial AA fluctuations in lactating goats, A-V differences for the EAA varied only slightly. However, Hanigan et al. (1992) found that as arterial AA concentrations increased, their A-V differences and extraction ratios increased linearly, whereas our data suggested that as arterial individual AA concentrations increased, the rate of extraction

by the gland decreased. Hanigan et al. (1992) used 21 cows with previous lactational performances ranging from 6,400 to 13,500 kg of milk/305 d and fed the same diets. Although the level of milk production and arterial AA concentrations of cows used in the current study were within the range reported by Hanigan et al. (1992), saturation of amino acid transporters may have occured when UIP was raised from 33 to 41 %.

Casper and Schingoethe (1989) observed that dietary fat decreased A-V differences for several EAA and total AA and proposed that dietary fat decreased AA extractions by the mammary gland due to low concentrations of bST in blood. Our data, like that of Cant et al. (1993a), could not substantiate this hypothesis because supplemental fat either increased or did not affect A-V differences and the percent extraction of plasma and whole blood AA (Table 4.3). We did observe that cows fed diets containing 41% UIP had lower extraction of Leu, Phe, Val, Ala, and Ser from plasma (but not whole blood), but it was strictly a manifestation of higher AA concentrations in combination with similar A-V differences.

Recall however, that nutrient supply to the mammary gland is not only a function of nutrient concentration in blood and transport efficiency, but also blood flow. Although we were able to manipulate arterial concentrations of AA by dietary treatments, changes in extraction by the mammary gland attenuated the responses so that A-V differences were not significantly affected by treatment. Additionally, we did not observe a significant treatment effect on calculated blood flow (Table 4.4). Therefore, plasma and whole blood AA uptakes also were not significantly affected by diet (excluding plasma Leu). However, despite a lack of significance, blood flow increased by nearly 15% when cows were fed FAT, UIP, and BOTH compared to CNTL. As a result, AA uptake increased in proportion to milk production and

accounted for carbon balance across the mammary gland, as noted in Chapter 3.

In the current study, estimated free AA uptakes contributed by red blood cells (whole blood minus plasma free AA uptake) were, with few exceptions, not statistically different from zero and not affected by dietary treatment (Table 4.9). Our findings suggest that the potential contribution of red blood cells to transport of AA into the gland (Hanigan et al. 1991, Koeln et al. 1993, McCormick and Webb 1982), may be obscured when the variability associated with calculating blood flow and A-V difference are considered.

To our knowledge, no one has previously tried to estimate whole blood peptide AA uptake by mammary gland of lactating dairy cows using A-V difference methodology. This is surprising because recent findings by Koeln et al. (1993) suggest that ~70% of the AA flux across the gastrointestinal tract of fed calves was in the form of peptide AA and that all but ~10% passed through the liver. Also, the plasma peptide AA pool in nonlactating ruminants was estimated to be from 50 to 80% of the available AA pool (DiRienzo 1990, Koeln et al. 1993, Seal and Parker 1991).

In the current study, whole blood peptide AA accounted for ~10% of the available AA pool. Even after correcting the AA values (Appendix Table 5) for degradation (Met, Hyl, Cys, Asp, and Tyr) or false enrichment (His and Ser) caused by the peptide hydrolysis procedure, the contribution of peptide AA residues to AA uptake generally was not different from zero (Met, Lys, and Cys) or was calculated to be less than zero (His, Asp + Asn, Glu + Gln, Ser, and Tyr) after accounting for uptake of free AA (Table 4.10). Only six EAA (Arg, Ile, Leu, Phe, Thr, and Val) and five NEAA (Ala, Gly, Hyl, Hyp, Pro) were quantitatively contributing to the peptide AA pool, and Gly residues accounted for nearly 64% of the total (Figure 4.1). Part of this total can be

explained by the fact that whole blood contains approximately 1 mM glutathione (Cant et al. 1993a), a tripeptide, which is ~24% Gly by weight. The N-terminal glycyl-derivatives appear to be more resistant to hydrolysis in vivo (Adibi et al. 1986), so the peptide fraction that we isolated from whole blood may have contained mostly peptides with at least one glycine residue. Cook (1973) had showed that uptake of glycine from glycylglycine was faster than from an equivalent amount of free glycine and that the processes of free AA and peptide AA transport involved different carriers.

Although the lactating mammary gland has the potential to utilize peptides as sources of AA (Backwell et al. 1993, Wang et al. 1993), we found negligible uptake or release of whole blood peptide AA by the lactating bovine mammary gland. The same effect was seen with plasma peptide AA (data not shown). Even if significant quantities of peptides are absorbed from the gastrointestinal tract of ruminants (Webb et al. 1992, and Webb et al. 1993), the level of circulating peptide AA in lactating dairy cows may reflect the hydrolysis of absorbed peptide AA into free AA prior to reaching the mammary gland, thereby indicating that free AA are the primary source of AA utilized by the mammary gland.

Mepham (1982) previously categorized free AA into three groups based on their uptake and utilization in the mammary gland. Group I contained AA that are stoichiometrically transferred from blood to milk, and include Met, Phe, Trp, and Try. Group II contained EAA taken up in excess of milk output and include Arg, His, Ile, Leu, Lys, Thr, and Val. Group III contained the remaining NEAA that are not taken from blood in sufficient quantities to account for their output in milk (for example Pro). In the current study, dietary treatments had no significant effect on the ratio of whole blood free AA uptakes to output in milk (Table 4.14). Based on whole blood estimations, it would appear that there were sufficient EAA (except for Thr) taken up to synthesize

the secreted proteins, with Thr uptake being marginal. Our data suggest that whole blood His and Thr would be categorized as Group I instead of Group II because, across treatments, His and Thr uptakes were similar to output. Similarly, Clark et al. (1974) found that when cows were fed formaldehyde treated soybean meal, plasma His, Phe, Met, Lys, and Thr could be identified as rate-limiting amino acids. However, DePeters and Cant (1992) recently summarized uptake to output ratios from six studies, and reported that only one (Peeters et al. 1979) observed low ratios of His and Thr.

Of particular interest is the high mammary gland uptake of arginine relative to its output in milk in the current study (Table 4.14) and others (Clark et al. 1974, Davis et al. 1978, Mepham and Linzell 1967). Mepham and Linzell (1967) showed that excess arginine taken up by goat mammary tissue is converted to proline by pathways involving the formation of ornithine and urea. These findings were later verified in the lactating dairy cow (Clark et al. 1975) and support our findings that proline output exceeded proline uptake (Tables 4.8 and 4.13).

Recently, Cant et al. (1993a) observed that diets supplemented with 4% yellow grease decreased milk protein percentage (3.32 versus 3.16) and tended to decrease mammary blood flow ($P < .07$) compared to diets containing 0% yellow grease. This reduction in blood flow was offset by increased free AA A-V differences so that total uptake of free AA was not affected. Although Cant et al. (1993a) suggested that the decreased EAA uptake relative to milk volume observed in their study was sufficient to reduce milk protein percentage, these effects were not significant. Our data suggest that 2.5% supplemental fat decreased milk protein percentage (3.30 vs 3.22) without significantly affecting mammary blood flow (Table 3.3 and Table 4.4), nor the proportion of blood per volume of milk produced. Additionally, it is interesting to

note that the extent of milk protein depression in our study was less than that of Cant et al. (1993a) even though the total crude fat content of our fat-supplemented diets was higher (6.0 versus 5.0% of diet DM). The means by which dietary fat may regulate blood flow to the gland and its subsequent affect on AA utilization warrants further study.

The decrease in milk protein percentage observed in this study (Chapter 3, Table 3.3) is in agreement with previous studies (DePeters et al. 1989, Jenkins and Jenny 1992, Khorasani et al. 1991). Generally, casein N, whey protein N, and NPN, constitute approximately 78, 17, and 5%, respectively, of the total N in milk (DePeters and Cant 1992). Across treatments, our casein N, whey N, and NPN constituted 72, 22, and 6%, respectively. DePeters and Cant (1992) have previously reported that nutrition, breed, parity, stage of lactation, environment, and disease could markedly affect the distribution of N-containing compounds in milk. As seen previously for fat-induced milk protein depression (DePeters and Cant 1992), when milk N was expressed as a percentage of total N, casein N tended to decrease and urea N increased. In the current study, the increase in casein N output from supplemental fat increased casein N output, is a manifestation of increased milk production.

Finally, the 88.3% overall efficiency of AA utilization, 86.5% carbon utilization, and the 72.8% AA nitrogen utilization by the mammary glands across treatments are similar to previously published estimations (Cant et al. 1993a). It is generally accepted that AA taken up in excess of milk output cannot be stored, and therefore must be used to synthesize NEAA, urea, or oxidized to $CO_2$ (Mepham 1982). Unlike Cant et al. (1993a), we did not observe a more efficient utilization of AA N and C by the mammary gland when cows were fed fat supplemented diets.

## Conclusions

Arterial concentrations of EAA were elevated in response to 41% dietary UIP, but not affected by 2.5% supplemental fat. Mammary arteriovenous differences, extraction, and uptakes of whole blood and plasma derived AA were not significantly affected by dietary treatments. The contribution of red blood cells to uptake of free AA into the mammary gland was negligible. Although peptide AA represented ~10% of AA in arterial whole blood, uptake of peptide AA by the lactating gland also was negligible. Depression in milk protein percentage when cows were fed fat-supplemented diets was not alleviated by elevated levels of arterial EAA and total AA in response to feeding 41% UIP. The ratio of AA uptake to output and the efficiency of AA C and N utilization observed in this study suggest that when supplemental fat is fed at current recommended levels, milk protein depression could not be shown to be a consequence of reduced mammary blood flow.

**TABLE 4.1.** Arterial concentrations of amino acids (AA) in plasma and whole blood.[1]

| | Plasma | | | | | Effect ( $P$ = ) | | | Whole blood | | | | | Effect ( $P$ = ) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| AA[2] | CNTL | FAT | UIP | BOTH | SEM | FAT | UIP | INTER | CNTL | FAT | UIP | BOTH | SEM | FAT | UIP | INTER |
| | --------- uM --------- | | | | | | | | ---------- uM --------- | | | | | | | |
| Arg | 71.7 | 66.1 | 64.2 | 62.5 | 2.6 | .28 | .13 | .54 | 53.5 | 53.9 | 51.8 | 51.4 | 2.4 | .99 | .44 | .87 |
| His | 38.5 | 38.9 | 57.8 | 52.8 | 1.4 | .51 | .01 | .45 | 81.0 | 86.6 | 106.2 | 100.8 | 2.4 | .97 | .02 | .30 |
| Ile | 97.5 | 84.6 | 85.6 | 89.3 | 4.5 | .36 | .47 | .15 | 90.7 | 84.8 | 83.0 | 89.1 | 3.1 | .98 | .63 | .15 |
| Leu | 148.5 | 143.4 | 225.6 | 223.1 | 7.1 | .59 | .01 | .85 | 146.6 | 149.6 | 222.5 | 224.7 | 5.7 | .36 | .01 | .87 |
| Lys | 74.2 | 60.0 | 64.9 | 66.3 | 3.8 | .33 | .80 | .25 | 96.2 | 94.9 | 90.5 | 95.8 | 3.5 | .46 | .39 | .26 |
| Met | 23.7 | 20.5 | 20.8 | 19.8 | 1.1 | .06 | .07 | .23 | 27.1 | 24.2 | 23.8 | 24.0 | 1.2 | .13 | .07 | .11 |
| Phe | 50.3 | 44.2 | 56.8 | 57.5 | 1.8 | .26 | .02 | .18 | 50.6 | 46.2 | 58.0 | 60.9 | 1.5 | .28 | .01 | .09 |
| Thr | 106.4 | 83.1 | 78.7 | 85.3 | 4.9 | .07 | .02 | .10 | 101.7 | 94.6 | 86.8 | 91.6 | 5.1 | .21 | .07 | .23 |
| Trp | 37.1 | 31.6 | 34.3 | 33.9 | 0.9 | .04 | .79 | .06 | 39.6 | 36.7 | 38.8 | 38.2 | .6 | .05 | .54 | .15 |
| Val | 201.7 | 189.1 | 259.0 | 269.3 | 7.0 | .91 | .01 | .30 | 190.0 | 187.0 | 252.8 | 266.0 | 5.5 | .38 | .01 | .20 |
| **EAA** | 849.3 | 761.2 | 947.5 | 959.3 | 28.0 | .15 | .01 | .10 | 876.7 | 858.3 | 1014 | 1042 | 22.9 | .60 | .01 | .07 |
| Ala | 278.7 | 273.6 | 278.4 | 250.3 | 7.0 | .17 | .29 | .30 | 259.9 | 264.7 | 259.8 | 241.7 | 8.3 | .58 | .37 | .38 |
| Asp | 4.2 | 4.4 | 4.6 | 3.9 | 0.3 | .28 | .70 | .07 | 23.7 | 24.4 | 24.6 | 22.8 | 1.3 | .74 | .85 | .52 |
| Asn | 75.2 | 65.1 | 72.7 | 66.7 | 2.5 | .02 | .79 | .28 | 69.3 | 66.3 | 69.8 | 65.8 | 2.3 | .16 | .98 | .79 |
| Cys | 31.1 | 36.7 | 36.2 | 33.6 | 4.4 | .26 | .43 | .04 | 21.8 | 28.0 | 28.2 | 22.5 | 1.9 | .54 | .54 | .26 |
| Glu | 48.5 | 45.6 | 42.4 | 40.2 | 1.3 | .28 | .06 | .86 | 122.7 | 115.1 | 109.1 | 111.8 | 2.1 | .54 | .11 | .26 |
| Gln | 237.3 | 242.6 | 258.5 | 229.2 | 5.6 | .11 | .51 | .14 | 175.3 | 182.7 | 190.9 | 170.3 | 5.3 | .29 | .78 | .08 |
| Gly | 280.9 | 287.2 | 287.8 | 266.9 | 8.0 | .22 | .25 | .07 | 332.2 | 365.1 | 357.0 | 333.4 | 7.9 | .57 | .63 | .03 |
| Hyl | 9.5 | 11.2 | 10.3 | 10.8 | 1.4 | .09 | .68 | .27 | 9.0 | 10.5 | 10.7 | 9.1 | .7 | .59 | .28 | .01 |
| Hyp | 10.4 | 9.3 | 7.1 | 7.6 | .9 | .86 | .27 | .69 | 8.9 | 8.7 | 8.0 | 7.2 | .9 | .70 | .43 | .84 |
| Pro | 95.1 | 93.8 | 131.0 | 116.5 | 3.6 | .22 | .01 | .29 | 112.6 | 115.9 | 147.7 | 138.5 | 3.4 | .66 | .02 | .35 |
| Ser | 101.8 | 89.4 | 103.3 | 90.9 | 2.4 | .01 | .46 | .99 | 104.2 | 97.1 | 107.2 | 96.1 | 2.2 | .05 | .76 | .52 |
| Tyr | 61.9 | 54.9 | 66.8 | 65.6 | 2.5 | .22 | .06 | .35 | 68.9 | 65.9 | 76.8 | 77.1 | 1.9 | .15 | .01 | .10 |
| **NEAA** | 1234 | 1213 | 1299 | 1182 | 27.0 | .01 | .26 | .12 | 1308 | 1344 | 1389 | 1296 | 26.0 | .22 | .44 | .04 |
| **Total AA** | 2084 | 1975 | 2246 | 2141 | 51.0 | .03 | .01 | .95 | 2185 | 2202 | 2404 | 2338 | 45.0 | .42 | .01 | .21 |
| Cit | 80.9 | 90.7 | 96.7 | 100.7 | 2.3 | .06 | .01 | .31 | 77.3 | 88.3 | 90.3 | 93.8 | 2.5 | .10 | .06 | .31 |
| Orn | 31.7 | 31.1 | 36.7 | 33.2 | 1.5 | .19 | .06 | .32 | 43.0 | 45.6 | 50.6 | 46.7 | 1.3 | .58 | .03 | .16 |
| Urea N | 2100 | 2382 | 2425 | 2392 | 60 | .41 | .29 | .21 | -- | -- | -- | -- | -- | -- | -- | -- |

[1]Dietary treatments and effects of treatments are defined in Table 3.3.
[2]Least squares means differed from zero ( $P < .05$ ).

**TABLE 4.2.** Mammary gland arteriovenous differences for amino acids (AA) in plasma and whole blood.[1]

| AA | Plasma | | | | | Effect ( $P$ = ) | | | Whole blood | | | | | Effect ( $P$ = ) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | CNTL | FAT | UIP | BOTH | SEM | FAT | UIP | INTER | CNTL | FAT | UIP | BOTH | SEM | FAT | UIP | INTER |
| | uM | | | | | | | | uM | | | | | | | |
| Arg | 40.3 | 36.0 | 34.1 | 32.0 | 2.2 | .43 | .24 | .77 | 27.7 | 30.3 | 27.1 | 27.6 | 2.2 | .70 | .68 | .79 |
| His | 10.4 | 8.4 | 7.0 | 9.3 | 1.6 | .92 | .37 | .17 | 7.1 | 7.6 | 8.5 | 7.2 | 2.7 | .93 | .91 | .83 |
| Ile | 36.0 | 31.9 | 34.8 | 31.9 | 2.9 | .22 | .80 | .81 | 28.8 | 29.3 | 31.1 | 32.3 | 2.6 | .74 | .34 | .90 |
| Leu | 65.2 | 62.9 | 79.2 | 71.5 | 5.3 | .37 | .10 | .62 | 36.6 | 37.3 | 42.4 | 47.8 | 4.7 | .60 | .22 | .69 |
| Lys | 46.4 | 37.9 | 39.6 | 42.3 | 2.8 | .56 | .80 | .30 | 33.4 | 36.5 | 30.2 | 34.4 | 3.3 | .44 | .56 | .91 |
| Met | 10.7 | 11.4 | 10.2 | 10.6 | .9 | .64 | .58 | .98 | 9.7 | 9.3 | 8.8 | 9.3 | .8 | .98 | .29 | .28 |
| Phe | 19.0 | 18.7 | 19.2 | 17.8 | 1.1 | .45 | .73 | .61 | 14.1 | 13.6 | 14.7 | 16.2 | 1.1 | .66 | .21 | .38 |
| Thr | 25.6 | 17.5 | 16.4 | 25.6 | 4.7 | .94 | .93 | .21 | 12.7 | 18.2 | 8.0 | 18.6 | 3.8 | .25 | .72 | .68 |
| Trp | 4.1 | 3.7 | 3.9 | 3.8 | .6 | .68 | .88 | .75 | 1.6 | 1.8 | 2.2 | 2.5 | .5 | .56 | .15 | .89 |
| Val | 50.3 | 47.3 | 54.5 | 47.9 | 4.3 | .43 | .67 | .76 | 33.4 | 34.7 | 39.1 | 46.2 | 4.4 | .35 | .11 | .50 |
| **EAA** | 307.8 | 275.2 | 298.8 | 292.7 | 18.6 | .52 | .88 | .65 | 205.1 | 218.4 | 212.0 | 241.9 | 18.0 | .43 | .57 | .75 |
| Ala | 34.3 | 33.1 | 27.6 | 21.7 | 2.9 | .10 | .13 | .13 | 32.6 | 28.9 | 23.3 | 24.8 | 6.6 | .19 | .16 | .21 |
| Asp | 1.1 | 1.5 | .5 | 1.2 | .6 | .24 | .29 | .72 | 2.1 | 2.4 | 2.1 | 1.0 | 1.1 | .41 | .18 | .18 |
| Asn | 18.1 | 13.7 | 16.9 | 14.1 | 1.9 | .19 | .86 | .73 | 15.6 | 15.5 | 15.3 | 15.4 | 1.9 | .99 | .75 | .89 |
| Cys | 5.6 | 8.9 | 9.8 | 9.2 | 3.6 | .46 | .24 | .31 | -8.9 | -11.7 | -13.1 | -3.5 | 5.0 | .56 | .73 | .32 |
| Glu | 34.5 | 33.0 | 25.7 | 28.5 | 1.8 | .79 | .05 | .36 | 28.0 | 26.3 | 26.0 | 24.3 | 2.1 | .55 | .49 | .99 |
| Gln | 40.7 | 32.6 | 43.4 | 40.6 | 5.7 | .54 | .55 | .76 | 34.2 | 27.1 | 23.4 | 32.6 | 4.4 | .73 | .39 | .06 |
| Gly† | 4.6 | -13.5 | -5.6 | -6.8 | 5.8 | .11 | .71 | .14 | .2 | -5.1 | -4.0 | -11.3 | 6.4 | .36 | .44 | .88 |
| Hyl† | 2.6 | 3.7 | 2.8 | 3.4 | 1.3 | .24 | .99 | .67 | -2.7 | -4.3 | -5.3 | -3.4 | 1.7 | .93 | .60 | .32 |
| Hyp† | -.3 | -1.1 | -1.0 | -.5 | .5 | .89 | .93 | .51 | -1.1 | -.5 | -.8 | -.9 | .7 | .85 | .96 | .80 |
| Pro | 11.6 | 14.4 | 19.0 | 12.4 | 2.3 | .22 | .13 | .03 | 13.7 | 12.8 | 14.1 | 16.7 | 2.8 | .63 | .28 | .36 |
| Ser | 15.7 | 17.4 | 15.5 | 16.4 | 1.9 | .45 | .72 | .79 | 14.6 | 13.5 | 10.4 | 13.4 | 1.9 | .43 | .14 | .16 |
| Tyr | 17.8 | 16.3 | 17.0 | 15.1 | 1.4 | .28 | .50 | .88 | 12.7 | 14.0 | 13.4 | 15.0 | 1.5 | .35 | .58 | .92 |
| **NEAA** | 186.3 | 159.8 | 171.7 | 155.2 | 18.7 | .18 | .39 | .99 | 141.0 | 119.0 | 111.9 | 124.0 | 19.2 | .52 | .15 | .11 |
| **Total AA** | 494.1 | 435.0 | 470.6 | 474.9 | 33.4 | .35 | .82 | .77 | 346.1 | 337.4 | 323.9 | 365.9 | 33.5 | .46 | .77 | .36 |
| Cit† | -.6 | .5 | 5.7 | 3.5 | 2.4 | .76 | .05 | .35 | 1.6 | 8.0 | 1.0 | 6.8 | 2.6 | .01 | .45 | .84 |
| Orn | 15.2 | 14.5 | 15.4 | 16.0 | 1.2 | .98 | .50 | .64 | 10.6 | 12.0 | 13.5 | 11.9 | 1.0 | .90 | .19 | .16 |
| Urea N† | 37.0 | 49.3 | 13.2 | 14.0 | 58.0 | .31 | .62 | .31 | -- | -- | -- | -- | -- | -- | -- | -- |

[1]Dietary treatments and effects of treatments are defined in Table 3.3.
†Least squares means in a row did not differ from zero ( $P > .05$ ).

**TABLE 4.3.** Mammary gland extraction of amino acids (AA) in plasma and whole blood.[1]

| AA | Plasma | | | | | Effect ( $P$ = ) | | | Whole blood | | | | | Effect ( $P$ = ) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | CNTL | FAT | UIP | BOTH | SEM | FAT | UIP | INTER | CNTL | FAT | UIP | BOTH | SEM | FAT | UIP | INTER |
| | ( % ) | | | | | | | | ( % ) | | | | | | | |
| Arg | 56.3 | 54.2 | 53.3 | 51.4 | 2.9 | .71 | .59 | .99 | 52.0 | 56.4 | 52.2 | 53.2 | 3.2 | .65 | .80 | .78 |
| His | 27.7 | 21.6 | 11.0 | 17.9 | 3.2 | .93 | .09 | .21 | 9.0 | 8.2 | 7.4 | 6.9 | 2.8 | .87 | .71 | .97 |
| Ile | 36.1 | 37.2 | 40.2 | 35.2 | 2.8 | .62 | .78 | .44 | 31.8 | 34.0 | 37.1 | 36.0 | 2.6 | .87 | .33 | .63 |
| Leu | 43.2 | 42.9 | 35.3 | 32.3 | 2.2 | .47 | .02 | .52 | 24.9 | 23.7 | 18.4 | 21.2 | 2.6 | .86 | .33 | .64 |
| Lys | 63.1 | 63.3 | 61.3 | 63.7 | 3.1 | .80 | .89 | .83 | 34.1 | 37.6 | 33.3 | 35.2 | 2.9 | .59 | .75 | .88 |
| Met | 46.3 | 57.2 | 48.8 | 55.6 | 3.3 | .22 | .94 | .75 | 36.9 | 38.7 | 36.9 | 39.1 | 2.7 | .44 | .94 | .94 |
| Phe | 38.8 | 41.8 | 33.8 | 31.1 | 1.8 | .94 | .02 | .19 | 28.7 | 28.9 | 25.0 | 26.6 | 1.8 | .69 | .24 | .77 |
| Thr | 24.6 | 12.2 | 19.1 | 30.3 | 5.4 | .95 | .44 | .20 | 11.6 | 16.5 | 7.1 | 18.1 | 5.6 | .23 | .80 | .60 |
| Trp | 10.6 | 10.5 | 11.5 | 11.1 | 1.7 | .93 | .76 | .97 | 4.1 | 4.7 | 5.6 | 6.5 | 1.3 | .51 | .19 | .89 |
| Val | 24.5 | 24.8 | 21.1 | 17.6 | 1.8 | .50 | .08 | .42 | 17.5 | 18.3 | 15.3 | 17.7 | 2.0 | .52 | .58 | .74 |
| **EAA** | 35.9 | 35.8 | 31.5 | 30.5 | 1.8 | .85 | .19 | .87 | 23.3 | 24.9 | 20.7 | 23.2 | 1.8 | .49 | .48 | .88 |
| Ala | 12.1 | 12.3 | 9.9 | 8.7 | 1.7 | .17 | .04 | .15 | 12.3 | 11.4 | 9.0 | 9.8 | 1.3 | .16 | .06 | .11 |
| Asp | 29.0 | 35.1 | 5.9 | 30.2 | 12.8 | .18 | .21 | .37 | 10.9 | 12.9 | 8.7 | .9 | 5.7 | .34 | .07 | .15 |
| Asn | 24.8 | 20.2 | 23.1 | 20.7 | 2.9 | .41 | .88 | .78 | 22.7 | 23.6 | 22.1 | 22.5 | 2.3 | .44 | .31 | .76 |
| Cys | 13.7 | 22.5 | 29.4 | 18.2 | 6.2 | .65 | .09 | .02 | -3.1 | 1.1 | -2.3 | 7.1 | 4.2 | .31 | .59 | .67 |
| Glu | 71.2 | 72.2 | 60.4 | 70.6 | 3.5 | .13 | .10 | .18 | 23.4 | 23.6 | 24.2 | 22.6 | 1.9 | .69 | .89 | .64 |
| Gln | 17.4 | 14.6 | 17.0 | 17.7 | 2.3 | .78 | .72 | .65 | 19.7 | 15.8 | 12.4 | 18.8 | 2.2 | .60 | .39 | .11 |
| Gly† | 1.4 | -4.5 | -2.0 | -2.8 | 2.0 | .12 | .61 | .20 | .1 | -1.3 | -1.3 | -4.0 | 2.0 | .35 | .35 | .72 |
| Hyl† | 16.3 | 26.1 | 20.6 | 15.3 | 9.0 | .73 | .61 | .28 | -.1 | .3 | -.2 | -1.6 | 1.6 | .56 | .28 | .34 |
| Hyp† | .6 | -6.7 | -1.6 | -7.0 | 5.4 | .13 | .55 | .36 | -3.1 | -1.0 | -3.3 | -1.9 | 3.7 | .61 | .88 | .92 |
| Pro | 13.0 | 15.7 | 14.3 | 10.7 | 2.0 | .68 | .18 | .16 | 12.5 | 12.1 | 9.7 | 12.6 | 1.7 | .35 | .38 | .24 |
| Ser | 15.8 | 18.9 | 14.8 | 17.9 | 1.8 | .63 | .05 | .42 | 14.3 | 14.0 | 9.4 | 13.7 | 1.7 | .24 | .15 | .20 |
| Tyr | 30.5 | 29.9 | 25.2 | 23.3 | 2.5 | .61 | .07 | .77 | 19.2 | 21.6 | 17.1 | 19.9 | 1.9 | .23 | .36 | .90 |
| **NEAA** | 15.0 | 13.5 | 13.3 | 13.1 | 1.4 | .41 | .33 | .81 | 10.7 | 9.1 | 8.1 | 9.4 | 1.4 | .36 | .20 | .18 |
| **Total AA** | 23.5 | 22.1 | 20.9 | 20.8 | 1.5 | .65 | .75 | .82 | 15.8 | 15.3 | 13.5 | 15.6 | 1.4 | .42 | .37 | .31 |
| Cit† | -.9 | .3 | 5.4 | 3.3 | 2.6 | .82 | .07 | .38 | 2.4 | 8.5 | .7 | 7.0 | 2.9 | .02 | .34 | .94 |
| Orn | 46.9 | 46.6 | 42.2 | 47.9 | 2.7 | .57 | .72 | .53 | 24.7 | 26.0 | 26.3 | 25.0 | 1.9 | .99 | .89 | .51 |
| Urea N† | -2.1 | 2.1 | .6 | .6 | 2.5 | .37 | .66 | .34 | -- | -- | -- | -- | -- | -- | -- | -- |

[1]Dietary treatments and effects of treatments are defined in Table 3.3.
†Least squares means in a row did not differ from zero ( $P > .05$ ).

**TABLE 4.4.** Mammary blood flow in relation to milk production.

| | Treatments | | | | | Effect ( *P* = ) [2] | | |
|---|---|---|---|---|---|---|---|---|
| | CNTL[1] | FAT | UIP | BOTH | SEM | FAT | UIP | INTER |
| Blood flow | | | | | | | | |
| L/min | 14.82 | 16.98 | 17.70 | 16.61 | .80 | .49 | .17 | .10 |
| L/kg of milk | 658 | 687 | 731 | 650 | 32 | .49 | .62 | .20 |

[1]Dietary treatments and effects of treatments are defined in Table 3.3.

**TABLE 4.5.** Mammary gland uptakes (mol/d) of amino acids (AA) from plasma.[1]

| | Treatments | | | | | Effect ( $P$ = ) | | |
|---|---|---|---|---|---|---|---|---|
| AA | CNTL | FAT | UIP | BOTH | SEM | FAT | UIP | INTER |
| | ------------ mol/d ------------ | | | | | | | |
| Arg | .61 | .63 | .61 | .53 | .05 | .57 | .35 | .30 |
| His | .16 | .14 | .12 | .16 | .03 | .58 | .62 | .19 |
| Ile | .54 | .56 | .61 | .52 | .06 | .19 | .60 | .07 |
| Leu | 1.00 | 1.10 | 1.45 | 1.22 | .01 | .21 | .01 | .12 |
| Lys | .70 | .66 | .72 | .71 | .05 | .73 | .66 | .81 |
| Met | .16 | .20 | .18 | .17 | .02 | .46 | .93 | .33 |
| Phe | .29 | .33 | .34 | .29 | .02 | .72 | .45 | .02 |
| Thr | .40 | .27 | .29 | .41 | .07 | .97 | .89 | .25 |
| Trp | .06 | .06 | .07 | .06 | .01 | .80 | .71 | .92 |
| Val | .76 | .84 | .98 | .79 | .09 | .36 | .20 | .09 |
| **EAA** | 4.67 | 4.80 | 5.38 | 4.87 | .37 | .59 | .29 | .37 |
| Ala | .51 | .59 | .53 | .52 | .10 | .16 | .08 | .08 |
| Asp | .02 | .03 | .01* | .02 | .01 | .35 | .41 | .73 |
| Asn | .27 | .24 | .29 | .23 | .04 | .18 | .92 | .62 |
| Cys | .09* | .16 | .21 | .18 | .07 | .54 | .09 | .17 |
| Glu | .52 | .57 | .48 | .49 | .03 | .29 | .09 | .57 |
| Gln | .60 | .56 | .74 | .67 | .11 | .74 | .48 | .93 |
| Gly† | .07 | -.26 | -.13 | -.13 | .12 | .11 | .70 | .12 |
| Hyl | .04 | .07 | .07 | .07 | .02 | .56 | .44 | .46 |
| Hyp† | -.01 | -.02 | -.03 | -.01 | .01 | .93 | .80 | .54 |
| Pro | .18 | .26 | .34 | .18 | .05 | .14 | .10 | .01 |
| Ser | .24 | .30 | .29 | .27 | .04 | .31 | .59 | .10 |
| Tyr | .27 | .29 | .31 | .25 | .03 | .27 | .99 | .06 |
| **NEAA** | 2.80 | 2.79 | 3.12 | 2.76 | .39 | .14 | .78 | .15 |
| **Total AA** | 7.47 | 7.59 | 8.50 | 7.62 | .68 | .33 | .56 | .26 |
| Cit | -.01* | .01* | .09 | .06* | .04 | .76 | .09 | .49 |
| Orn | .23 | .25 | .28 | .28 | .02 | .70 | .06 | .44 |

[1]Dietary treatments and effects of treatments are defined in Table 3.3.
*Individual least squares mean did not differ from zero ( $P > .05$ ).
†Least squares means in a row did not differ from zero ( $P > .05$ ).

**TABLE 4.6.** Mammary gland uptakes (g/d) of amino acids (AA) from plasma.[1]

| AA | Treatments | | | | SEM | Effect ( *P* = ) | | |
|---|---|---|---|---|---|---|---|---|
| | CNTL | FAT | UIP | BOTH | | FAT | UIP | INTER |
| | ------------- g/d ------------- | | | | | | | |
| Arg | 105.7 | 110.7 | 107.1 | 93.0 | 8.4 | .57 | .35 | .30 |
| His | 24.1 | 21.8 | 18.7 | 24.3 | 4.5 | .58 | .62 | .19 |
| Ile | 70.9 | 74.2 | 80.1 | 68.2 | 7.7 | .19 | .60 | .07 |
| Leu | 131.4 | 144.4 | 189.9 | 160.7 | 13.3 | .21 | .01 | .12 |
| Lys | 102.4 | 95.9 | 104.7 | 103.6 | 7.5 | .73 | .66 | .81 |
| Met | 23.6 | 29.7 | 26.8 | 25.9 | 2.7 | .46 | .93 | .33 |
| Phe | 47.3 | 54.3 | 56.2 | 47.9 | 3.9 | .72 | .45 | .02 |
| Thr | 47.0 | 32.7 | 34.9 | 48.4 | 8.7 | .97 | .89 | .25 |
| Trp | 12.4 | 11.8 | 14.0 | 12.7 | 2.3 | .80 | .71 | .92 |
| Val | 89.1 | 98.5 | 115.2 | 92.7 | 10.8 | .36 | .20 | .09 |
| **EAA** | 654.1 | 674.1 | 747.7 | 677.6 | 50.8 | .59 | .29 | .37 |
| Ala | 45.4 | 52.8 | 47.1 | 46.5 | 9.0 | .16 | .08 | .08 |
| Asp | 2.4* | 3.4 | 1.2* | 2.8 | 1.3 | .35 | .41 | .73 |
| Asn | 36.3 | 31.7 | 38.6 | 30.2 | 4.9 | .18 | .92 | .62 |
| Cys | 11.0* | 19.5 | 25.4 | 21.4 | 8.7 | .54 | .09 | .17 |
| Glu | 77.2 | 84.3 | 70.6 | 73.1 | 4.6 | .29 | .09 | .57 |
| Gln | 88.2 | 81.9 | 108.6 | 98.2 | 16.7 | .74 | .48 | .93 |
| Gly† | 5.0 | -19.7 | -9.6 | -10.1 | 8.8 | .11 | .70 | .12 |
| Hyl | 6.7* | 10.8 | 11.3 | 10.9 | 4.0 | .56 | .44 | .46 |
| Hyp† | -.9 | -2.4 | -3.3 | -1.4 | 1.3 | .93 | .80 | .54 |
| Pro | 20.6 | 29.8 | 39.2 | 21.1 | 5.6 | .14 | .10 | .01 |
| Ser | 24.8 | 31.9 | 30.7 | 28.2 | 3.9 | .31 | .59 | .10 |
| Tyr | 48.5 | 52.4 | 55.4 | 45.0 | 5.3 | .27 | .99 | .06 |
| **NEAA** | 365.1 | 376.5 | 415.3 | 365.8 | 44.8 | .14 | .78 | .15 |
| **Total AA** | 1019.2 | 1050.5 | 1163.1 | 1043.4 | 87.9 | .33 | .56 | .26 |
| Cit | -1.1* | 1.4* | 15.4 | 9.8* | 6.8 | .76 | .09 | .49 |
| Orn | 30.5 | 32.7 | 36.9 | 36.1 | 2.8 | .70 | .06 | .44 |

[1]Dietary treatments and effects of treatments are defined in Table 3.3.
*Individual least squares mean did not differ from zero ( $P > .05$ ).
†Least squares means in a row did not differ from zero ( $P > .05$ ).

**TABLE 4.7.** Mammary gland uptakes (mol/d) of amino acids (AA) from whole blood.[1]

| AA | Treatments | | | | SEM | Effect ( *P* = ) | | |
|---|---|---|---|---|---|---|---|---|
| | CNTL | FAT | UIP | BOTH | | FAT | UIP | INTER |
| | ------------ mol/d ------------ | | | | | | | |
| Arg | .58 | .73 | .68 | .64 | .06 | .54 | .98 | .32 |
| His | .13 | .19 | .22 | .18 | .07 | .97 | .71 | .64 |
| Ile | .61 | .70 | .77 | .76 | .06 | .54 | .17 | .44 |
| Leu | .77 | .87 | 1.02 | 1.10 | .11 | .52 | .14 | .91 |
| Lys | .69 | .89 | .74 | .78 | .09 | .37 | .82 | .56 |
| Met | .21 | .22 | .21 | .21 | .02 | .85 | .84 | .17 |
| Phe | .30 | .32 | .35 | .37 | .03 | .56 | .15 | .87 |
| Thr | .26 | .41 | .20 | .38 | .11 | .24 | .74 | .88 |
| Trp | .04 | .04 | .05 | .05 | .01 | .89 | .20 | .87 |
| Val | .71 | .81 | .96 | 1.08 | .10 | .37 | .08 | .93 |
| **EAA** | 4.29 | 5.18 | 5.22 | 5.55 | .40 | .37 | .34 | .66 |
| Ala | .66 | .65 | .56 | .57 | .16 | .15 | .18 | .23 |
| Asp | .05* | .05* | .07 | .02* | .03 | .23 | .77 | .13 |
| Asn | .34 | .36 | .38 | .35 | .05 | .94 | .56 | .41 |
| Cys | -.20* | -.33 | -.36 | -.08* | .13 | .61 | .75 | .20 |
| Glu | .59 | .64 | .69 | .58 | .07 | .61 | .77 | .24 |
| Gln | .72 | .62 | .51 | .75 | .12 | .18 | .36 | .12 |
| Gly† | .02 | -.16 | -.10 | -.36 | .19 | .25 | .38 | .82 |
| Hyl | -.06* | -.11 | -.14 | -.09 | .05 | .91 | .42 | .25 |
| Hyp† | -.02 | -.01 | -.02 | -.01 | .02 | .99 | .89 | .84 |
| Pro | .29 | .29 | .33 | .37 | .08 | .66 | .24 | .59 |
| Ser | .31 | .31 | .26 | .30 | .05 | .64 | .40 | .67 |
| Tyr | .26 | .33 | .33 | .35 | .04 | .29 | .34 | .50 |
| **NEAA** | 2.96 | 2.64 | 2.52 | 2.75 | .48 | .48 | .22 | .16 |
| **Total AA** | 7.25 | 7.83 | 7.74 | 8.29 | .72 | .40 | .85 | .77 |
| Cit | .04* | .18 | .02* | .17 | .07 | .01 | .62 | .92 |
| Orn | .22 | .29 | .34 | .27 | .03 | .92 | .12 | .15 |

[1]Dietary treatments and effects of treatments are defined in Table 3.3.
*Individual least squares mean did not differ from zero ( *P* > .05 ).
†Least squares means in a row did not differ from zero ( *P* > .05 ).

**TABLE 4.8.** Mammary gland uptakes (g/d) of amino acids (AA) from whole blood.[1]

| | Treatments | | | | | Effect | | |
|---|---|---|---|---|---|---|---|---|
| AA | CNTL | FAT | UIP | BOTH | SEM | FAT | UIP | INTER |
| | ------------- g/d ------------ | | | | | | | |
| Arg | 100.4 | 127.4 | 117.9 | 110.7 | 9.9 | .54 | .98 | .32 |
| His | 20.5 | 29.0 | 34.6 | 27.3 | 11.6 | .97 | .71 | .64 |
| Ile | 79.3 | 92.4 | 101.5 | 99.7 | 8.1 | .54 | .17 | .44 |
| Leu | 101.0 | 114.9 | 135.0 | 144.7 | 13.8 | .52 | .14 | .91 |
| Lys | 100.8 | 129.6 | 107.6 | 114.6 | 13.3 | .37 | .82 | .56 |
| Met | 30.6 | 32.7 | 32.0 | 30.8 | 2.8 | .85 | .84 | .17 |
| Phe | 49.2 | 52.5 | 58.6 | 60.7 | 4.4 | .56 | .15 | .87 |
| Thr | 31.6 | 48.7 | 24.2 | 45.9 | 13.6 | .24 | .74 | .88 |
| Trp | 7.1 | 8.0 | 10.9 | 11.0 | 2.6 | .89 | .20 | .87 |
| Val | 83.4 | 95.2 | 112.4 | 126.3 | 12.4 | .37 | .08 | .93 |
| **EAA** | 603.8 | 730.6 | 734.8 | 771.6 | 55.4 | .37 | .34 | .66 |
| Ala | 58.9 | 57.6 | 50.7 | 51.1 | 4.9 | .15 | .18 | .23 |
| Asp | 6.1* | 7.1* | 9.4 | 2.3* | 3.8 | .23 | .77 | .13 |
| Asn | 44.3 | 47.8 | 50.9 | 46.7 | 6.2 | .94 | .56 | .41 |
| Cys | -24.1* | -40.3 | -43.4 | -9.9* | 15.8 | .61 | .75 | .20 |
| Glu | 86.7 | 93.8 | 84.9 | 101.7 | 10.5 | .61 | .77 | .24 |
| Gln | 105.6 | 91.0 | 75.0 | 109.5 | 17.0 | .18 | .36 | .12 |
| Gly† | 1.3 | -12.0 | -7.6 | -26.7 | 14.0 | .25 | .38 | .82 |
| Hyl | -10.3* | -17.9 | -24.1 | -14.7 | 7.4 | .91 | .42 | .25 |
| Hyp† | -2.4 | -1.7 | -2.0 | -3.0 | 2.5 | .99 | .89 | .84 |
| Pro | 34.0 | 33.3 | 37.8 | 43.0 | 8.6 | .66 | .24 | .59 |
| Ser | 32.7 | 32.8 | 27.8 | 31.0 | 5.1 | .64 | .40 | .67 |
| Tyr | 47.4 | 60.5 | 59.3 | 62.9 | 6.4 | .29 | .34 | .50 |
| **NEAA** | 380.2 | 352.1 | 318.7 | 393.9 | 51.2 | .48 | .22 | .16 |
| **Total AA** | 984.0 | 1082.6 | 1053.5 | 1165.5 | 88.6 | .40 | .85 | .77 |
| Cit | 6.3* | 32.1 | 3.9* | 29.4 | 11.4 | .01 | .62 | .92 |
| Orn | 29.7 | 38.2 | 45.2 | 35.8 | 4.1 | .92 | .12 | .15 |

[1]Dietary treatments and effects of treatments are defined in Table 3.3.
*Individual least squares mean did not differ from zero ( $P > .05$ ).
†Least squares means in a row did not differ from zero ( $P > .05$ ).

**TABLE** 4.9. Contribution of red blood cells to free amino acid (AA) uptakes.[1]

| AA | Whole blood minus plasma AA | | | | SEM | Effect ( *P* = ) | | |
|---|---|---|---|---|---|---|---|---|
| | CNTL | FAT | UIP | BOTH | | FAT | UIP | INTER |
| | ------------ g/d ------------ | | | | | | | |
| Arg | -5.39 | 16.74 | 10.84 | 17.67 | 12.16 | .45 | .64 | .67 |
| His | -3.67 | 7.18 | 15.85 | 2.95 | 13.70 | .95 | .64 | .48 |
| Ile | 8.40 | 18.21 | 21.39 | 31.49# | 13.42 | .31 | .21 | .99 |
| Leu | -30.41 | -29.42 | -54.97 | -15.98 | 22.66 | .32 | .76 | .34 |
| Lys | -1.64 | 33.74# | 2.93 | 11.05 | 15.30 | .37 | .68 | .55 |
| Met | 6.99 | 2.93 | 5.17 | 4.85 | 4.75 | .60 | .99 | .65 |
| Phe | 1.89 | -1.74 | 2.35 | 12.69 | 6.99 | .39 | .11 | .13 |
| Thr | -15.44 | 15.97 | -10.64 | -2.48 | 12.47 | .11 | .48 | .27 |
| Trp | -5.33 | -3.79 | -3.08 | -1.75 | 4.47 | .76 | .65 | .99 |
| Val | -5.64 | -3.32 | -2.78 | 33.62 | 20.93 | .32 | .31 | .37 |
| **EAA** | -50.22 | 56.49 | -12.93 | 94.10 | 77.31 | .27 | .67 | .99 |
| Ala | 13.46 | 4.81 | 3.65 | 4.60 | 21.29 | .11 | .44 | .07 |
| Asp | 3.76 | 3.71 | 8.23 | -.46 | 4.46 | .20 | .96 | .20 |
| Asn | 8.01 | 16.12 | 12.24 | 16.45 | 10.15 | .47 | .78 | .81 |
| Cys | -35.09 | -59.77# | -68.86# | -31.30 | 22.95 | .72 | .88 | .15 |
| Glu | 9.53 | 9.48 | 31.09 | 11.81 | 11.68 | .31 | .23 | .31 |
| Gln | 17.31 | 9.01 | -33.55 | 11.29 | 30.65 | .41 | .29 | .26 |
| Gly | -3.68 | 7.64 | 2.01 | -16.52 | 20.19 | .83 | .58 | .39 |
| Hyl | -16.97 | -28.65# | -35.36# | -25.64# | 11.04 | .91 | .40 | .27 |
| Hyp | -1.53 | .72 | 1.23 | -1.55 | 3.27 | .97 | .97 | .69 |
| Pro | 13.46 | 3.50 | -1.38 | 21.93 | 13.50 | .21 | .70 | .12 |
| Ser | 7.85 | .84 | -2.88 | 2.85 | 8.02 | .86 | .27 | .15 |
| Tyr | -1.06 | 8.16 | 4.16 | 17.88 | 10.42 | .22 | .39 | .79 |
| **NEAA** | 15.04 | -24.44 | -96.63 | 28.11 | 88.26 | .39 | .27 | .04 |
| **Total AA** | -35.17 | 32.05 | -109.56 | 122.21 | 147.37 | .20 | .98 | .37 |
| Cit | 7.31 | 30.65 | -11.55 | 19.60 | 15.46 | .04 | .12 | .62 |
| Orn | -.79 | 5.46 | 8.34 | -.26 | 5.16 | .75 | .65 | .12 |

[1]Dietary treatments and effects of treatments are defined in Table 3.3.
#Individual least squares mean differed from zero ( $P < .05$ ).

**TABLE 4.10.** Peptide contribution (uM) to the available pool of amino acids (AA) in arterial blood.[1,2]

| | Treatments | | | | | Effect | | |
|---|---|---|---|---|---|---|---|---|
| AA | CNTL | FAT | UIP | BOTH | SEM | FAT | UIP | INTER |
| | ------------- uM ------------- | | | | | | | |
| Arg | 4.4 | 3.8 | 7.0 | 7.6 | 2.0 | .99 | .40 | .87 |
| His | -10.6 | -9.4 | -16.1 | -14.8 | 1.1 | .58 | .08 | .98 |
| Ile | 2.7 | 1.0* | 2.3 | 3.2 | .8 | .65 | .41 | .23 |
| Leu | 9.7 | 4.5 | 8.0 | 10.5 | 2.3 | .66 | .49 | .25 |
| Lys | 5.2* | 3.1* | 7.7 | 1.7* | 3.3 | .17 | .83 | .45 |
| Met | -2.7* | -6.2 | -7.2 | -3.2* | 2.4 | .87 | .62 | .07 |
| Phe | 5.4 | 1.5* | 4.0 | 4.3 | 1.6 | .32 | .67 | .25 |
| Thr | 17.8 | 1.1* | 19.9 | 17.8 | 5.9 | .39 | .36 | .21 |
| Val | 12.0 | 5.0 | 12.2 | 14.7 | 2.4 | .61 | .29 | .31 |
| Ala | 22.6 | 11.8 | 26.6 | 22.6 | 4.4 | .14 | .14 | .41 |
| Asp + Asn | -30.5 | -29.2 | -36.6 | -31.8 | 3.1 | .13 | .07 | .33 |
| Cys† | .5 | 4.3 | 3.5 | .6 | 4.1 | .98 | .83 | .41 |
| Glu + Gln | -30.4 | -34.6 | -37.0 | -22.8 | 7.4 | .18 | .42 | .05 |
| Gly | 144.6 | 153.4 | 170.1 | 160.0 | 6.7 | .86 | .02 | .08 |
| Hyl | 4.6 | 4.9 | 5.4 | 2.9 | 1.2 | .48 | .67 | .40 |
| Hyp | 5.5 | 7.1 | 8.3 | 9.0 | .8 | .58 | .28 | .82 |
| Pro | 11.5 | 7.0 | 14.5 | 18.1 | 3.0 | .89 | .09 | .24 |
| Ser | -14.4 | -17.5 | -17.1 | -13.7 | 3.0 | .98 | .92 | .50 |
| Tyr | -5.9* | -9.2 | -11.5 | -13.1 | 4.3 | .22 | .06 | .64 |

[1]Dietary treatments and effects of treatments are defined in Table 3.3.
[2]Hydrolyzed arterial whole blood (uM) minus free arterial whole blood (uM).
*Individual least squares mean did not differ from zero ( $P > .05$ ).
†Least squares means in a row did not differ from zero ( $P > .05$ ).

**TABLE 4.11.** Mammary gland uptakes of peptide amino acids (AA) from whole blood.[1]

| | Plasma | | | | | Effect ( *P* = ) | | |
|---|---|---|---|---|---|---|---|---|
| AA | CNTL | FAT | UIP | BOTH | SEM | FAT | UIP | INTER |
| | ------------- g/d ------------- | | | | | | | |
| Arg† | -5.20 | -2.49 | 1.96 | -1.68 | 5.23 | .96 | .67 | .74 |
| His | -12.63 | -9.38 | -4.36* | -12.22 | 3.43 | .53 | .47 | .19 |
| Ile† | .26 | -1.95 | -2.00 | -3.45 | 3.01 | .55 | .54 | .90 |
| Leu† | 5.28 | -1.06 | 2.39 | -6.78 | 2.75 | .59 | .76 | .92 |
| Lys† | 11.35 | 1.11 | 4.77 | -6.49 | 6.65 | .22 | .38 | .95 |
| Met | .81* | -15.99 | -13.35 | .96* | 4.98 | .65 | .62 | .01 |
| Phe† | 7.54 | -.53 | 6.34 | -1.44 | 5.32 | .27 | .87 | .99 |
| Thr† | 2.63 | -7.82 | 16.94 | 6.32 | 8.36 | .35 | .23 | .99 |
| Val† | 8.57 | -2.96 | 3.25 | -3.47 | 8.39 | .37 | .76 | .80 |
| | | | | | | | | |
| Ala† | 2.78 | -17.17 | 2.39 | 5.33 | 9.82 | .58 | .48 | .47 |
| Asp + Asn | -13.56 | -19.01 | -38.98 | -24.91 | 7.39 | .56 | .10 | .23 |
| Cys† | 4.99 | 10.11 | 4.71 | 2.48 | 8.90 | .90 | .72 | .73 |
| Glu + Gln† | -40.80 | -29.86 | -84.17 | -44.54 | 28.90 | .54 | .48 | .72 |
| Gly† | -10.33 | -7.42 | -7.65 | -.15 | 12.13 | .80 | .81 | .91 |
| Hyl† | -1.20 | -3.59 | 3.88 | -1.81 | 3.54 | .34 | .40 | .67 |
| Hyp† | -.24 | 1.78 | 2.34 | 1.60 | 2.39 | .87 | .76 | .73 |
| Pro | 6.16* | -13.58* | 6.20* | 19.67 | 7.74 | .77 | .18 | .18 |
| Ser | .43* | -10.64 | -.32* | -4.25 | 4.86 | .26 | .64 | .56 |
| Tyr† | 16.25 | -11.21 | -5.43 | 9.11 | 14.47 | .68 | .97 | .23 |

[1]Dietary treatments and effects of treatments are defined in Table 3.3.
†Least squares means in a row did not differ from zero ( *P* > .05 ).
*Individual least squares mean did not differ from zero ( *P* > .05 ).

**TABLE 4.12.** Nitrogen fractions in milk.[1]

| N fraction | Treatments | | | | SEM | Effect ( P = ) | | |
|---|---|---|---|---|---|---|---|---|
| | CNTL | FAT | UIP | BOTH | | FAT | UIP | INTER |
| | -------- g/100 g milk -------- | | | | | -------- P< -------- | | |
| Total | .516 | .510 | .518 | .500 | .003 | .03 | .27 | .14 |
| Casein | .379 | .370 | .376 | .343 | .007 | .83 | .42 | .17 |
| Whey | .107 | .108 | .100 | .130 | .011 | .26 | .55 | .29 |
| Urea | .017 | .019 | .016 | .024 | .001 | .05 | .23 | .10 |
| Other[2] | .013 | .012 | .026 | .003 | .003 | .10 | .69 | .11 |
| | ----------- g/d ------------ | | | | | | | |
| Total | 168.2 | 180.2 | 182.0 | 182.6 | 4.0 | .21 | .14 | .26 |
| Casein | 123.0 | 131.0 | 132.3 | 125.6 | 1.3 | .04 | .16 | .02 |
| Whey | 35.4 | 38.3 | 35.1 | 47.1 | 4.2 | .18 | .40 | .37 |
| Urea | 5.7 | 6.5 | 5.5 | 8.8 | .6 | .05 | .18 | .13 |
| Other | 4.0 | 4.4 | 9.2 | 1.2 | 1.6 | .11 | .62 | .10 |
| | ------ g/100 g total N ------ | | | | | | | |
| Casein | 73.2 | 72.9 | 72.7 | 68.7 | 1.7 | .60 | .63 | .32 |
| Whey | 21.0 | 21.1 | 19.3 | 25.8 | 1.9 | .20 | .50 | .21 |
| Urea | 3.4 | 3.6 | 3.0 | 4.8 | .3 | .04 | .22 | .07 |
| Other | 2.4 | 2.5 | 5.0 | .7 | 1.0 | .11 | .69 | .10 |

[1]Dietary treatments and effects of treatments are defined in Table 3.3.
[2]Other = Total N - Casein N - whey N - urea N.

**TABLE 4.13.** Mammary gland outputs of amino acids (AA).[1]

| AA[2] | Treatments | | | | | Effect ( $P =$ ) | | |
|---|---|---|---|---|---|---|---|---|
| | CNTL | FAT | UIP | BOTH | SEM | FAT | UIP | INTER |
| | ------------- g/d ------------- | | | | | | | |
| Arg | 34.2 | 38.1 | 38.1 | 37.6 | .3 | .05 | .05 | .03 |
| His | 24.5 | 27.1 | 26.9 | 26.9 | .3 | .11 | .15 | .11 |
| Ile | 63.7 | 70.8 | 64.7 | 68.3 | 1.2 | .16 | .85 | .63 |
| Leu | 95.8 | 106.6 | 96.9 | 102.5 | 1.7 | .15 | .79 | .62 |
| Lys | 88.7 | 101.1 | 78.5 | 89.1 | 2.8 | .12 | .15 | .90 |
| Met | 25.9 | 29.1 | 26.9 | 27.5 | .4 | .13 | .83 | .28 |
| Phe | 49.4 | 51.9 | 58.6 | 60.5 | 1.4 | .16 | .47 | .84 |
| Thr | 35.5 | 45.4 | 34.6 | 47.2 | .8 | .28 | .22 | .56 |
| Val | 63.8 | 71.2 | 67.9 | 69.5 | .8 | .13 | .61 | .30 |
| **EAA** | 481.5 | 541.3 | 493.2 | 529.0 | 3.4 | .14 | .74 | .57 |
| Ala | 21.4 | 24.4 | 24.4 | 23.3 | .1 | .03 | .03 | .01 |
| Asp | 55.3 | 63.1 | 63.6 | 60.8 | .5 | .07 | .04 | .01 |
| Cys | 5.8 | 5.9 | 5.6 | 6.6 | .3 | .47 | .68 | .59 |
| Glu | 207.6 | 233.0 | 231.7 | 229.2 | 1.8 | .05 | .07 | .04 |
| Gly | 12.2 | 13.8 | 13.6 | 13.2 | .1 | .10 | .19 | .03 |
| Pro | 77.5 | 88.0 | 86.8 | 84.0 | .7 | .05 | .12 | .02 |
| Ser | 41.5 | 46.5 | 46.5 | 46.3 | .4 | .09 | .10 | .10 |
| Tyr | 47.4 | 60.7 | 58.9 | 62.7 | .9 | .13 | .54 | .30 |
| **NEAA** | 468.6 | 535.3 | 531.1 | 526.2 | 3.4 | .04 | .07 | .03 |
| **Total AA** | 950.1 | 1076.6 | 1024.6 | 1055.2 | 10.8 | .09 | .68 | .20 |

[1]Dietary treatments and effects of treatments are defined in Table 3.3.
[2]Least squares means differed from zero ( $P < .05$ ).

**TABLE** 4.14. Ratio of uptake to output of essential amino acids (EAA) by the mammary gland.[1,2]

| | Plasma | | | | | Effect ( $P$ = ) | | | Whole blood | | | | | Effect ( $P$ = ) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| AA | CNTL | FAT | UIP | BOTH | SEM | FAT | UIP | INTER | CNTL | FAT | UIP | BOTH | SEM | FAT | UIP | INTER |
| | --- uptake/output --- | | | | | | | | --- uptake/output --- | | | | | | | |
| Arg | 3.09 | 2.91 | 2.81 | 2.47 | .28 | .35 | .07 | .20 | 2.94 | 3.35 | 3.10 | 2.94 | .31 | .95 | .50 | .64 |
| His | .98 | .80 | .70 | .90 | .19 | .24 | .49 | .39 | .84 | 1.07 | 1.29 | 1.01 | .50 | .82 | .75 | .27 |
| Ile | 1.11 | 1.05 | 1.24 | 1.00 | .14 | .01 | .28 | .08 | 1.24 | 1.30 | 1.57 | 1.46 | .16 | .98 | .07 | .42 |
| Leu | 1.37 | 1.35 | 1.96 | 1.57 | .17 | .21 | .30 | .10 | 1.05 | 1.08 | 1.39 | 1.41 | .20 | .90 | .19 | .70 |
| Lys | 1.15 | .95 | 1.33 | 1.16 | .09 | .28 | .51 | .96 | 1.14 | 1.28 | 1.37 | 1.29 | .16 | .64 | .42 | .35 |
| Met | .91 | 1.02 | 1.00 | .94 | .11 | .52 | .46 | .24 | 1.18 | 1.12 | 1.19 | 1.12 | .13 | .11 | .90 | .83 |
| Phe | .96 | 1.05 | .96 | .80 | .07 | .32 | .42 | .23 | 1.00 | 1.01 | 1.00 | 1.00 | .09 | .25 | .29 | .74 |
| Thr | 1.32 | .72 | 1.01 | 1.03 | .24 | .64 | .44 | .20 | .89 | 1.07 | .92 | .97 | .35 | .84 | .72 | .87 |
| Val | 1.40 | 1.38 | 1.70 | 1.33 | .21 | .38 | .71 | .18 | 1.31 | 1.34 | 1.66 | 1.82 | .21 | .85 | .28 | .89 |
| **EAA**[3] | 1.33 | 1.22 | 1.49 | 1.26 | .12 | .20 | .58 | .22 | 1.24 | 1.33 | 1.47 | 1.44 | .11 | .99 | .23 | .62 |

[1]Dietary treatments and effects of treatments are defined in Table 3.3.
[2]Uptake to output ratio = uptake (g/d)/output (g/d).
[3]Tryptophan was destroyed during hydrolysis step of amino acid derivititation and therefore is not included in total.

**TABLE 4.15.** Amino acid (AA) carbon and nitrogen utilization by the bovine mammary gland.[1]

| Item[2] | Treatments | | | |
|---|---|---|---|---|
| | CNTL | FAT | UIP | BOTH |
| | g/d | | | |
| AA Uptake, g/d | 1058 | 1191 | 1144 | 1271 |
| AA Output, g/d | 950 | 1076 | 1024 | 1055 |
| Efficiency, % | 90 | 90 | 90 | 83 |
| AA Carbon uptake, g/d | 496 | 564 | 552 | 607 |
| AA Carbon output, g/d | 442 | 501 | 478 | 493 |
| Efficiency, % | 89 | 89 | 87 | 81 |
| AA Nitrogen uptake, g/d | 166 | 186 | 173 | 190 |
| AA Nitogen output, g/d | 120 | 136 | 129 | 133 |
| Efficiency, % | 72 | 74 | 75 | 70 |

[1]Dietary treatments and effects of treatments are defined in Table 3.3.
[2]Determined from chemical analyses of output and carbon percentage composition.

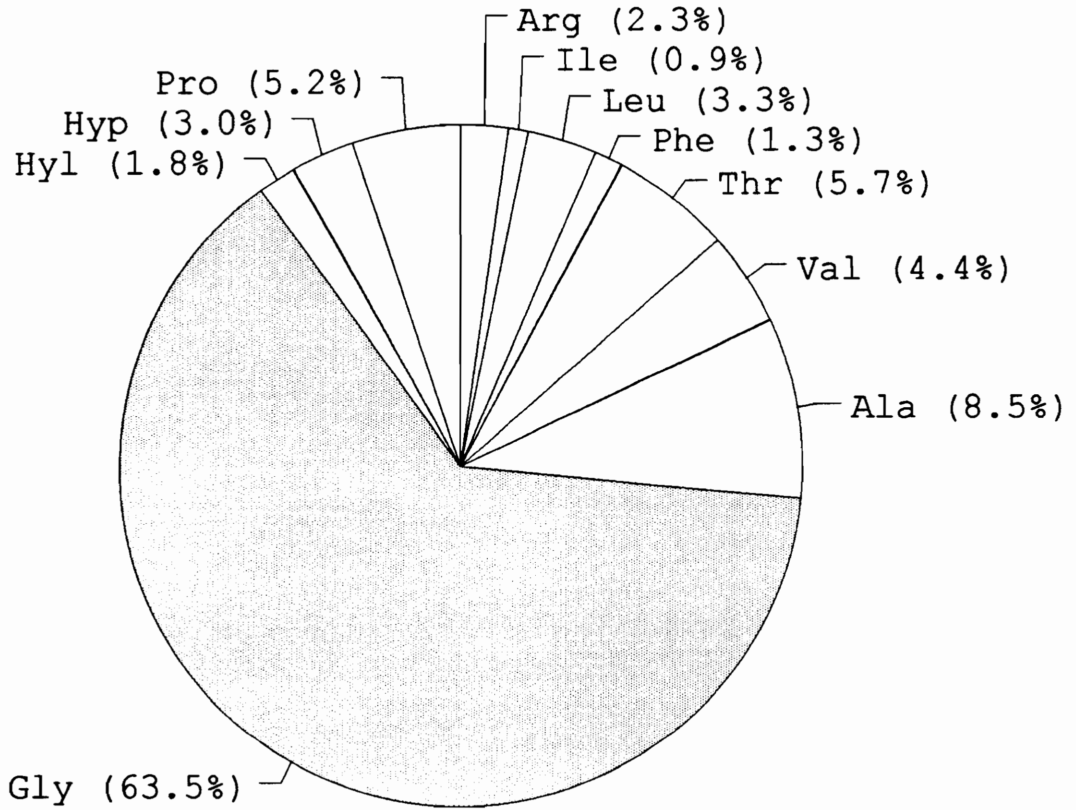



Figure 4.1. Average percent composition of the arterial peptide AA pool.

## EPILOGUE

Lactation can be best characterized by coordinated changes in metabolism of mammary and extramammary tissues to ensure a sufficient supply of substrate to the mammary gland for milk synthesis. Much of our current understanding of ruminant mammary gland metabolism comes from the combined research efforts of the following scientists: Linzell, Mepham, Bickerstaffe, Davis, Collier, Baldwin, and Bauman. Although their work has defined and shaped our fundamental understanding of nutrient utilization by the gland, much of their work was completed between 1960 and 1980.

Advances in diet formulation, genetics, biotechnology products, and management practices have superseded our ability to address all of the changing metabolic requirements of the lactating mammary gland. For example, 10 to 15 yr ago researchers and producers did not have to formulate diets for 20,000 to 30,000 lb rolling herd averages, fat-induced milk protein depression, and, most recently, the additional nutritional requirements for successfully implementing bST technology. Although current research efforts by investigators at the University of California-Davis (including DePeters, Cant, Miller, Hanigan, and Chow) have provided additional understanding of mammary gland metabolism, their progress has been limited by the complexity of this type of research. The data presented in this dissertation represent our first attempt within the Dairy Nutrition group at Virginia Tech to "tackle" a comprehensive in vivo study of nutrient metabolism by the lactating mammary gland. Our findings represent the hard work of many individuals, and provided a sound basis for future experiments. The following is an evaluation of our results in relation to current concepts regarding milk synthesis.

Utilization of nutrients by the mammary gland is controlled by many factors. One of the most important aspects of determining nutrient uptake by the gland is accurate measurement of blood flow. This project was one of few documented experiments to utilize ultrasonic blood flow probe technology to measure minute to minute variations in blood flow over extended periods of time. However, we found that while the probes were precise in measuring changes in blood flow, they did not accurately measure the magnitude of flow. Since completion of our study, Metcalf et al. (1992) reported similar results. Together, these results represent the first documented findings that question the accuracy of the ultrasonic probes during chronic implantation in high producing dairy cows. Estimating nutrient uptake by the Fick equation, however, enabled us to account for nearly 100% of the nutrients required to synthesize milk components. This was remarkable considering the number of nutrients analyzed and the inherent variation in estimating blood flow and nutrient concentrations in blood and milk.

Another finding of importance was the manner by which dietary fat increased the efficiency of milk production. It has been speculated that additional dietary fatty acids would "spare" glucose for synthesis of additional lactose (i.e. increased milk volume) by decreasing the requirements for de novo fatty acid synthesis. Only our work and that of Cant et al. (1993a and 1993b) have categorized total nutrient flux across the gland to verify this hypothesis. In addition, Cant et al. (1993a and 1993b), found that fat-induced milk protein depression resulted from a perturbation in AA uptake due to reduced blood flow to the mammary gland. Our results suggested, however, that there was sufficient blood flow and uptake of AA from blood.

Finally, this research represented one of the few in vivo experiments that have addressed the potential role of peptide AA in the lactating mammary gland. Previously, the plasma peptide AA pool in

nonlactating ruminants was shown to account for 50 to 80% of the available AA pool (DiRienzo 1990, Koeln et al. 1993, Seal and Parker 1991). However, we found that only 10% of whole blood AA were circulating as peptide AA in the lactating cow, and could not confirm peptide utilization by the lactating gland as shown by in vitro studies utilizing mammary epithelial cells (Wang et al. 1993) and muscle cells (Pan et al. 1993). This raises some interesting questions regarding the physiological state of an animal with regard to the "pool" of circulating peptides and the role of peptides as sources of AA for protein synthesis by the mammary gland in vivo.

Our results could not explain the cause of fat-induced milk protein depression observed in this study. As milk production increased, milk protein production increased but not at the same rate when cows consumed the fat-supplemented diets. However, uptake of free AA and mammary blood flow did not appear to be limiting. Therefore, some unknown factor(s) related to fat supplementation limits expression of the genetic potential of dairy cows to produce milk protein.

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## Appendix Tables

**TABLE A1.** Blood flow to the mammary gland estimated using the Fick principle or measured by Transonic® flow probes.[1,2,3]

| Blood flow | Treatments | | | | SEM | Effect ( *P* = ) | | |
|---|---|---|---|---|---|---|---|---|
| | CNTL | FAT | UIP | BOTH | | FAT | UIP | INTER |
| | ------------ L/min ------------ | | | | | | | |
| Fick | 14.82 | 16.98 | 17.70 | 16.61 | .80 | .49 | .17 | .10 |
| Flow probe | 8.40 | 8.24 | 8.28 | 8.05 | .25 | .48 | .22 | .64 |
| Flow probe fraction | -------------- % -------------- | | | | | | | |
| of Fick | 56.68 | 48.53 | 46.78 | 48.46 | | | | |

[1]CNTL= 0% added fat and 33% UIP; FAT= 2.5% added fat and 33% UIP; UIP= 0% added fat and 41% UIP; BOTH= 2.5% added fat and 41% UIP.

[2]Probability of effects due to 0% versus 2.5% FAT, 33% versus 41% UIP, and the interaction (INTER) between FAT and UIP.

[3]Transonic® flow probes (Transonic Systems Inc., Ithaca, NY).

**Table A2.** Molecular weight (MW) and percent composition of selected energy metabolites.

| Item | MW | %C |
|---|---|---|
| Glucose | 180.16 | 40.00 |
| Lactate | 90.08 | 40.00 |
| Pyruvate | 88.06 | 40.92 |
| | | |
| β-Hydroxybutyrate | 104.10 | 46.15 |
| Acetoacetate | 101.00 | 47.52 |
| | | |
| Acetate | 60.05 | 40.00 |
| Propionate | 74.08 | 48.64 |
| Butyrate | 88.10 | 54.43 |
| Isovalerate | 102.13 | 58.80 |
| | | |
| Oxygen | 15.99 | 0.00 |
| Carbon dioxide | 44.01 | 27.29 |
| Bicarbonate | 61.01 | 19.60 |
| Sodium | 22.98 | 0.00 |
| Potassium | 39.10 | 0.00 |
| | | |
| **Fatty acids** | | |
| 6:0 | 116.16 | 62.04 |
| 8:0 | 144.21 | 66.23 |
| 10:0 | 172.26 | 69.72 |
| 12:0 | 200.31 | 71.95 |
| 14:0 | 228.36 | 73.63 |
| 14:1 | 226.34 | 74.37 |
| 16:0 | 256.42 | 74.94 |
| 16:1 | 254.40 | 75.51 |
| 18:0 | 284.47 | 75.99 |
| 18:1 | 282.45 | 76.54 |
| 18:2(n-6) | 280.44 | 77.09 |
| 18:3(n-3) | 278.42 | 77.65 |

**TABLE A3.** Arterial concentrations, arteriovenous differences (A-V), mammary gland extractions, and uptakes of carbon and nitrogen as determined from CHN analyses.[1,2,3]

| | Treatments | | | | | Effect ( *P* = ) | | |
|---|---|---|---|---|---|---|---|---|
| Item | CNTL | FAT | UIP | BOTH | SEM | FAT | UIP | INTER |
| **Arterial** | ----------- g/L ------------- | | | | | | | |
| Carbon | 38.6 | 39.4 | 39.0 | 39.1 | .4 | .70 | .95 | .79 |
| Nitrogen | 10.2 | 10.4 | 10.5 | 10.4 | .2 | .99 | .74 | .67 |
| **A-V** | ----------- g/L ------------- | | | | | | | |
| Carbon | -.02* | -.03* | .01* | .61 | .26 | .30 | .26 | .29 |
| Nitrogen | -.03* | -.06* | -.04* | .13 | .06 | .41 | .33 | .28 |
| **Extraction** | ------------- % -------------- | | | | | | | |
| Carbon | -.005* | -.080* | -.008* | 1.525 | .667 | .31 | .27 | .27 |
| Nitrogen | -.192* | -.498* | -.372* | 1.310 | .661 | .41 | .34 | .26 |
| **Uptake** | ----------- kg/d ------------ | | | | | | | |
| Carbon | 3.81* | 2.20* | 2.22* | 12.48 | 4.5 | .22 | .21 | .12 |
| Nitrogen† | -.39 | -.92 | -.52 | 2.51 | 1.3 | .40 | .29 | .26 |

[1]CNTL= 0% added fat and 33% UIP; FAT= 2.5% added fat and 33% UIP; UIP= 0% added fat and 41% UIP; BOTH= 2.5% added fat and 41% UIP.

[2]Probability of effects due to 0% versus 2.5% FAT, 33% versus 41% UIP, and the interaction (INTER) between FAT and UIP.

[3]PE 2400 Series 11 CHNS/O Analyzer (Perkin Elmer, Norwalk, CT).

*Individual least squares mean did not differ from zero ($P > .05$).

†Least squares means in a row did not differ from zero ($P > .05$).

**Table A4.** Molecular weight (MW) and percent composition of amino acids and selected proteins.

| | MW | %C | %N |
|---|---|---|---|
| Arg | 174.20 | 41.36 | 32.16 |
| His | 155.16 | 46.44 | 27.08 |
| Ile | 131.17 | 54.94 | 10.68 |
| Leu | 131.17 | 54.94 | 10.67 |
| Lys | 146.19 | 42.29 | 19.16 |
| Met | 149.21 | 40.25 | 9.39 |
| Phe | 165.19 | 65.43 | 8.48 |
| Thr | 119.12 | 40.33 | 11.76 |
| Trp | 204.22 | 64.69 | 13.72 |
| Val | 117.15 | 51.26 | 11.96 |
| Ala | 89.09 | 40.44 | 15.72 |
| Asp | 133.10 | 36.09 | 10.52 |
| Asn | 132.12 | 36.36 | 21.20 |
| Cys | 121.16 | 29.74 | 11.56 |
| Glu | 147.13 | 40.81 | 9.52 |
| Gln | 146.15 | 41.09 | 19.17 |
| Gly | 75.07 | 32.00 | 18.66 |
| Hyp | 131.13 | 45.79 | 10.68 |
| Pro | 115.13 | 52.16 | 12.17 |
| Ser | 105.09 | 34.28 | 13.33 |
| Tyr | 181.19 | 59.66 | 7.75 |
| Cit | 175.19 | 41.13 | 23.99 |
| Orn | 132.16 | 45.44 | 21.20 |

## VITA

| | |
|---|---|
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| | |
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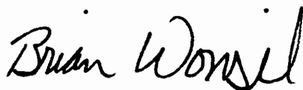
Gamma Sigma Delta, The Honor Society of Agriculture
American Dairy Science Association
American Society of Animal Science
Knights of Columbus



Brian J. Wonsil