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RELATIONSHIP OF NITROGEN METABOLISM CAPACITY, CARCASS QUALITY, AND EXPRESSION OF GLUTAMATE TRANSPORTERS AND METABOLIZING ENZYMES IN POLYPAY AND PERCENTAGE WHITE DORPER LAMBS

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ABSTRACT OF THESIS

RELATIONSHIP OF NITROGEN METABOLISM CAPACITY, CARCASS QUALITY, AND EXPRESSION OF GLUTAMATE TRANSPORTERS AND METABOLIZING ENZYMES IN POLYPAY AND PERCENTAGE WHITE DORPER LAMBS

Two studies were conducted to compare nitrogen (N) and glutamate metabolism in Polypay and percentage White Dorper lambs.

First, a two-phase digestion/N metabolism trial was conducted with 18 wether lambs of three genetic types: Polypay (PP), ½ White Dorper ½ Polypay (½ D), and ¾ White Dorper ¼ Polypay (¾ D). Six lambs of each genetic type were fed a high roughage diet (HR; Phase 1) or high concentrate diet (HC; Phase 2). DM and N digestion was higher for ½ D than PP or ¾ D fed HC. N retention was highest for ½ D regardless of diet.

The second study analyzed the effect of genetic type on glutamate transporter and metabolizing enzyme expression in liver, kidney, longissimus dorsi muscle (LD muscle), and subcutaneous fat (Sub Q Fat) tissue of 18 wether lambs of three genetic types: PP, ½ D, and $^{15}/_{16}$ White Dorper $^{1}/_{16}$ Polypay ($^{15}/_{16}$ D). Tissue samples were analyzed for protein and mRNA content of GS, GDH, ALT, EAAC1, and GLT-1. Glutamate transport and metabolism capacity was lowest for the heavier muscled $^{15}/_{16}$ D lambs.

The results suggest genetic type has an effect on N metabolism due to differential expression of glutamate transporters and metabolizing enzymes.

KEYWORDS: Nitrogen metabolism, glutamate transporters, glutamate metabolizing enzymes, genetic type, lambs

Andrea K. Lunsford

July 16, 2007

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July 16, 2007

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THESIS

Andrea Karen Lunsford

The Graduate School

University of Kentucky

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THESIS

A thesis submitted in partial fulfillment of the
requirements for the degree of the Master of Science in the
College of Agriculture
at the University of Kentucky

By

Andrea Karen Lunsford

Lexington, Kentucky

Director: Dr. Donald G. Ely, Professor of Animal Sciences

Lexington, Kentucky

2007

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CHAPTER I

Introduction

Nutrient requirements of ruminant animals are met by using a wide range of feedstuffs that fall into two categories: roughage and concentrate. The ratio of roughage to concentrate provided depends on the physiological state and production system of the animals. Regardless of the feedstuff consumed, ruminants must receive energy, amino acids, minerals, and vitamins at the cellular level in order to sustain life. Feedstuffs that meet these basic needs will need to supply the five classes of nutrients; carbohydrates, protein, fats, minerals, and vitamins.

The goal of livestock producers is to determine the most suitable biological type (i.e. genotype) for their given production setting, and to provide these animals with diets that will meet their basic needs in the most efficient and cost effective manner. On a weight basis, protein (nitrogen) is the most expensive and most limiting nutrient in growing animal diets. Therefore, a primary producer concern is the efficient use of dietary protein. Protein not only contributes to the nitrogen (N) requirement, but also supplies carbon skeletons for energy metabolism (Van der Walt, 1993). Furthermore, it is central to all metabolic processes. In fact, proteins (enzymes and enzyme systems) are organized in a unique manner in each organism, organ, cell, and even cell organelle in order to control metabolic events. Amino acids from protein molecules contribute to carbohydrate synthesis via gluconeogenesis, to fat synthesis or energy production via acetyl-CoA, and to the synthesis of special N compounds such as catecholamines (neurotransmitters), thyroid hormones, creatine (-phosphate), and the protoporphyrin ring (heme). Amino acids contribute to nucleic acid and phospholipid synthesis as N group donors (Nissim, 1999). According to Asplund (1994), one wise observer once said, “life is a drama played out on the surface of protein molecules.” After all, it is protein (N) that is required for the development of muscle, which is ultimately the product to be marketed (Van der Walt, 1993).

CHAPTER II

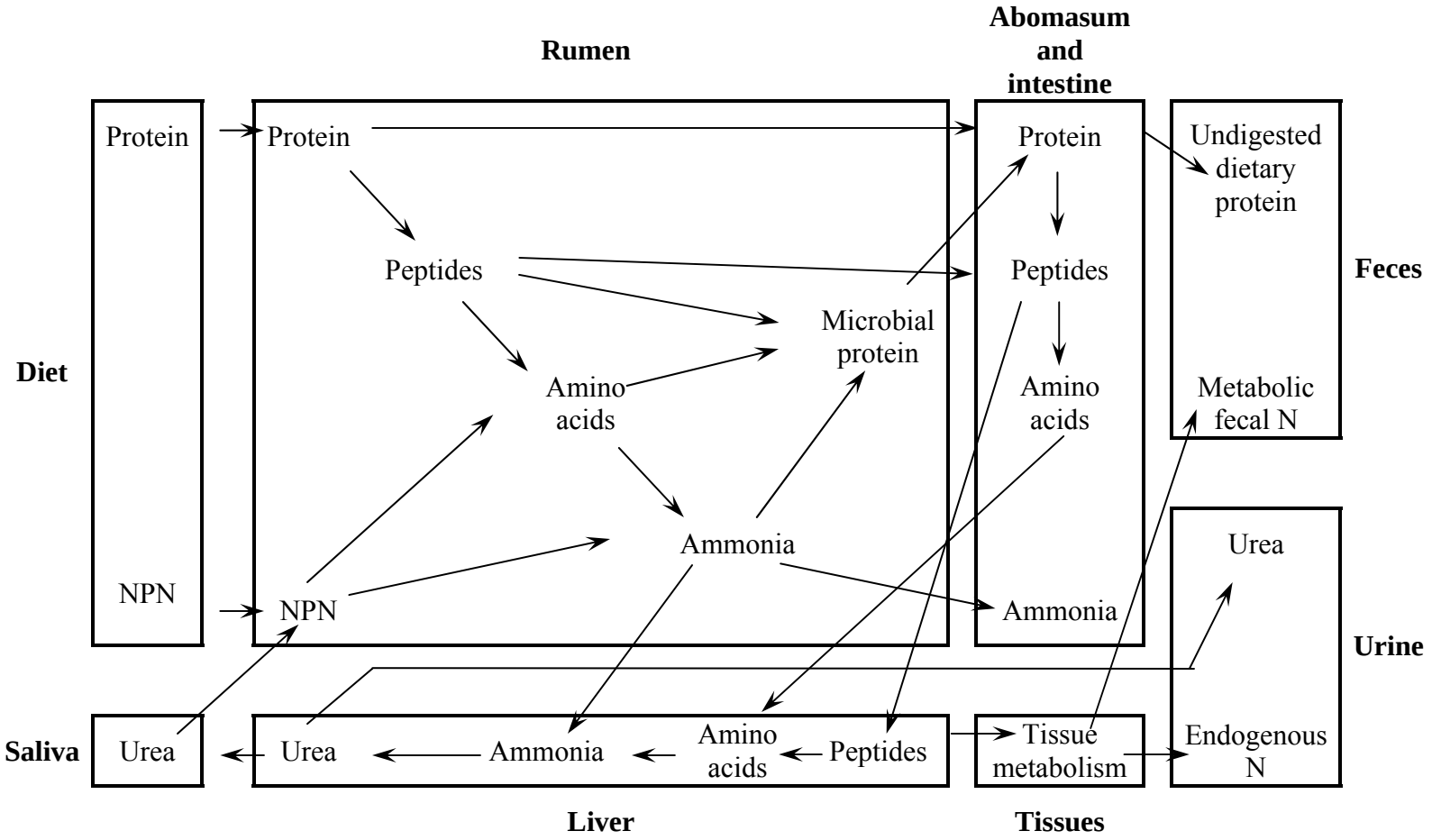
Literature Review

Nitrogen Metabolism in the Ruminant

To fully appreciate the importance of protein, an understanding of N metabolism is necessary. In nonruminants, N requirements are met by ingestion of proteins that are enzymatically degraded in the stomach and small intestine and, then, absorbed as peptides or free amino acids (Annison and Lewis, 1959). The situation in ruminants differs in that ingested proteins are subject to degradation by the rumen microorganism population prior to absorption by the animal.

Rumen. Dietary protein can be rapidly degraded, in the rumen, to amino acids which can be further degraded to ammonia and ketoacids and utilized for protein synthesis by the rumen microorganisms (Lewis, 1955). Rumen microbes can modify and supplement the amino acids of the ingested protein and, therefore, alter the amount of N that becomes available to the ruminant animal (Purser and Buechler, 1966). Because of this modification process, protein quality is dependent on the availability of amino acids leaving the rumen rather than in the ingested diet. Furthermore, it is a distinct advantage for the ruminant animal to be able to utilize N from both dietary protein and nonprotein nitrogen (NPN) sources. Three common NPN compounds found in the ruminant diet are urea, biuret, and nitrate, with urea being the most important (Fejes et al., 1983). NPN sources are rapidly degraded to ammonia and will also contribute to the microbial protein pool (Krishnamoorthy et al., 1983). Leng and Nolan (1984) estimated 60 to 90% of the daily N intake of the ruminant may be converted to ammonia, with 50 to 70% of bacterial N being derived from ammonia. Some NPN may enter the rumen via the diet, but some will come from urea that has been recycled through the body. This urea enters the rumen via saliva or directly by diffusion through the rumen wall (Nolan et al., 1976; Kennedy and Milligan, 1978; Varady et al., 1979). In the rumen, protein may take one of two paths depending on whether it is soluble or insoluble in the rumen liquor (Figure 2.1).

Figure 2.1. Nitrogen metabolism in the ruminant[1]

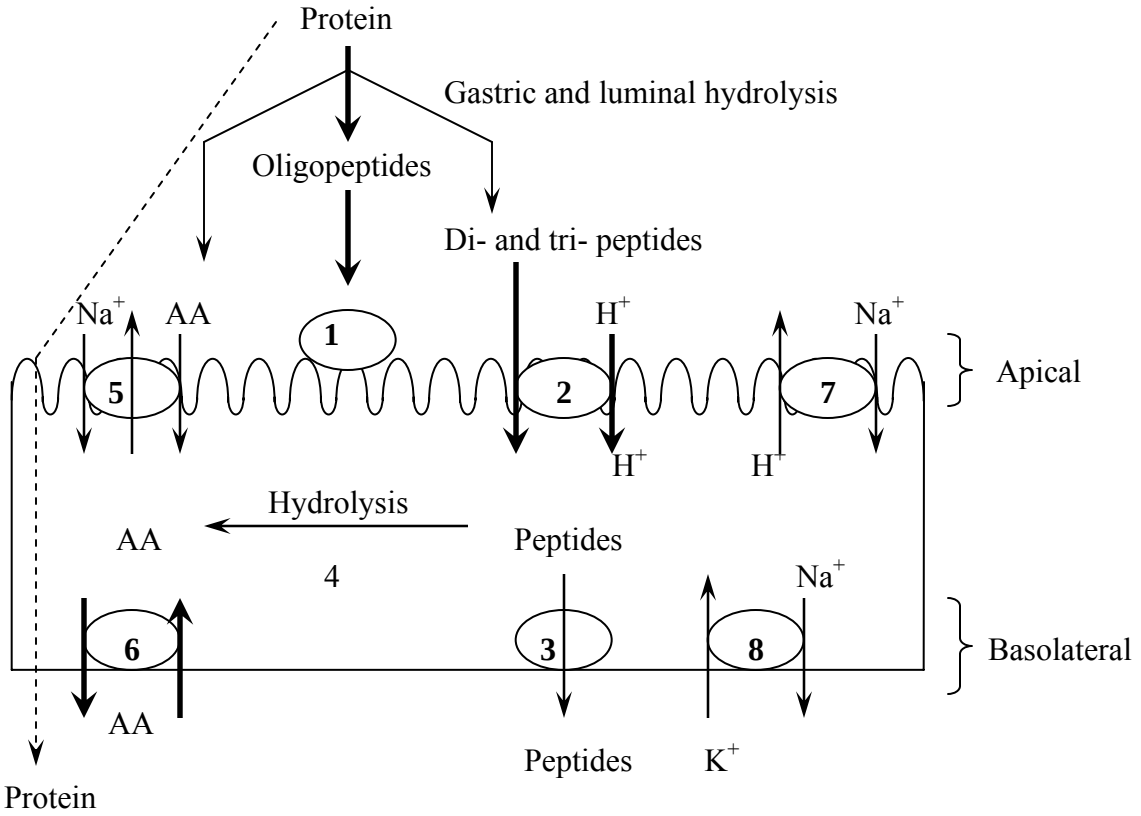


[1] Adapted from Annison and Lewis (1959) and incorporating data by Krehbiel and Matthews (2003).

Soluble protein will be metabolized by proteolytic microbes to peptides, amino acids, and ammonia (Lewis, 1955). A considerable amount of ammonia will be incorporated into microbial cells, but some will be absorbed across the ruminal epithelium (Huntington, 1986). When expressed in terms of metabolic mass, the amounts of ruminally absorbed ammonia are similar in sheep and cattle, ranging from 0.04 to 0.25 g N/day/kg BW, which corresponds to N intakes of 0.45 to 2.65 g N/day/kg BW (Nolan and Stachiw, 1979; Bunting et al., 1987; Bunting et al., 1989). Ammonia absorbed from the rumen will enter the portal blood and travel to the liver where it will be further metabolized. In addition to the ammonia, leucine, isoleucine, lysine, and methionine, as well as some dipeptides, can be absorbed from the rumen (Lewis and Emery, 1962; Liebholtz, 1971a, 1971b; Krehbiel and Matthews, 2003). On the other hand, the insoluble protein fraction passes through the rumen escaping the fermentation processes. Nitrogen fractions that exit the rumen include the insoluble protein fraction, ammonia, free amino acids, peptides, and microbial protein. Passage will be through the reticulum and omasum to the abomasum and small intestine for further degradation (Pilgrim et al., 1970; Smith, 1979; Storm et al., 1983; Storm and Orskov, 1984).

Small Intestine. The small intestine is the most important site of terminal digestion and absorption of the end products of rumen fermentation (Tagari and Bergman, 1978). Nitrogenous compounds enter the small intestine via the digesta flowing from the abomasum, the bile and pancreatic juices, or sloughed cells from the gastrointestinal tract (Van der Walt and Meyer, 1988). A large portion (64 to 69%) of this N will be in the α-amino linked form (Clarke et al., 1966), which can be absorbed across the small intestinal epithelium. Although it was initially assumed that N was assimilated from the small intestine solely in the form of free amino acids, research has shown peptides can also be absorbed across the small intestine wall (Armstrong and Hutton, 1975; Krehbiel and Mattews, 2003). Small intestinal cells have transport systems on both the luminal-facing and blood-facing sides to facilitate absorption of both peptides and free amino acids (Krehbiel and Matthews, 2003) (Figure 2.2).

Figure 2.2. Peptide and amino acid uptake for protein assimilation[1]



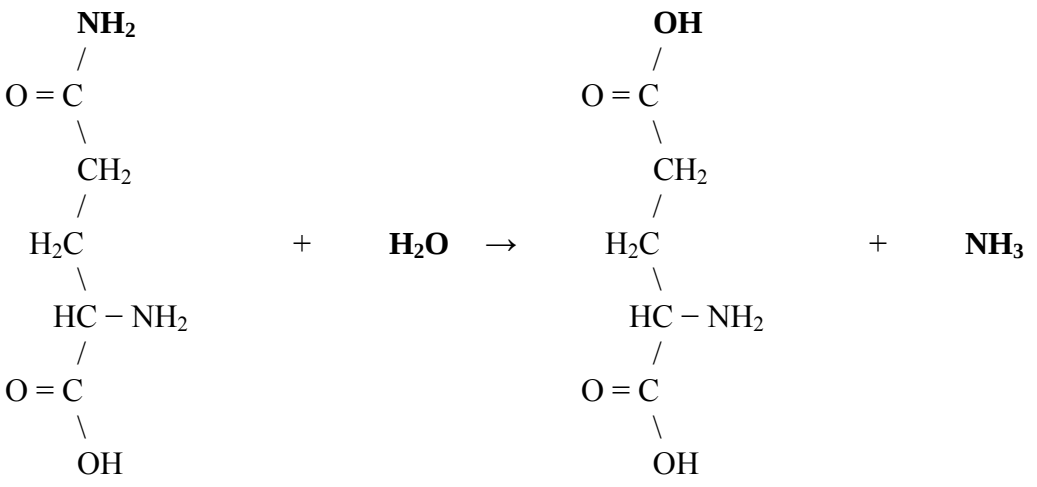
[1] Adapted from Krehbiel and Matthews (2003).

Amino acids appear to be absorbed mainly from the mid to lower ileum (Johns and Bergen, 1973), although the highest rate of absorption occurs in the mid jejunum (Ben-Ghedalia et al., 1974). Peptides and amino acids undergo extensive metabolic changes as they pass through the wall of the small intestine. Burrin and Reeds (1997) and Wu and Knabe (1996) showed both nonessential and essential amino acids are extensively metabolized by the intestinal mucosa. Macrae (1978) and Tagari and Bergman (1978) found up to 67 to 71% and 55 to 57% of absorbed amino acids were metabolized in the gut wall of sheep fed 19.8 and 15.6% CP diets, respectively.

There is a net absorption of most amino acids from the small intestine. For example, Wolff and Bergman (1972) and Heitmann and Bergman (1978) found glutamine was removed in large quantities from the small intestine of sheep fed at maintenance. Burrin et al. (1991) reported similar findings when growing lambs were fed at maintenance and ad libitum. Reynolds et al. (1988) also found small intestinal glutamine removed in Holstein cows 8 wk postpartum. Mucosal cells of the small intestine absorb glutamine from both the lumen and the arterial blood supply (Krehbiel and Matthews, 2003). Souba (1991) concluded the rate of uptake is equal to that of glucose and it is even more important than glucose as an oxidative fuel.

In the small intestine, glutamate serves as a major source of carbons for oxidation derived metabolic energy and for endogenous synthesis of alanine and other amino acids (Wu, 1998). Glutamate is a dietary nonessential amino acid. However, it is essential in supporting whole body energy metabolism and maintaining N homeostasis throughout the body (Heitmann and Bergman, 1981; Fahey and Berger, 1988; Wu, 1998; Nissim, 1999). Glutamate shares a number of functions with glutamine, also a dietary nonessential amino acid. Many metabolic functions can be fulfilled by either substrate, because they are metabolically interconvertible (Snyderman et al., 1962). Glutaminase catalyzes the conversion of glutamine to glutamate (Kvamme et al., 1985) (Figure 2.3), whereas glutamine synthetase catalyzes the reverse reaction (Meister, 1985) (Figure 2.4). Energy derived from the oxidation of glutamate is used to support intestinal ATP-dependent metabolic processes, such as active nutrient transport and high rates of intracellular protein turnover (Burrin and Reeds, 1997).

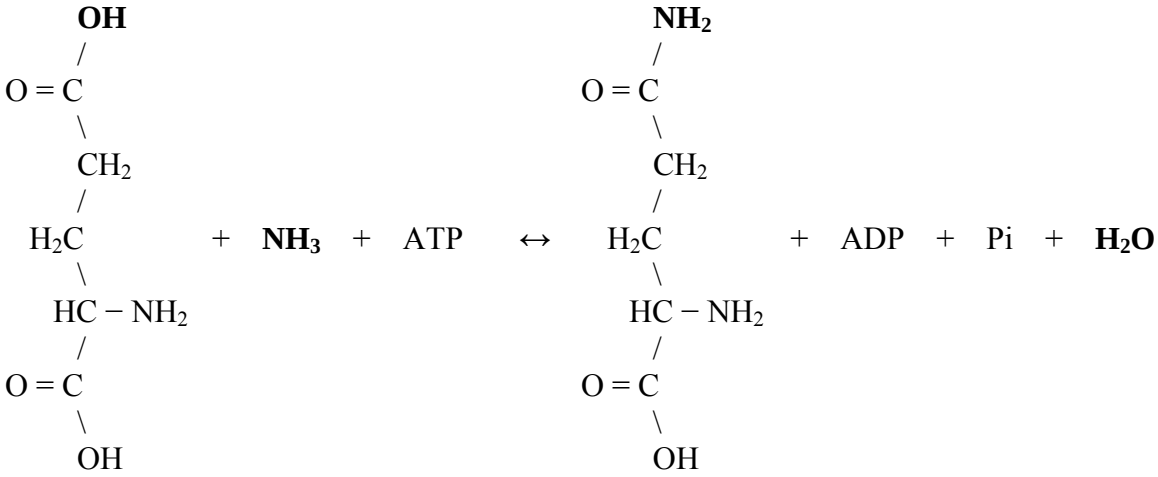
Figure 2.3. Hydrolysis reaction catalyzed by glutaminase[1]



Glutamine ($C_5H_{10}N_2O_3$) + Water → Glutamate ($C_5H_9NO_4$) + Ammonia

[1] Adapted from Kvamme et al. (1985).

Figure 2.4. Condensation reaction catalyzed by glutamine synthetase[1]



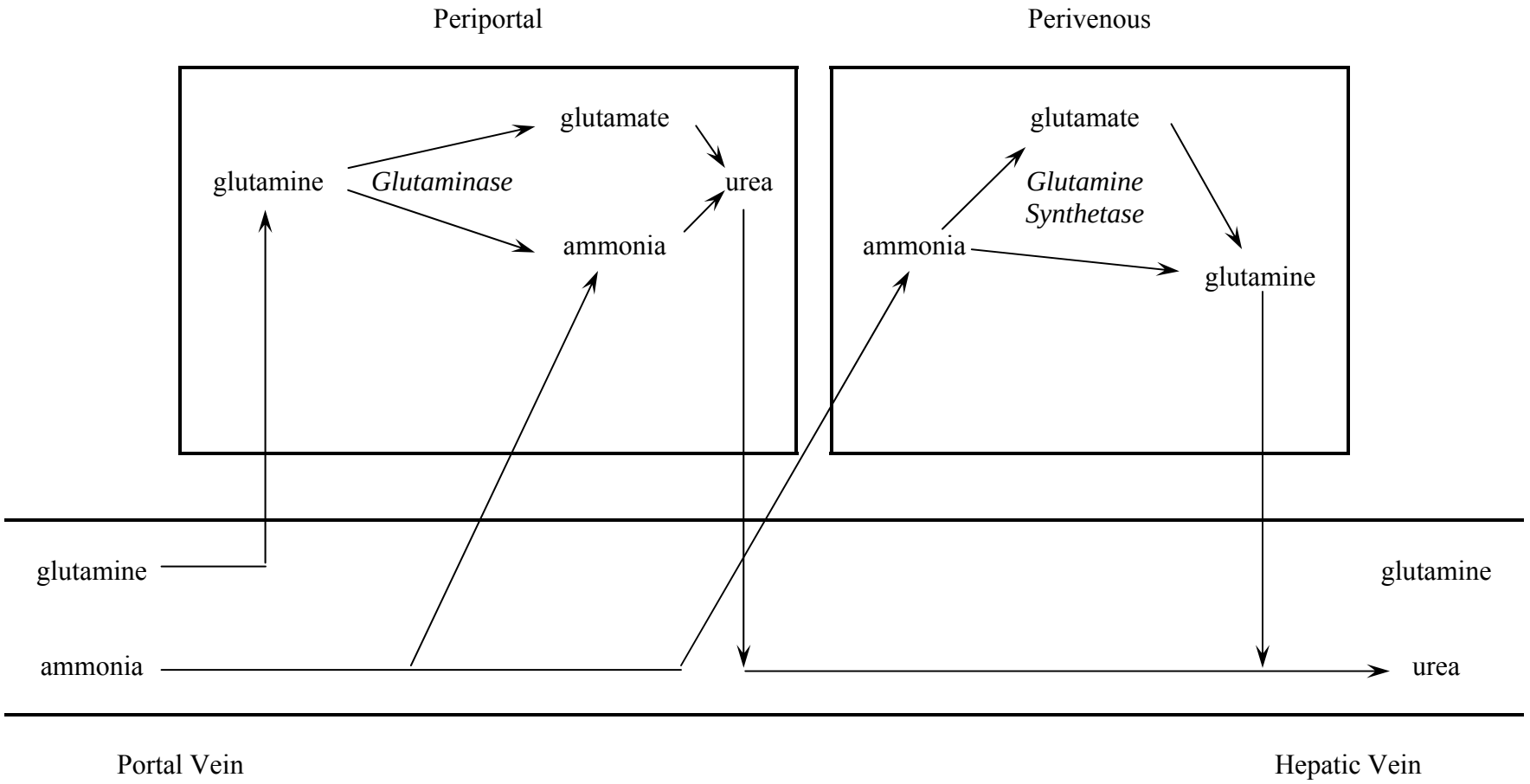
Glutamate + Ammonia + Adenosine ↔ Glutamine + Adenosine + Phosphate
($C_5H_9NO_4$) triphosphate ($C_5H_{10}N_2O_3$) diphosphate

[1] Adapted from Meister (1985).

Luk et al. (1980) found ornithine, a by-product of glutamine metabolism, to be the immediate precursor for polyamine synthesis. According to Luk et al. (1980), this synthesis is essential for proliferation, differentiation, and repair of intestinal epithelial cells. Furthermore, glutamate is a precursor for the synthesis of glutathione, a tripeptide critical for defending the intestinal mucosa against toxic and peroxidative damage (Reeds et al., 1997). A study conducted by Windmueller (1982) demonstrated the small intestine extracted 25 to 33% of the arterial glutamine in a single pass, which accounts for 30% of whole body glutamine utilization in the postabsorptive rat. In contrast to glutamine, the uptake of arterial glutamate in the small intestine is nonsignificant (Windmueller, 1982). However, intra-luminally delivered glutamate and glutamine are metabolized extensively by the small intestine mucosa. Windmueller and Spaeth (1975) found 66 to 98% of luminal glutamine and glutamate was catabolized in a single pass of the adult rat small intestine. The carbon skeletons of the glutamine were metabolized to CO_2 (64%) and lactate (11%), while the N was exported as ammonia (38%), citrulline (28%), and alanine (24%), thus providing a major energy source for the small intestine (Windmueller and Spaeth, 1974, 1975, 1980; Windmueller, 1982). It is likely that similar reactions occur in the ruminant small intestine, since research with sheep has shown a net export of alanine and citrulline from portal drained viscera (Wolff and Bergman, 1972; Heitmann and Bergman, 1978; Burrin, et al., 1991). Ultimately, digested protein will enter the portal blood as peptides and free amino acids (Krehbiel and Matthews, 2003). Once in the portal blood, these components will travel to the liver for further metabolism.

Liver. The liver is positioned so all metabolites taken from the gastrointestinal tract must pass through it before circulating into the rest of the body. Thus, the liver plays a central role in coordinating whole body energy and N metabolism (Matthews and Sipe, 2006). It is the major organ for amino acid disposal and the only organ with a urea cycle (Brosnan, 2003). Van der Walt (1993) classified liver cells (hepatocytes) into two categories, periportal and perivenous, depending on their location along the portal-venous gradient (Figure 2.5). The periportal hepatocytes comprise 95% of the hepatic cells along the acinus and reside from the terminal portal venules to deep within the acinus.

Figure 2.5. Hepatic arrangement of the intercellular glutamate/glutamine cycle[1]



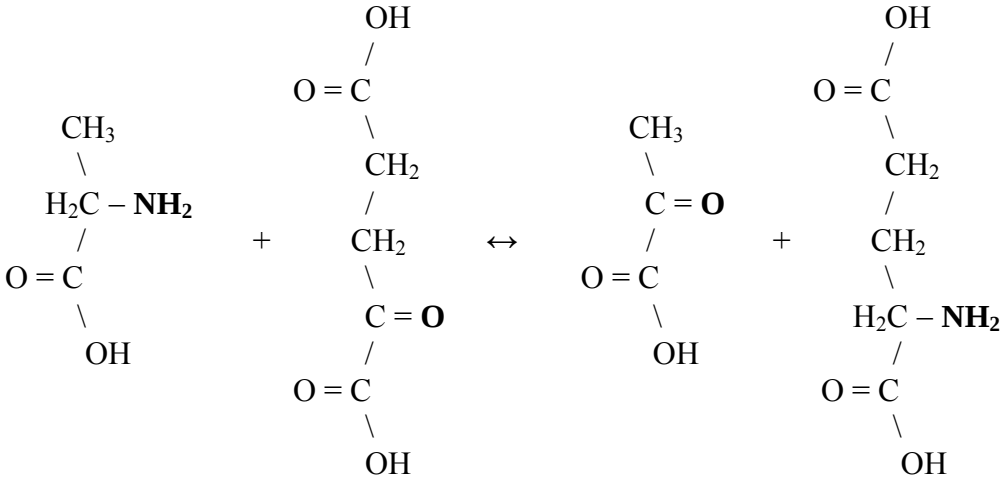
[1] Adapted from Haussinger et al. (1985), Watford (2000), and Brosnan (2003).

The perivenous hepatocytes comprise the remaining 5% of the hepatocytes along the acinus and are localized within a small population of hepatocytes surrounding the terminal hepatic venules at the outflow of the sinusoidal bed. Haussinger and Gerok (1983) demonstrated the heterogeneity of glutamate transport and glutamate metabolism along the hepatic acinii was key to glutamate metabolism, which allows for its role in the glutamate/glutamine cycle. Haussinger et al. (1985) and Guder et al. (1987) found glutaminase and the enzymes of the urea cycle to be localized to the periportal cells, while glutamine synthetase was confined to the perivenous cells. This arrangement of enzymes allows the levels of portal blood ammonia to control the activity of periportal glutaminase (Souba, 1991). Glutamine in portal blood will be taken up by the periportal cells and contribute additional ammonia to the intra-mitochondrial pool. This ammonia activates the urea cycle, whereas the glutamine synthetase system in the perivenous cells is a high affinity, low capacity system which scavenges any ammonia overflow from the urea cycle (Van der Walt, 1993).

The collective concentration of free amino acids in plasma is ~2 mM, with glutamine being the most abundant (Brosnan, 2003). Excess dietary amino acids entering the liver via the portal vein will largely be converted to glucose (Van der Walt, 1993). The main pathway for the deamination of amino acids in hepatocytes starts by transferring the α-amino group to α-ketoglutarate (ketoacid of glutamate) by means of a transaminase in the cytoplasm. Alanine transaminase catalyzes the conversion of alanine and α-ketoglutarate to pyruvate and glutamate (Figure 2.6). The resulting glutamate can then be taken up by the mitochondria where it is oxidatively deaminated to α-ketoglutarate by glutamate dehydrogenase, an enzyme exclusive to mitochondria (McGivan and Chappell, 1975) (Figure2.7). This set of reactions is the route of deamination for most amino acids (Krebs, 1972; Torchinsky, 1987).

In contrast, ammonia entering the liver through the portal blood is largely converted to urea via the urea cycle (Van der Walt, 1993) (Figure 2.8). However, this ammonia can provide N for synthesis of amino acids and ultimately tissue protein synthesis, heme formation, pyrimidine and purine synthesis (nucleotide precursors), ketone body formation, carbohydrate synthesis, and de novo synthesis of nonessential amino acids (Van der Walt, 1993).

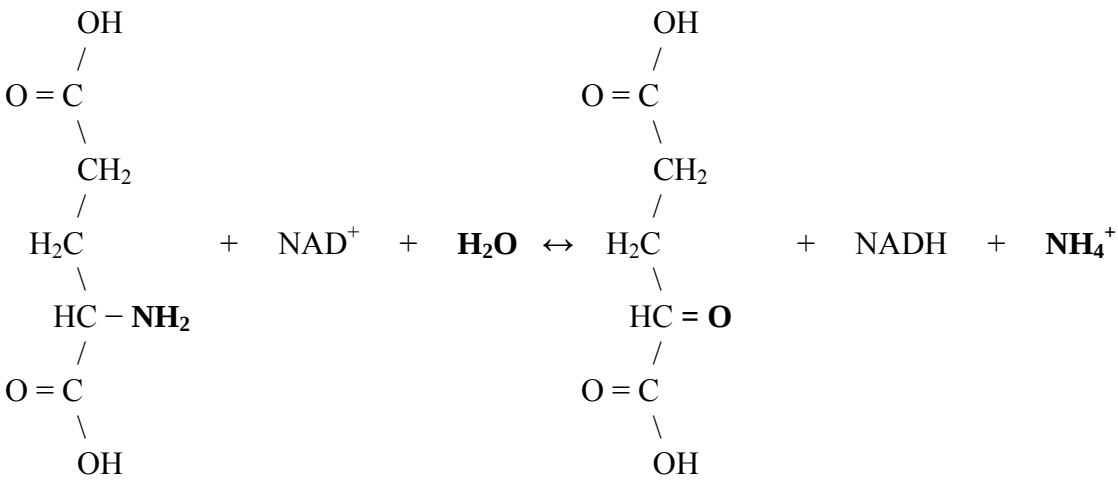
Figure 2.6. Transamination reaction catalyzed by alanine transaminase[1]



Alanine	+	α-Ketoglutarate	↔	Pyruvate	+	Glutamate
($C_3H_7NO_2$)		($C_5H_6O_5$)		($C_3H_4O_3$)		($C_5H_9NO_4$)

[1] Adapted from Cooper (1985).

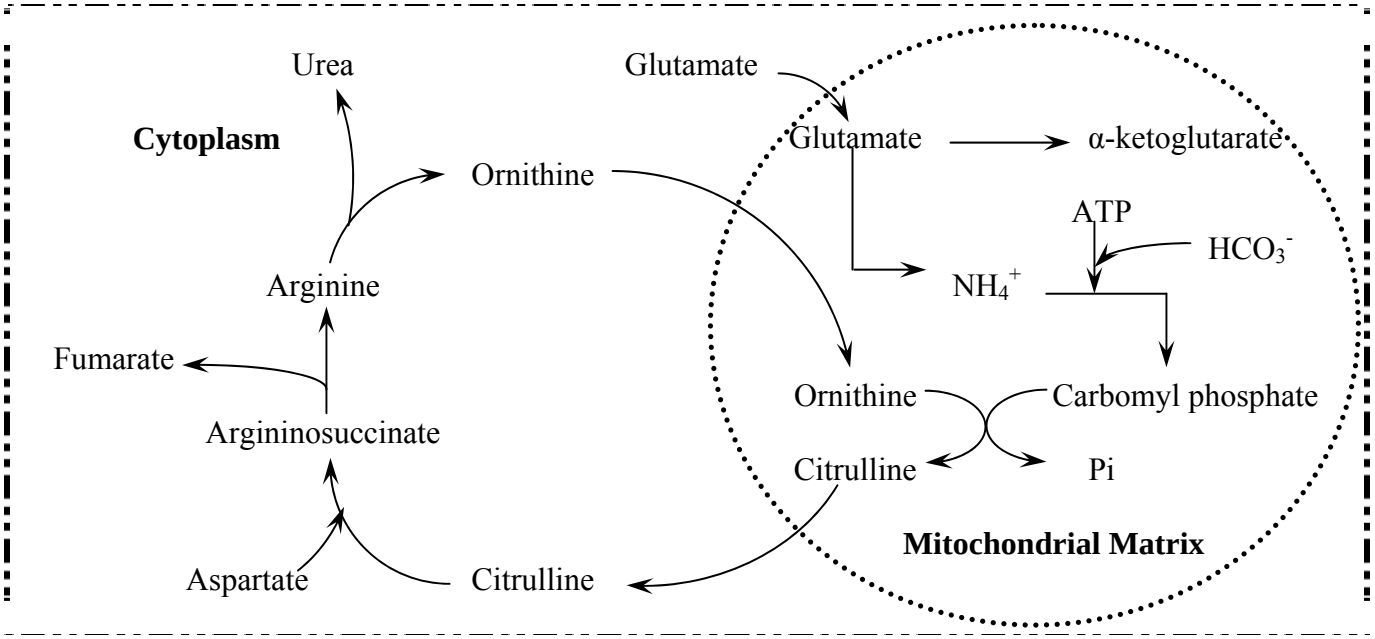
Figure 2.7. Mitochondrial specific reaction catalyzed by glutamate dehydrogenase[1]



Glutamate + NAD^+ + Water ↔ α-Ketoglutarate + NADH + Ammonia
($C_5H_9NO_4$) ($C_5H_6O_5$)

[1] Adapted from Fisher (1985).

Figure 2.8. Urea cycle in the ruminant hepatocyte



This metabolism of portal blood ammonia prevents an accumulation that can become highly toxic. In ruminants, normal plasma ammonia levels are 30 to 80 μM (Hemingway et al., 1972; Davidovich et al., 1977; Redmond et al., 1993).

The operation of an intercellular glutamine/glutamate cycle to partition ammonia uptake between ureagenesis and glutamine synthesis assists in the control of whole body pH balance (Van der Walt, 1993; Matthews and Sipe, 2006) (Figure 2.5). Glutamate is a central substrate for hepatic ureagenesis, gluconeogenesis, glutathione production, de novo protein synthesis, and N shuttling via glutamine (Meijer et al., 1990; Matthews, 2000; Watford, 2000). Haussinger et al. (1989) demonstrated that glutamine anabolism and catabolism occurred simultaneously. This researcher showed $^{14}CO_2$ was produced from [1-^{14}C]glutamine in the presence of a net glutamate release from intact rat liver, switching to a net glutamate uptake at portal glutamate concentrations above 0.3 mM (Haussinger and Gerok, 1983). Because of the extensive utilization of dietary glutamate by the intestine (Tagari and Bergman, 1978; Reeds et al., 1996; Wu, 1998), the primary source of circulating glutamate is the liver. Under normal physiological conditions, the liver is the principal source of glutamate for peripheral tissue use. Based on this information, the liver makes all other systems function. In fact, hormonal control makes sure animals in a protein deprived state first degrade proteins from nonessential organs like skeletal muscle, while liver enzymes for gluconeogenesis and the urea cycle are enhanced. This allows free amino acid levels and blood plasma proteins to be maintained at constant levels despite fluctuations in dietary intake and tissue demands.

Kidney. Renal ammoniagenesis is a major regulator of acid-base balance in the body. Pitts (1972) found ammonia was excreted, as an ammonium ion, in response to acid loads. Most of the newly formed ammonia comes from the amide and amino N atoms of glutamine (Van Slyke et al., 1943). In the kidney, plasma and kidney synthesized glutamate acts to regulate ammoniagenesis, serves as a precursor for incorporation into glutathione and extracellular matrix protein, and serves as a source of carbons for mitochondrial oxidation derived energy (Welbourne and Matthews, 1999; Brosnan, 2003). The kidney generates high fluxes of glutamate without any net utilization or production (Hediger and Welbourne, 1999; Patterson et al., 2002) and, like the liver,

possesses a well defined glutamate/glutamine cycle (Nissim, 1999). In addition, Heitmann and Bergman (1981) determined the kidney has gluconeogenic capacity, providing significant amounts of glutamate derived glucose in fasted and acidotic animals.

Skeletal Muscle. Most of the body's protein is in the form of muscle protein. Therefore, muscle plays an important role in intra-organ amino acid metabolism. For example, during starvation, amino acids are released from muscle tissue to help support bodily functions. Glutamine and alanine account for approximately 50% of the amino acids released during this type of scenario (Brosnan, 2003). Glutamine is derived from the metabolism of intramuscular glutamate or metabolism of aspartate, asparagine, valine, or isoleucine (Brosnan, 2003). Biolo et al. (1995) and Vesli et al. (2002) showed skeletal muscle also plays a key role in whole body ammonia recovery and detoxification. Glutamate is central to many of these metabolic processes in skeletal muscle (Graham et al., 2000). Not only does glutamate participate in the transamination of branched chain amino acids, it is pivotal to the formation of ammonia, aspartate, alanine, and glutamine (Mourtzakis and Graham, 2002). In skeletal muscle, the production of glutamine, from plasma glutamate, is thought to constitute an important route for whole body ammonia recovery and detoxification (Biolo et al., 1995; Vesli, 2002). Through this route, muscle becomes a net user of glutamate and a producer of glutamine and alanine for use by other organs and cell types. Although glutamate is abundant in the skeletal muscle free amino acid pool, it is the primary amino acid consumed by resting and active muscle (Mourtzakis and Graham, 2002). Skeletal muscle uptake of glutamate, intracellular conversion to glutamine, and subsequent export to the plasma is essential to the proper functioning of intestinal epithelia and the immune system (Newsholme and Parry-Billings, 1990; Hack et al., 1996).

Adipose Tissue. Adipose tissue was once considered a rather inert storehouse for excess energy. In contrast, it is now recognized to have a dynamic pattern of metabolism (Frayn et al., 1991). Information pertaining to adipose tissue amino acid metabolism is limited (Kowalski and Watford, 1994), but knowledge is being gained by arteriovenous flux

studies that employ microdialysis sampling techniques (Summers et al., 1998; Rolinski et al., 2001; Lange et al., 2002; Patterson et al., 2002). From these studies, Frayn et al. (1991) identified human subcutaneous adipose tissue as a net producer of alanine and glutamine and net utilizer of glutamate. In contrast, Kowalski and Watford (1994) found the rat inguinal fat pad was a net producer of glutamine, but not alanine, and net utilizer of glutamate. In addition, adipose tissue is thought to be a major site of whole body glutamine synthesis. Evidence of glutamine exchanges between blood and adipose tissue in vivo suggests adipose tissue plays a significant role in whole body glutamine turnover (Ritchie et al., 2001).

Amino Acid Transport Systems

Given the central role of glutamate to support tissue and whole body growth and maintenance, knowledge regarding the functional properties of biochemically and molecularly characterized glutamate transport systems is critical. In fact, these transport systems are composed of transporter proteins that allow a cell or organelle to selectively bind and acquire compounds from other substrates. Typically, transport activities that demonstrate relatively low affinities have high capacities for transport, whereas those that display high affinities have low capacities (Matthews, 2000). In mammals, mediated absorption of anionic amino acids (glutamate and aspartate) occurs by Na^+- dependent (X^-_{AG}, X^-_A, ASC, or B^o) or Na^+-independent (x^-_c or x^-_G) biochemical activities (Matsuo et al., 2002; Matthews and Sipe, 2006). High affinity and concentrative uptake of anionic amino acids occurs principally in Na^+- dependent X^-_{AG} and X^-_A transport systems, which recognize only anionic amino acids and some of their analogues. System X^-_{AG} activity is biochemically defined as a high affinity (K_m=1 to 50 μM), Na^+- dependent transporter of L-glutamate or L-aspartate and is inhibited by D-aspartate. In contrast to system X^-_{AG} activity, system X^-_A does not transport glutamate or its analogues (Matsuo et al., 2002). In addition, Na^+- dependent transport systems ASC and B^o transport L-glutamate and L-aspartate, but only in acidic environments (pH < 5.5). Na^+-independent uptake of anionic amino acids is mediated by systems x^-_c or x^-_G. System x^-_c transport is an obligate exchange activity that typically mediates the exchange of L-glutamate for L-cystine, but also recognizes D-glutamate. In contrast, system x^-_G only transports L-glutamate. The

limited knowledge about tissue specific distribution patterns of these anionic amino acid transport activities in ruminant tissues has been reviewed elsewhere (Matthews, 2000). However, recent advances in the biochemical, molecular, and regulatory properties of system X^-_{AG} transporters, with particular focus on ruminant systems, are discussed below.

System X^-_{AG} Transport Proteins

System X^-_{AG} is likely the principal anionic amino acid transport system responsible for glutamate based N metabolism in ruminants, given the widespread distribution of system X^-_{AG} protein transporters in sheep and cattle (Howell et al. 2001, 2003; Matthews and Sipe, 2006). Five mammalian proteins have been cloned that are capable of system X^-_{AG} transport activity. Originally identified in nonhumans, the transporter orthologs (and genes) are known as EAAC1 (SLC1A1), GLT-1 (SLC1A2), GLAST1 (SLC1A3), EAAT4 (SLC1A6), and EAAT5 (SLC1A7). All are members of the Solute Carrier Family 1 (Kanai and Hediger, 2004) and are differentially expressed by a number of tissues. EAAC1 mRNA and protein have been detected in the heart (Nakayama et al., 1996), small intestine (Kanai and Hediger, 1992; Gissendanner, 2004; Sipe, 2004), skeletal muscle (Velaz-Faircloth et al., 1996), and liver (McGivan and Nicholson, 1999; Howell et al., 2001, 2003; Gissendanner, 2004; Sipe, 2004). GLT-1 mRNA has been detected in the kidney (Welbourne and Matthews, 1999) and liver (Howell et al., 2001, 2003; Kim et al., 2003). GLAST1 mRNA has been detected in the heart (Nakayama et al., 1996). EAAC1, GLAST1, and GLT-1 mRNA and protein are expressed in placental tissue (Matthews et al., 1998a, 1998b; Danbolt, 2001). In the brain, only GLAST1 and GLT-1 proteins (Torp et al., 1994; Schmitt et al., 1996; Torp et al., 1997; Berger and Hediger, 1998) are expressed in astrocytes, whereas EAAC1 and EAAT4 are the predominant transporters in neurons (Rothstein et al., 1994; Kanai et al., 1995; Berger and Hediger, 1998). Furthermore, EAAT4 and EAAT5 are predominantly expressed in the cerebellum (Fairman et al., 1995; Nagao et al., 1997) and the retina (Arriza et al., 1997), respectively. Howell et al. (2001) found EAAC1 was expressed in homogenates of forestomach (rumen, omasum), small intestinal epithelium (duodenum, jejunum, ileum), large intestinal epithelium (cecum, colon), liver, kidney, and pancreatic

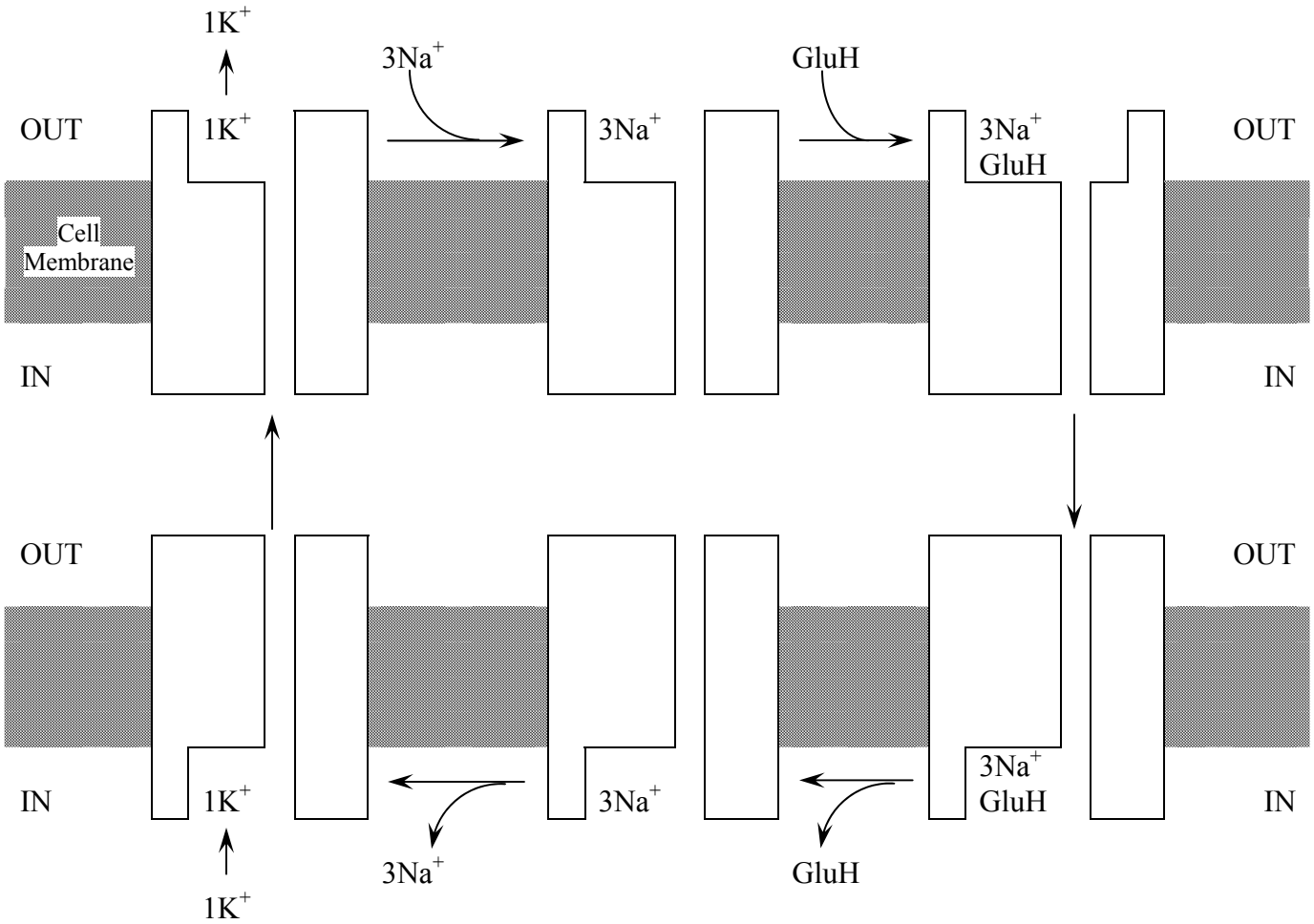
tissues of sheep and cattle. Matthews (2000) concluded EAAC1 was the primary system X^-_{AG} isoform responsible for supplying anionic amino acids used in general metabolic processes.

System X^-_{AG} can transport glutamate against a steep electrochemical gradient (Kanai and Hediger, 2004). By utilizing the stored energy in the electrochemical gradients of Na^+ and K^+, glutamate can be concentrated in tissue. The mechanism of this action involves the co-transport (extracellular to intracellular) of three Na^+ molecules and one glutamate molecule. Re-orientation of the transporter to the extracellular face of the membrane is driven by the counter transport (intracellular to extracellular) of one K^+ molecule (Kanai and Hediger, 2004). In addition, either one OH^- molecule is counter transported or one H^+ molecule is co-transported (Danbolt, 2001) (Figure 2.9). EAAT4 and EAAT5 also have a large inward chloride ion flux associated with their function. This flux may aid in the re-establishment of membrane potential by influencing cellular chloride permeability and has led these transporters to be the most accurately described as that of glutamate gated chloride channels (Arriza et al., 1997). Although system X^-_{AG} transporters typically transport Na^+ and anionic amino acids from the extracellular fluid into the cytosol, they are capable of reverse transport when the typically high intracellular K^+ levels are too low (Zerangue and Kavanaugh, 1996; Levy et al., 1998).

Enzymes Associated with Glutamate Metabolism

Glutamine synthetase (GS) is responsible for two key functions in ruminant N metabolism: ammonia assimilation and glutamine biosynthesis. GS is present in perivenous cells of the liver where it catalyzes the condensation of glutamate to glutamine (Figure 2.4). Also present in the kidney, GS helps limit the release of ammonia, generated by the renal cells, into both the urine and renal vein (Conjard et al., 2003). Thus, GS is important in the detoxification of ammonia, interorgan N flux, and contributes to the regulation of whole body acid-base balance (Haberle et al., 2005).

Figure 2.9. Generalized diagram of a system X^-_{AG} transporter protein[1, 2]



[1] Adapted from Kanai and Hediger (2004).
[2] GluH = glutamate with an attached H^+.

Ammonia incorporation in animal tissue occurs through the actions of both GS and glutamate dehydrogenase (GDH). GDH, located exclusively in mitochondria (Smith et al., 1975), catalyzes the oxidative deamination of glutamate to produce α-ketoglutarate (Figure 2.7). Although the GDH reaction is reversible, glutamate synthesis is strongly favored at equilibrium (Hudson and Daniel, 1993).

Another important enzyme in glutamate metabolism and N shuttling throughout the body is alanine transaminase (ALT). ALT catalyzes the reaction of alanine and α-ketoglutarate to produce pyruvate and glutamate (Figure 2.6). Alanine, like glutamine, is a major storage molecule for glutamate N (Erecinska, et al., 1994), making it an important player in N metabolism and glutamate and glutamine cycling.

Regulation of Glutamate Transporter Proteins

Ferrer-Martinez et al. (1995) used a cellular model (MDBK/NBL-1 cell line) to show hypertonic stress was the cause of an up regulation in EAAC1 transport activity and mRNA expression. Matthews et al. (1999) demonstrated endocrine hormones affected the regulation of certain glutamate transporters. These workers used transgenic mice placental tissue to demonstrate over expression of bovine growth hormone can increase mRNA and protein expression for the GLT-1 transporter. In mice that did not express the IGF-II gene, an increase in EAAT4 protein and mRNA expression was shown. However, the same mice showed a decrease in GLT-1 protein expression, but an increase in GLT-1 mRNA expression. This difference was attributed to a post transcriptional mode of regulation. Howell et al. (2003) analyzed the effects of growth on glutamate transporter regulation in the ruminant. EAAC1 and GLT-1 were up regulated with growth and this up regulation occurred in different tissue types.

There is ongoing research exploring the mechanisms that control GS activity, because this enzyme catalyzes the production of glutamine from glutamate and ammonia. Feng et al. (1990) demonstrated the transcription of GS can be stimulated by lowered levels of glutamine. Furthermore, signaling of the nuclear transcription factor, β-catenin, has been shown to cause over expression of GS and GLT-1 (Cadoret et al., 2002). Clearly, further research is needed to understand the mechanisms that regulate glutamate uptake by the cell.

Regulation of Whole Animal Nitrogen Metabolism

In contrast to the limited amount of research concerning glutamate regulation, many studies have been conducted that analyzed the mechanisms involved in regulating N metabolism and digestibility. Of particular importance for this study is direct genetic type comparisons of differences in N metabolism and digestibility. Comparison of the digestive physiology of cattle, sheep, goats, and deer has shown digestibility and N metabolism differs among ruminants and can be influenced by diet selection, ruminal fermentation rate, and digesta passage rate (Huston et al., 1986). However, information on the effects of genetic type, within a species, on nutrient digestibility is limited. It is hypothesized that genetic types have adapted to different nutritional environments and, therefore, have evolved with different digestive mechanisms and possibly different efficiencies of digestion and N metbolism. For example, within cattle, a number of digestibility differences have been found when comparing *Bos indicus* with *Bos taurus* species. Research has shown *Bos indicus* cattle have a higher digestive efficiency and higher rumen fermentation rate than *Bos taurus* (Hungate et al., 1960; Howes et al., 1963; Hunter and Siebert, 1985). However, this was a species rather than a genetic type within species comparison. Similar studies, using goats, have shown differences in digestive capacities, digesta passage rates, and ruminal fermentation rates among breeds of goats (Hart et al., 1993; Sahlu et al., 1993; Silanikove et al., 1993). Angora (mohair) goats had higher protein and energy requirements than Alpines (milk) and Nubians (dual purpose). The difference was attributed to the unique fiber production of the Angora breed (Hart et al., 1993). Within sheep, only a few studies have been conducted that specifically determined the effect genetic type has on nutrient utilization. A comparison of Cheviot and Suffolk sheep showed breed affected digestibility to a higher degree than did age or body weight (Givens and Moss, 1994). Although breed produced significant ($P < 0.01$) differences in all digestibility values for two diets, neither age nor body weight produced statistically significant differences. This study also showed Cheviot had a higher digestive efficiency than Suffolk and the difference in digestibility tended to be greater for the less digestible diet (Givens and Moss, 1994). Ranilla et al. (1997) found dry matter degradability was more efficient in the Churra than Merino sheep. This difference was attributed to greater ruminal microbial activity in the Churra. An important

conclusion that came from both studies, and later studies, was that differences in digestibility between breeds of sheep tend to be greater when less digestible feedstuffs are consumed (Quick and Dehority, 1986; Ragland, 1990; Ranilla et al., 1998). The two parent breeds of sheep used in this study, Polypay and White Dorper, were developed for different production qualities and have become adapted to different production conditions.

The Polypay, a traditional wool breed, was developed at the U.S. Sheep Experiment Station in Dubois, ID. Dr. C. V. Hulet had five primary goals in mind when leading the development of this breed: high lifetime prolificacy, large lamb crop at one year of age, ability to lamb more frequently than once per year, rapid growth rate of lambs, and desirable carcass quality (American Polypay Sheep Association). The Polypay is a maternal breed with exceptional mothering ability and high prolificacy rates. They are a range sheep most appropriate for high potential feed producing areas of the world. Thus, the Polypay are derived from sheep selected to perform well in temperate zone conditions. The Polypay is a combination of the Finnish Landrace (prolificacy), Polled Dorset (growth), Rambouillet (out-of-season breeding), and Targhee (wool quantity) (SID, 2002).

In contrast, the White Dorper breed was developed in South Africa by crossing the Black Head Persian (hair breed adapted to harsh, arid environmental conditions) and the British Dorset Horn (wool breed selected for growth characteristics). The goal was to create an adapted breed capable of producing fast growing lambs with good carcass characteristics under grazing conditions (Milne, 2000). The White Dorper was imported into the U. S. in 1995. They have gained popularity in the U.S. because management requirements of these hair composite sheep are relatively low compared with the traditional wool breeds. Shearing is unnecessary and treatment for gasto-intestinal tract nematode parasites tends to be reduced due to resistance to infection (Courtney et al., 1985; Zajac et al., 1990; Gamble and Zajac, 1992; Wildeus, 1997; Burke and Miller, 2002, 2004). They thrive under range conditions where other breeds can barely exist and can even raise lambs under severe conditions. The White Dorper is more hardy, earlier maturing, and has a more muscular body shape than the Polypay.

Objectives

Due to the differences in genetic makeup of each breed, the Polypay and White Dorper may have different protein digestion, absorption, and metabolism which could cause differences in N metabolic capacities. This may result from a differential capacity for expression of transporters and enzymes critical for N metabolism. The overall goal of this study was to answer the following questions:

1) Does genetic type have an effect on digestion and N metabolism in growing lambs?
2) Is there a relationship between the relative amount of glutamate transporters and metabolizing enzymes expressed by lambs of different genetic types exposed to the same dietary regimen?

CHAPTER III

Nutrient Utilization in Polypay and Percentage White Dorper Lambs Fed a High Roughage and a High Concentrate Diet

Introduction

Although there are over 200 breeds of sheep in the world, not one is the most productive in all possible production situations. Even though Givens and Moss (1994) concluded that breed of sheep had a larger effect on nutrient utilization than either age or body weight, there is still limited knowledge on the effects genetic makeup has on digestibility and nutrient utilization in ruminants. Variable digestive efficiencies and N metabolism capacities have been noted in comparisons of different species of cattle (Hungate et al., 1960; Howes et al., 1963; Hunter and Siebert, 1985) and different breeds of goats (Hart et al., 1993; Sahlu et al., 1993; Silanikove et al., 1993). Ultimately, it is differences in genetic makeup that cause animals to perform differently in the same environment. These differences may produce variation in digestive tract anatomy, rumen microbial populations, amounts and kinds of enzymes secreted – all of which affect diet digestibility. The objective of this trial was to determine the effect of three genetic types on nutrient utilization of two divergent diets fed to sheep.

Materials and Methods

Animals and Diets

Research protocols were approved by the University of Kentucky Institutional Animal Care and Use Committee. A two-phase nutrient digestion/N retention trial was conducted with six wether lambs of each genetic type: Polypay (PP), ½ White Dorper ½ Polypay (½ D), and ¾ White Dorper ¼ Polypay (¾ D). The PP is a traditional wool breed, while the White Dorper is a composite hair sheep. All lambs were born in the University of Kentucky sheep flock. Lambs averaged 35 kg and were 5 mo of age at the

start of the study. Animals were housed in individual pens for diet adjustment and digestion crates for collection of feces and urine. Lambs always had ad libitum access to fresh water.

Diets were high roughage (HR; Phase 1) and high concentrate (HC; Phase 2). The HR diet consisted of 60% ground grass hay and 40% concentrate. The HC diet contained 90% concentrate and 10% ground grass hay. Both diets were balanced to meet nutrient requirements (NRC, 1985) and were fed at 2% BW (as-fed), based on individual lamb weights at the start of the study. Each lamb received equal amounts of its daily dietary allotment twice daily at 0800 and 1600 h. Diet samples were taken at each feeding during the 7-d fecal and urine collection periods and composited to represent an average ingredient and chemical composition (Table 3.1).

Phase 1: Digestibility and Nitrogen Metabolism of a High Roughage Diet

To begin the study, 18 lambs were removed from a flock that was consuming pasture plus a 90:10 concentrate: roughage supplement (1% BW daily). Lambs were assigned to individual pens at the Animal Research Center Sheep Unit. The HR diet (Table 3.1) replaced the 90:10 supplement and was gradually increased to 2% BW during a 7-d diet adjustment period (Figure 3.1). Lambs were then moved to individual digestion crates (Figure 3.2) and allowed 7 d to adjust to the crates. During this time, lambs continued to receive the HR diet at 2% BW daily in equal amounts at 0800 and 1600 h. Crates were fitted to each lamb so feces and urine could be collected in separate collection buckets. In addition, a 10- x 10-cm of 2-mm plastic screen was placed in each urine funnel to prevent fecal, wool, and/or hair contamination (Figure 3.3).
Total feces were collected and weighed for each lamb at 1600 h for 7 consecutive days (d 15 through 21). A 10% aliquot (by weight) was taken from each day's collection, dried at 50°C in a forced air oven for 24 h, air-equilibrated, and composited for each lamb to provide a representative sample of the 7-d fecal output.

Table 3.1. Chemical composition of high roughage (HR) and high concentrate (HC) diets fed to lambs

	Diet	
	HR	HC
Dry Matter (DM), %	93.5	92.2
	% of DM	
Organic Matter (OM)	92.4	94.5
Crude Protein (CP)	16.5	17.0
Neutral Detergent Fiber (NDF)	52.4	22.7
Acid Detergent Fiber (ADF)	23.5	5.1
Acid Detergent Lignin (ADL)	2.8	0.3

Figure 3.1. Timeline of events in a two-phase nutrient digestion/nitrogen retention trial with lambs fed a high roughage (HR) and a high concentrate (HC) diet

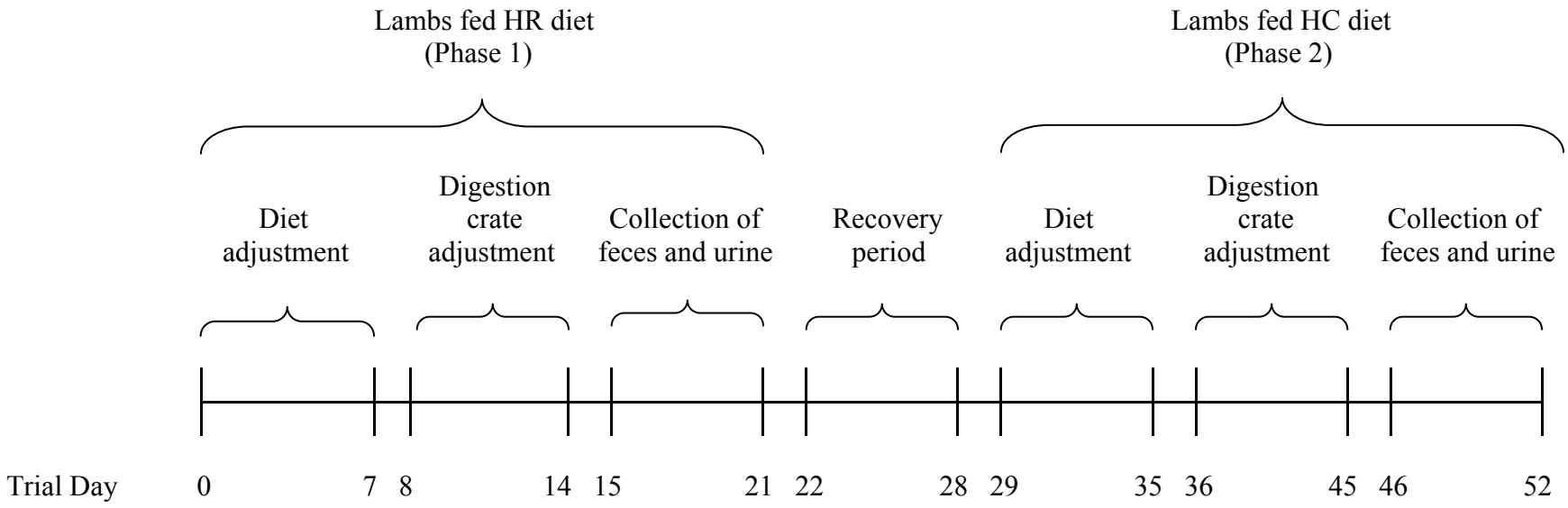
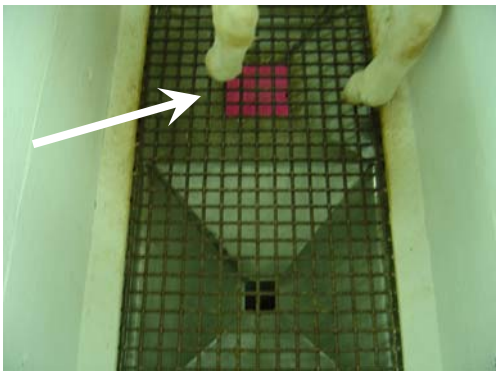


Figure 3.2. Front (a) and rear (b) view of lamb in digestion crate for collection of feces and urine

(a)  (b) 

Figure 3.3. Feces and urine collection funnels with 10- x 10-cm plastic screen insert (indicated by arrow) in urine funnel



Urine was also collected daily for 7 d (d 15 through 21). All urine collection buckets were acidified with 10 mL of 50 % HCl prior to the start of collection and after each daily collection. Total urine was weighed daily at 1600 h. Urine output of each lamb was stirred to ensure homogeneity and a 1% aliquot (by weight) was taken daily, composited by lamb, and frozen until analyzed for N content.

Phase 2: Digestibility and Nitrogen Metabolism of a High Concentrate Diet

Following Phase 1, all lambs returned to individual pens. The HR diet was gradually replaced with the HC diet over a 7-d adjustment period as daily intake was maintained at 2% BW. Lambs were returned to their original digestion crate on d 8 and continued to consume the HC diet at 2% BW daily in equal amounts at 0800 and 1600 h for 7 d. Feces and urine were collected daily at 1600 h for 7 d (d 15 through 21). Sampling procedures were the same as described for Phase 1.

Laboratory Analyses

All diet and fecal samples were ground in a Wiley mill (Thomas Scientific, Swedesboro, NJ) to pass through a 1-mm screen and stored in sealed plastic bags at room temperature until chemically analyzed. Dry matter and ash content of diet and fecal samples were determined by AOAC (1999) procedures. Organic matter (OM) was calculated as 100 minus ash content. Neutral detergent fiber (NDF) analyses followed the procedures of Robertson and Van Soest (1981). Acid detergent fiber (ADF) concentrations were determined by the procedures of Goering and Van Soest (1970) modified for use in an Ankom 200 Fiber Analyzer (Ankom Co., Fairport, NY). Heat stable alpha-amylase was added for the neutral detergent fiber analyses of all feed and fecal samples to degrade starch which could inhibit filtration. Feed, feces, and urine samples were analyzed for N using the automated Kjeldahl method described in AOAC (1999).

Experimental Design and Statistical Analysis

Each phase was conducted as a generalized randomized complete block design with a one-way treatment structure (genetic type). Three environmentally controlled rooms were used to house lambs while in digestion crates. Two lambs of each genetic type were randomly assigned to each room. Rooms (3) constituted experimental blocks.

Data were analyzed by phase using PROC GLM of SAS (Windows version 5.1.2600, SAS Inst., Inc., Cary, NC). The statistical model included effects of room, genetic type, and their interaction. All effects were considered to be fixed. Differences among genetic types were compared using the PDIFF option (all possible t-tests) of PROC GLM. Initial age and weight were included in preliminary analyses, but were found to be nonsignificant and were omitted from the final model. Significant differences were determined at $P < 0.1$, 0.05, and 0.01 levels of probability.

Results and Discussion

Genetic differences may be expressed in a number of ways and many may be influenced by uncontrolled or uncontrollable environmental and management factors such as type of diet, age, or weight (Warwick and Cobb, 1975). Knowledge of sheep nutrition is probably greater than any other aspect of sheep production; however, direct investigation of genetic variation in sheep nutrition is rare. Little is known about the effect of genetic differences among sheep on nutrient requirements for production. For this study, lambs of the same sex, age, and weight were fed the same diet and exposed to the same environmental elements differing only by genetic makeup.

Phase 1: Digestibility and Nitrogen Metabolism of a High Roughage Diet

Genetic type did not alter digestibility of the high roughage diet fiber components (DM, NDF, ADF) (Table 3.2) among lambs used in this trial. Other studies (Givens and Moss, 1994; Ranilla et al., 1997; Lopez et al., 2001) have shown similar digestibility percentages when lambs consumed diets of similar ingredient and chemical composition. These results agree with Ragland's (1990) conclusion that there is no breed advantage associated with fiber digestibility.

Table 3.2. Dry matter, neutral detergent fiber, and acid detergent fiber digestibility of a high roughage diet consumed by Polypay (PP), ½ White Dorper ½ Polypay (½ D), and ¾ White Dorper ¼ Polypay (¾ D) lambs[1]

	Genetic Type			
	PP	½ D	¾ D	SEM
Digestibility, %				
DM	67.9	68.9	69.3	1.6
NDF	69.6	70.4	70.6	2.1
ADF	63.8	65.8	64.8	2.1

[1] Values are least squares means and pooled SEM.

Table 3.3. Daily nitrogen utilization by Polypay (PP), ½ White Dorper ½ Polypay (½ D), and ¾ White Dorper ¼ Polypay (¾ D) lambs fed a high roughage diet[1]

	Genetic Type			
	PP	½ D	¾ D	SEM
DNI, g	17.2	17.2	17.1	
DFN, g	5.8	5.6	5.5	0.5
ND, %	66.3	67.4	67.8	2.3
DUN, g	10.2	9.3	9.6	3.1
DNR, g	1.3	2.3	2.0	3.3
DNR, % of DNI	7.6	13.4	11.7	19.1
DNR, % of ND	11.4	19.8	17.2	27.5

[1] Values are least squares means and pooled SEM.
[2] DNI=Daily nitrogen intake, DFN=Daily fecal nitrogen, ND=Nitrogen digestibility, DUN=Daily urinary nitrogen, DNR=Daily nitrogen retained.

Although fiber digestion was unaffected by genetic type for lambs fed the HR diet, N utilization tended to be improved in the ½ D lambs (Table 3.3). All lambs consumed a constant amount of N at approximately 17 g/ hd/ d. Daily fecal N excretion rates (5.5 to 5.8 g) were similar for PP, ½ D, and ¾ D lambs, as were N digestibilities (66.3, 67.4, and 67.8% for PP, ½ D, and ¾ D, respectively). However, daily urinary N excretion rates tended to be lowest in the ½ D lambs, ranging from 9.3 (½ D) to 10.2 g (PP). The ½ D lambs retained numerically more N (2.3 g/d) while the PP lambs retained the least (1.3 g/d). Furthermore, N retention, expressed as a percent of DNI and as a percent of ND, was numerically highest for the ½ D lambs. Although the ½ D lambs tended to be more efficient than the PP and ¾ D lambs at retaining dietary N, no significant differences were found among genetic types when the HR diet was consumed.

Phase 2: Digestibility and Nitrogen Metabolism of a High Concentrate Diet

Dry matter digestibility (Table 3.4) was higher ($P < 0.1$) for ½ D lambs than for PP and ¾ D lambs, contradicting the conclusions of Notter et al. (1984) that DM digestibility did not differ due to genetic type in sheep. However, NDF and ADF digestion remained unaffected by genetic type agreeing with other studies conducted with lambs consuming a diet of similar chemical composition (Swanson et al., 2004; Atkins et al., 2006; Haddad and Obeidat, 2006).

As in Phase 1, all lambs consumed approximately 17 g N/hd/d (Table 3.5). The amount of N excreted in the feces was similar among genetic types and comparable to fecal N excretion in lambs consuming a similar diet (Swanson et al., 2004). Digestibility of N in the HC diet was higher ($P < 0.05$) in the ½ D lambs than the PP, yet similar to the ¾ D lambs. These differences imply that ½ D lambs may be more efficient in retaining dietary N. This is indicated in the numerically lower urinary N excretion and larger amount of N retained in the ½ D lambs compared with the PP and ¾ D lambs. N retention values for lambs consuming the HC diet, as a percent DNI, ranged from 33.3 (¾ D) to 40.8 (½ D) and, as a percent of ND, from 42.6 (¾ D) to 51.1 (½ D). When the HC diet was consumed, the ½ D lambs digested more N and tended to retain more N than the ¾ D lambs when expressed either as a percent of DNI or as a percent of ND.

Table 3.4. Dry matter, neutral detergent fiber, and acid detergent fiber digestibility of a high concentrate diet consumed by Polypay (PP), ½ White Dorper ½ Polypay (½ D), and ¾ White Dorper ¼ Polypay (¾ D) lambs[1, 2]

	Genetic Type			
	PP	½ D	¾ D	SEM
Digestibility, %				
DM	83.1[a]	84.8[b]	83.6[a]	1.2
NDF	72.8	76.5	72.4	5.0
ADF	50.2	57.2	46.9	11.3

[1] Values are least squares means and pooled SEM.
[2] Least squares means within a row with different superscripts differ ($P < 0.1$).

Table 3.5. Daily nitrogen utilization by Polypay (PP), ½ White Dorper ½ Polypay (½ D), and ¾ White Dorper ¼ Polypay (¾ D) lambs fed a high concentrate diet[1, 2, 3]

	Genetic Type			
	PP	½ D	¾ D	SEM
DNI, g	17.1	17.4	17.4	
DFN, g	3.9	3.5	3.8	0.3
ND, %	77.2[a]	79.9[b]	78.2[a, b]	1.8
DUN, g	7.2	6.8	7.8	3.6
DNR, g	6.0	7.1	5.8	3.7
DNR, % of DNI	35.1	40.8	33.3	20.8
DNR, % of ND	45.5	51.1	42.6	26.5

[1] Values are least squares means and pooled SEM.
[2] DNI=Daily nitrogen intake, DFN=Daily fecal nitrogen, ND=Nitrogen digestibility, DUN=Daily urinary nitrogen, DNR=Daily nitrogen retained.
[3] Least squares means within a row with different superscripts differ ($P < 0.05$).

Comparison of Phase 1 and Phase 2

Statistical comparisons of Phase 1 and Phase 2 were not possible because trials were conducted consecutively rather than tandemly. Overall DM digestibilities were greater with the HC than with the HR diet (80^{+} vs. 60^{+} %) (Tables 3.2 and 3.4). In fact, all digestibility values were higher when the HC diet was fed because it contained lower levels of NDF, ADF, and ADL (Table 3.1). Comparing the NDF and ADF digestibilities in Table 3.4 with those of the HR diet in Table 3.2, larger numerical differences were found when the HC diet was fed. Relatively large numerical differences without showing statistical significance is probably a result of the small amount of NDF and ADF provided in the HC diet (Table 3.1). Small variation in laboratory analyses could have magnified digestibility differences with the HC diet.

Similar to Phase 1 (HR diet), lambs fed the HC diet in Phase 2 consumed approximately 17 g of N/d (Table 3.5). However, lambs excreted only 66% as much fecal N, resulting in higher N digestibility coefficients (70^{+} vs. 60^{+} %). Overall, daily urinary N loss was less when lambs consumed the HC diet (Table 3.5) than when the HR diet (Table 3.3) was consumed. Consequently, N retention values (g, percent of intake, and percent of digested) were greater when the HC rather than the HR diet was consumed. Although, genetic type differences for N retention were statistically nonsignificant in both trials, from a practical standpoint, ½ D lambs may be able to utilize dietary N from a HC diet more efficiently than PP or ¾ D lambs.

More differences may have been observed in this study if the lambs had possessed larger genetic differences or had larger differences in their production type. Hart et al. (1993) found differences in digestive capacities, digesta passage rates, and ruminal fermentation rates using different breeds of goats that were also different production types (fiber, milk, meat and milk). Both the PP and the White Dorper breed can be traced back to the Dorset breed of sheep (Polled Dorset in the PP and British Dorset Hornin the White Dorper) (SID, 2002). Therefore, the genetics from each parent breed used in this study cannot be considered unique. Furthermore, the model for this study was intended to represent the physiological state and nutritional status of young growing lambs in a production setting, rather than, for example, the "starved" model used by Heitmann and Bergman (1981) who were able to show larger differences in digestibility among

treatment groups Additionally, several studies (Hart et al., 1993; Sahlu et al. 1993; Silanikov et al., 1993) have demonstrated differences in digestive efficiency and nutrient utilization among breeds of goats, but only when low digestibility diets were fed. Even though digestibility of the HR diet was lower than the HC diet for all components across genetic types, both diets were considered to be high quality. The HR diet contained high CP (16.5%) and highly digestible hay (vegetative state). The HC diet also contained high CP (17.0%), but lower amounts of fiber. The CP content in each diet exceeded the requirements for growing wether lambs (NRC, 1985). The small differences noted among genetic types in this study could be in part due to the high quality of both diets used. Had a lower quality diet been fed, genetic type differences may have been greater (Hunter and Siebert, 1985; Huston et al., 1986; Quick et al., 1986; Givens and Moss, 1994; Ranilla et al., 1997; Ranilla et al., 1998). However, from a practical standpoint, growing lambs will not be fed a low quality diet. Therefore, differences in nutrient digestibility in the rumen, and through the entire gastrointestinal tract, of high quality diets fed to lambs of different genetic makeups may be so subtle that gross measures (digestive coefficients) will not identify. To produce differences in digestibilities between genetic types, diets probably have to be low quality (high fiber-low N). Furthermore, differences will probably be a result of N content rather than fiber digestion, as indicated by the results of this study.

Conclusions

When the HR diet was consumed, the ½ D lambs had a tendency to retain a larger amount of dietary N than either the PP or the ¾ D lambs, with the PP retaining the least amount. When the HC diet was consumed, the ½ D lambs had significantly higher digestibility values for DM and N leading to larger numerical differences in N retention for the ½ D lambs over the PP and ¾ D lambs. Different from when the HR diet was consumed, ¾ D, rather than the PP lambs, tended to be the least efficient at digesting and retaining dietary N from the HC diet. Regardless of diet consumed (HR or HC) the ½ D lambs had tendencies to be more efficient at digesting and retaining N than either the PP

or the ¾ D lambs. Furthermore, these data could indicate ¾ D lambs are more efficient users of a HR diet than PP lambs, but the PP lambs are more efficient N users when a HC diet is fed.

Theoretically, the superior performance of the ½ D compared with the PP and ¾ D lambs could translate into increased weight gains. Furthermore, it can be concluded that genetic type does influence utilization of dietary nutrients in sheep and analysis of the merits of different genetic types should be conducted by the producer if maximum profits are to be attained.

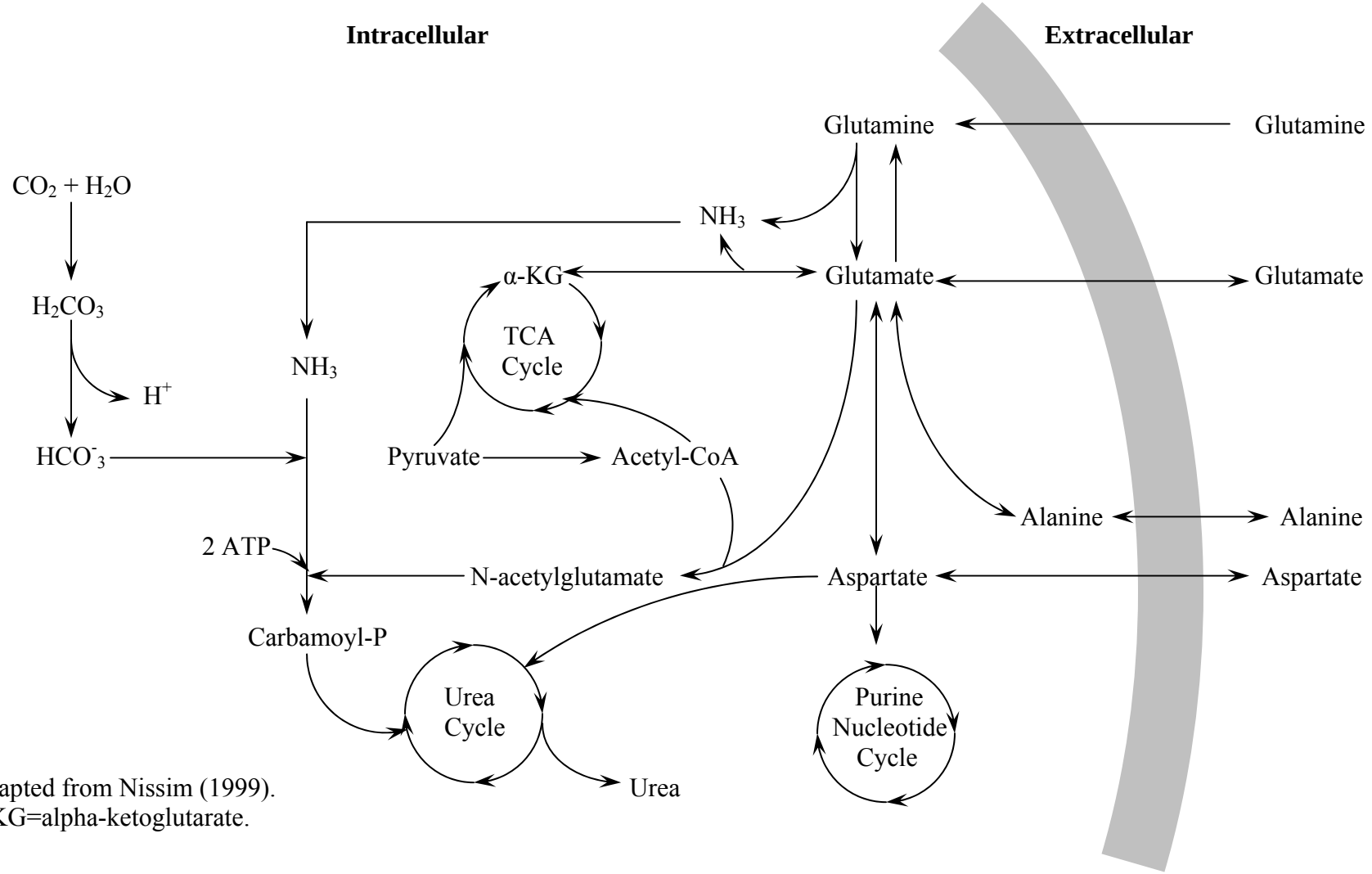
CHAPTER IV

Expression of Glutamine Synthetase, Glutamate Dehydrogenase, Alanine Transaminase, and EAAC1 and GLT-1 Glutamate Transporters by Liver, Kidney, Longissimus Dorsi Muscle, and Subcutaneous Fat Tissues of Growing Polypay and Percentage White Dorper Lambs

Introduction

The previous study showed that genetic type may alter dietary nutrient utilization in sheep. Furthermore, variation among genetic types was a result of modified N metabolism rather than differences in fiber digestibility. In both ruminants and nonruminants, glutamate and glutamine are known to be an amino acid couplet essential in supporting whole body energy metabolism and maintaining N homeostasis throughout the body (Heitmann and Bergman, 1981; Fahey and Berger, 1988; Wu, 1998; Nissim, 1999). Both amino acids share a number of functions that can be fulfilled by either substrate, since they are metabolically interconvertible (Snyderman et al., 1962). Krebs (1935) identified glutamine as quantitatively the most significant N transporter between tissues. In ruminants, glutamate plays a key role in the urea, tricarboxylic acid (TCA), and purine nucleotide metabolic cycles (Nissim, 1999) (Figure 4.1). Glutamate can serve as a source of oxidizable fuel, precursor for other amino acid synthesis, carbon receptor for α-amino and ammonia N derived from the catabolism of other amino acids, or substrate for de novo protein synthesis. Compared with nonruminants, glutamate has a heightened role in both N and carbon metabolism in ruminant animals because of its importance as a gluconeogenic amino acid and the large number of microbe derived nucleotides available for digestion and absorption. Therefore, knowledge regarding the expression of glutamate transporters and metabolizing enzymes is needed to better understand the effect genetic type has on nutrient utilization and N metabolism. The objective of the current study was to determine the effect of genetic type, specifically a traditional wool breed (PP) versus a composite hair breed (White Dorper), on the tissue expression of glutamate transporters and metabolizing enzymes in growing lambs.

Figure 4.1. Illustration of glutamate and glutamine metabolism and their interaction with the tricarboxylic acid (TCA) cycle, urea cycle, and transamination reactions[1, 2]



[1] Adapted from Nissim (1999).
[2] α-KG=alpha-ketoglutarate.

Materials and Methods

Animals and Diet

Research protocols were approved by the University of Kentucky Institutional Animal Care and Use Committee. Animals for this trial were managed from birth until the conclusion of the growth phase as part of a larger Polypay flock that was being graded up to White Dorper at the University of Kentucky Animal Research Center Sheep Unit. Ewes and lambs were maintained on orchardgrass and bluegrass mix pasture until weaning at approximately 70 DOA. Lambs continued to graze orchardgrass and bluegrass mixed pasture and were supplemented with a grain mixture at 2% BW after weaning. The ingredient and chemical compositions of the grain supplement are shown in Tables 4.1 and 4.2. Lambs were weighed at birth, approximately 30 DOA, weaning, and every 3 weeks post-weaning until slaughter. At approximately 153 DOA (d 0) (Figure 4.2), 18 wether lambs were identified for slaughter. Six were selected from each genetic type: Polypay (PP), ½ White Dorper ½ Polypay (½ D), and $^{15}/_{16}$ White Dorper $^{1}/_{16}$ Polypay ($^{15}/_{16}$ D). Lambs were selected from each genetic group based individual lamb gains from birth to 153 DOA. Selected lambs were heaviest and had the fastest rate of gain, and thus were expected to reach slaughter weight (50.0 kg) sooner than their contemporaries. Treatment groups were the three genetic types: PP, ½ D, and $^{15}/_{16}$ D. Lambs were weighed weekly after assignment to treatment group to ensure lambs were slaughtered as close to 50.0 kg as possible. A common final weight was taken for each lamb on d 35, 1 d prior to the first slaughter date.

Blood Analyses

Blood was collected from lambs by jugular venipuncture into heparinized vacutainer tubes 5 h after the morning feeding on d 21 and 35. Samples were immediately placed on ice and transported to an onsite laboratory. Due to problems with laboratory equipment, blood samples collected on d 28 were not taken 5 h after the morning feeding as outlined in the blood collection protocol for this trial.

Table 4.1. Ingredient composition of grain supplement fed to lambs

Item	% (as-fed)
Ground shelled corn	77.33
Soybean meal (47.5% CP)	15.00
Distillers dried grains with solubles	5.00
Ground limestone	1.00
TMS + Se	1.00
Ammonium chloride	0.50
Vitamin E[1]	0.13
Vitamin A, D, and E premix[2]	0.05

[1] Vitamin E=9091 IU/kg of premix.

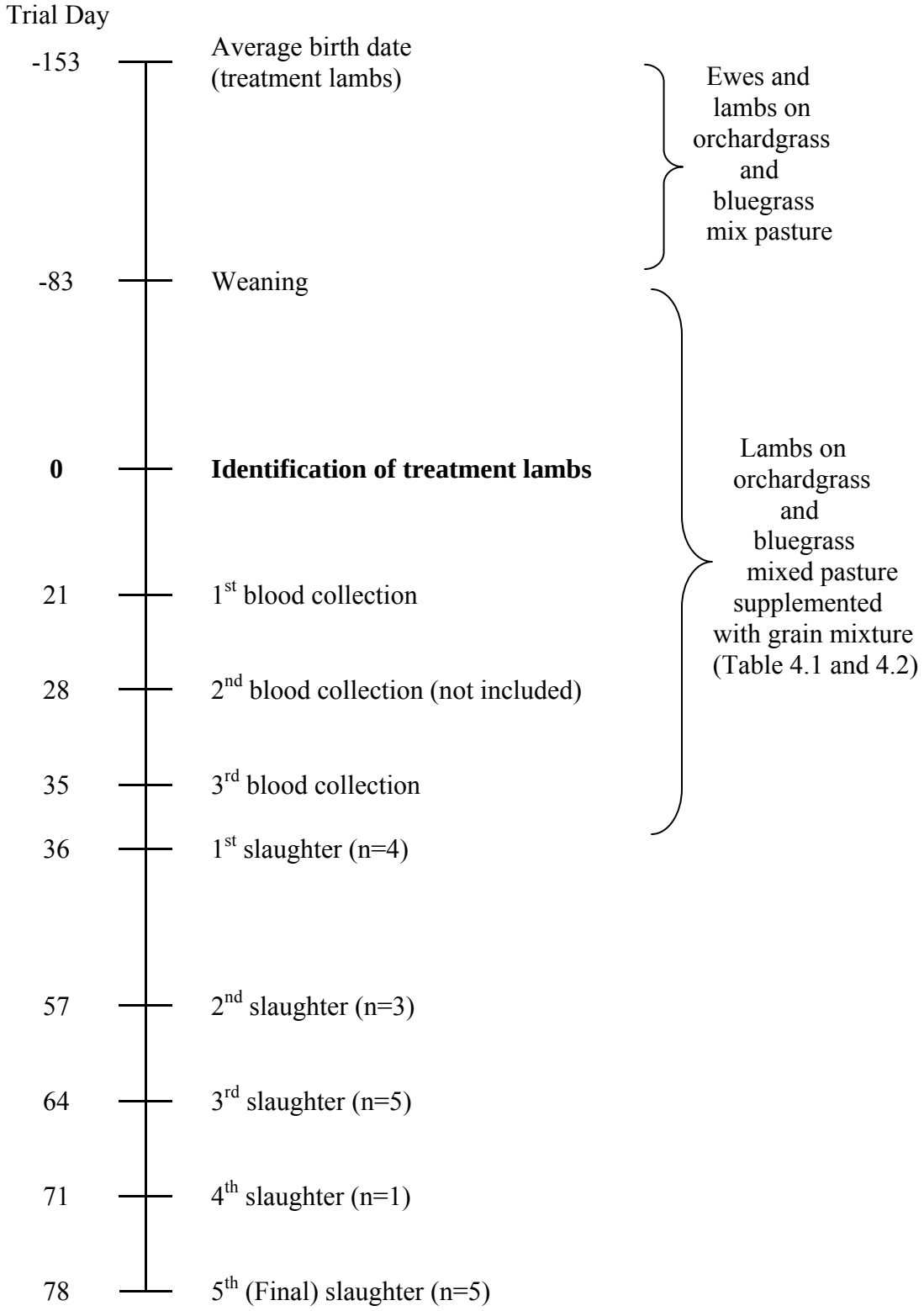
[2] Vitamin A=1,818,182 IU/kg; vitamin D=363,636 IU/kg; vitamin E=227 IU/kg of premix.

Table 4.2. Chemical composition of grain supplement fed to lambs[1]

Item	As-fed
Crude protein, %	14.91
Nonprotein nitrogen, %	0.80
Lysine, %	0.69
Methionine, %	0.31
Crude fat, %	3.35
Acid detergent fiber, %	4.03
Calcium, %	0.45
Phosphorus, %	0.36
Salt, %	0.96
Magnesium, %	0.14
Potassium, %	0.58
Copper, ppm	3.00
Selenium, ppm	0.90
Zinc, ppm	35.00
Vitamin A, IU/kg	909.09

[1] NRC (1985).

Figure 4.2. Timeline of events for a glutamate transporter and metabolizing enzyme expression trial in growing lambs



Several studies have shown blood plasma metabolite levels have a distinct pattern of change associated with time of feeding (Thye et al., 1970; Mondal et al., 2005; Hackl et al., 2006), and therefore, d 28 plasma data were not included in the blood analysis data for this trial. Fresh blood was centrifuged at 3,000 x g for 15 min at 4°C to yield plasma. Approximately 2 mL of plasma was pipetted into 5 mL glass test tubes to immediately determine plasma glutamate and glutamine concentrations using the automated YSI (Yellow Springs Instrument, Model 2700, Yellow Springs, OH) enzymatic system. Specifically, glutamate was measured with L-glutamate oxidase (membrane No. 2754), whereas glutamine was measured with co-immobilized glutaminase and L-glutamate oxidase (membrane No. 2735). Assays were validated using standard curves of 0 to 0.2 mM for glutamate and 0 to 0.6 mM for glutamine. The remainder of the collected plasma was frozen at -80°C in 6 mL scintillation vials until further analyzed for plasma ammonia N concentration using the Konelab 20XTi clinical chemistry analyzer (Thermo Electron Corp., Vantaa, Finland).

Carcass Measurements and Tissue Collections

Tissue collection began after rendering the animals unconscious with electrical stunning, exsanguination, and skinning. Hot carcass weights (HCW) were collected on the slaughter floor within 10 min after slaughter and dressing percentages (DP) were calculated by dividing HCW by live weights taken at the time of slaughter. The entire liver, one kidney, and an approximately 2-cm thick cross section of the longissimus dorsi (LD) muscle (collected between the 12^{th} and 13^{th} ribs) with accompanying subcutaneous (Sub Q) fat were harvested from each animal. Tissues were collected in order to facilitate preservation of protein and mRNA integrity. The total weights of the liver (with gall bladder removed) and one kidney were recorded. Two representative 1-g samples of the liver (caudal lobe), kidney (cortex and medulla), LD muscle, and Sub Q fat were collected for protein and mRNA extraction. Tissue samples for protein extraction were homogenized in 7.5 mL of 4°C SEB (Sample Extraction Buffer) solution [0.25mM sucrose, 10.0 mM HEPES-KOH pH 7.5, 1.0 mM EDTA, and 50 μL protease inhibitor (Sigma, St. Louis, MO)]. Tissue samples for mRNA extraction were homogenized in the same way in 10 mL of 4°C TRI Reagent® (Molecular Research Center, Inc., Cincinnati,

OH). The resulting homogenates were frozen at -80°C. After a 24-h chill, cold carcass weights were collected and all carcasses were measured for 12th-rib fat (backfat), body wall thickness (BWT), loineye area (LEA), and yield grade. Weights were also recorded for the leg, boneless leg, loin, rack, shoulder, boneless shoulder, and trim. Percentage of boneless closely trimmed retail cuts (BCTRC) was calculated by the equation developed by Staab et al. (2000): 49.936-(0.0848 x HCW, kg)-(4.376 x 12th rib fat, cm)-(3.530 x BWT, cm)+(2.456 x LEA, cm^2).

Extraction of Tissue Protein and Immunoblot Analysis

Homogenized tissue samples were solubilized in 0.2% (w/v) SDS/0.2 M NaOH and protein was quantified using a modified Lowry assay with Bovine Serum Albumin as a standard (Lowry et al., 1951). After protein quantification, 50 μg of tissue homogenate were separated by 12% SDS-PAGE and electrotransferred to a 0.45-μm nitrocellulose membrane (Bio-Rad, Hercules, CA). Immunoblot analyses for the presence of GS, GDH, ALT, EAAC1, and GLT-1 were performed as described by Matthews et al. (1998a, 1998b, 1999) and Howell et al. (2001, 2003). The relative tissue content of GS protein expression was determined by hybridization with anti-sheep GS polyclonal primary antibody (1:200; BD Biosciences, San Jose, CA) and blocking solution (5% nonfat dry milk in 10mM Tris-Cl, 100mM NaCl, 0.1% Tween-20, pH 7.5) for 1 h at room temperature. Immunoreactive bands for GS were detected with goat anti-mouse IgG:HRPO HRP secondary antibody (1:5000; BD Biosciences, San Jose, CA) and blocking solution for 30 min at 37°C with agitation. The relative tissue content of GDH was determined by hybridization with anti-bovine GDH polyclonal primary antibody (1:1000; US Biological) and blocking solution (1% nonfat dry milk in 30 mM Tris-Cl, 200mM NaCl, 0.1% Tween-20, pH 7.5) for 1.5 h at room temperature. The relative tissue content of ALT was determined by the same procedures as GDH using anti-pig ALT polyclonal primary antibody (1:250; US Biological) and blocking solution (1.5% nonfat dry milk in 30mM Tris-Cl, 200mM NaCl, 0.1% Tween-20, pH 7.5). Immunoreactive bands for GDH and ALT were detected with donkey anti-rabbit IgG HRP secondary antibody (1:7500; Amersham Biosciences) or rabbit anti-sheep IgG HRP secondary antibody (1:5000; Santa Cruz Biotechnology) and respective blocking

solutions for 1 h at room temperature with gentle rocking on a rocking platform. The relative tissue contents of EAAC1 and GLT-1 were determined using stripped nitrocellulose membranes following procedures previously described. EAAC1 and GLT-1 relative tissue contents were determined by hybridization with EAAC1 polyclonal primary antibody (1:200; Santa Cruz Biotechnology) or GLT-1 polyclonal primary antibody (1:1000; Affinity BioReagents, Golden, CO) following the same protocol as GDH determination. Immunoreactive bands for EAAC1 and GLT-1 were detected from the same protocols as GDH. All detected immunoreactive bands were visualized with a chemiluminescence kit (Pierce, Rockford, IL). After exposure to autoradiographic film (Amersham), digital images of the radiographic bands were recorded and quantified using the Biorad Versadoc imaging system (Yamin et al., 1996; Dehnes et al., 1998; Ding et al., 1998) and the Quantity One Program (Version 4.2.3, BioRad). Densitometric data were corrected for unequal loading and/or transfer of proteins by normalization to relative amounts of Fast-Green-Stained (Fisher Scientific, Pittsburgh, PA) proteins common to all immunoblot lanes/samples (Swanson et al., 2000; Howell et al., 2003). The relative amount of stained protein per lane for each sample of the blots was also determined as described above via densitometric analysis. All densitometric results were normalized to control animals (PP), by obtaining on average control densitometric value and dividing all other results by this value (Howell et al., 2003; Fan et al., 2004).

Isolation of Tissue mRNA and Relative Real Time Reverse Transcriptase Polymerase Chain Reaction (Relative Rl-T RT-PCR) Analyses

Total mRNA was obtained by an acidic phenol-chloroform extraction as per instruction of the manufacturer (Invitrogen). Chloroform (2mL or 0.2mL per mL TRI Reagent®) was added to each TRI Reagent® homogenate sample in sterile 50 mL polypropylene centrifuge tubes (Fisher Scientific) and samples were shaken vigourosly. After 3 min incubation at room temperature, samples were centrifuged at 12,000 x g for 10 min at 4°C. The aqueous phase containing mRNA was transferred to a fresh, sterile, 50 mL polypropylene centrifuge tube. Isopropyl alcohol was added and mixed with the aqueous phase in a 1:1 ratio to precipitate the mRNA. Samples were allowed to incubate at room temperature for 10 min and at -20°C for another 1 h to increase mRNA yield.

After thawing, samples were centrifuged at 12,000 x g for 10 min at 4°C. After the supernatant was removed, a mRNA pellet remained. Each pellet was gently washed with 75% ethanol (10mL or 1mL per 1mL of TRI Reagent® used in homogenization) and centrifuged at 7,500 x g for 5 min at 4°C. The supernatant was removed as before and tubes were inverted to allow the mRNA pellets to air dry for 10 min. After drying, the pellets were resuspended in DNase-RNase-free water (500 µL), vortexed at a low speed, transferred to a 1.5 mL microcentrifuge tubes, and stored at -80°C.

Total mRNA samples were purified using an RNeasy kit (Qiagen) following manufacturer instructions. The RLT buffer (350 µL) and ethanol (250 µL) were added and mixed with 100 µL of the total mRNA sample. After mixing, samples (700 µL) were immediately transferred, by pipetting, to an RNeasy Mini spin column placed in a 2-mL collection tube and centrifuged at 8,000 x g for 15 s. The flow-through was discarded. Then, the RNeasy spin column membrane was washed with RPE buffer, centrifuged at 8,000 x g for 15 s, rewashed with RPE buffer, and centrifuged again at 8,000 x g for 2 min. The flow-through was discarded after each wash. Finally, the RNeasy spin column was placed in a 1.5 mL collection tube and 30 µL of RNase free water was pipetted directly on the spin column membrane to release the mRNA. The mRNA sample was then centrifuged at 8,000 x g for 1 min to elute the mRNA.

Total mRNA for relative RT-PCR was treated with DNase I enzyme (amplification grade) immediately prior to conducting the reverse transcription procedure, as described by Invitrogen, to ensure that no DNA was present. Three micrograms of mRNA, in a total volume of 5.09 µL, was combined with 0.64 µL DNase I reaction buffer (10x) and 0.64 µL of DNase I (Invitrogen) and incubated at room temperature for 15 min. Then, 0.64 µL of 25 mM EDTA was added to the DNase-treated total mRNA and incubated at 65 °C for 10 min to stop the reaction.

Analysis of relative amount of mRNA expression was performed as a two-step reverse transcription polymerase chain reaction (RT-PCR) protocol. The entire volume (7 µL) of DNase treated total mRNA was used in the reverse transcription reaction described by Invitrogen. A solution of hexamers (50 ng/µL; Invitrogen) and oligo dT (50 µM; Invitrogen) primers were added to each reaction tube (1 µL each) and incubated at 70°C for 10 min. The reaction was quick chilled on ice for at least 1 min. Then, a

solution containing 2 µL RT reaction buffer (10x), 2 µL DTT (0.1 M), 4 µL $MgCl_2$ (25 mM), 1 µL dNTP mix (10 mM), and 1 µL RNase Out was added to the reaction tube and incubated at 37°C for 2 min. One microliter of reverse transcriptase (SuperScript III RT, 200 U/µL) was added to the reaction tube and incubated at room temperature for 10 min, at 50°C for 50 min, and finally at 70°C for 10 min. Following incubation, reaction tubes were quick chilled on ice and stored at -20°C until used in the Rl-T PCR reaction.

Rl-T PCR reactions were assayed in triplicate with an ABI PRISM 7000 (Applied Biosystems) using Universal PCR MasterMix. The following PCR conditions were used for amplification: initial denaturing (95ºC for 10 min), followed by 40 cycles of a two stage amplification of denaturing (95ºC for 15 sec) and annealing/extension (60ºC for 1 min), with a melting curve program (60ºC to 95ºC), heating rate of 0.15ºC/sec and continuous fluorescence measurements.

Relative quantification of GS, GDH, and ALT mRNA expression was conducted using the relative standard curve method and serial dilution of RT cDNA. The standard curves for each target gene mRNA and 18S were constructed with a common basis cDNA sample consisting of a pool of all cDNA samples generated for quantification. For each mRNA quantified, ribosomal 18S mRNA was used as an endogenous control to normalize variations in input mRNA and RT reaction efficiencies. Specifically, LD muscle and Sub Q fat total mRNA were reverse transcribed, the cDNA product was serially diluted (2.5x, 5x, 25x, 125x, 625x, 3125x, 15625x, 78125x, 390625x), and the linear range for target established to ascertain the appropriate amount of cDNA to be used for a standard curve. GS, GDH, ALT, and 18S cDNA were amplified using 25x, 25x, 5x, and 15625x dilutions, respectively of cDNA. The minimal threshold (CT) values detected, using these dilutions of cDNA were, 22 to 25 and 26 to 35 for the control and target genes, respectively. The level of each gene expressed was determined using the relative standard curve method (User Bulletin #2, ABI PRISM 7700 Sequence Detection System, Applied Biosystems). CT values were normalized for unequal loading by calculating the ratios of target gene CT to 18S CT. These 18S normalized ratios were used for statistical analysis of target gene mRNA expression.

Experimental Design and Statistical Analysis

This experiment was conducted as a completely randomized design with a one-way treatment classification. Treatments were fixed effects and gradient in nature. Treatments consisted of three genetic types, Polypay (PP), ½ White Dorper ½ Polypay (½ D), and $^{15}/_{16}$ White Dorper $^{1}/_{16}$ Polypay ($^{15}/_{16}$ D). Replications consisted of six lambs of each genetic type. Data were analyzed using PROC GLM of SAS (Windows version 5.1.2600, SAS Inst., Inc., Cary, NC). The statistical model included the effect of genetic type with individual animals serving as the experimental unit. Differences among genetic types were compared using the PDIFF option (all possible t-tests) of PROC GLM. Initial age and weight were included in preliminary analyses, but were found to be nonsignificant and were omitted from the final model. Significance was indicated at the $P < 0.1$, 0.05, and 0.01 levels.

Results and Discussion

Utilization of glutamate is essential for normal whole body N and carbon metabolism. In mammals, it is high amounts of glutamate uptake by liver (perivenous hepatocytes), skeletal muscle, and fat tissues that support N and ammonia recycling (Hediger and Welbourne, 1999). The importance of amino acid derived glucose and the role of glutamate as a primary source of glucogenic amino acid derived hydrocarbons in ruminants are well established (Heitmann and Bergman, 1981; Fahey and Berger, 1988; Moores et al., 1994). In forage-fed lactating dairy cows, the extent of glutamate oxidation has been shown to approximate the total of acetate, propionate, and butyrate (Black et al., 1990). In sheep, the liver is the major tissue responsible for the production of glucose from glutamate. During fasting or acidosis, however, ⅓ to ½ of the total glucose produced from glutamate is synthesized by the kidney (Heitmann and Bergman, 1981). Despite obvious differences in the amount and metabolic fate of glutamate used by tissues throughout the body, surprisingly little is known about perceived differences in the type and relative quantity of glutamate transporters expressed by these tissues to support their individual functions. Furthermore, identifying the relationships that exist among glutamate transporters, enzymes that utilize glutamate, and the growth of tissues

as animals develop is critical. Relative to nonruminants, this knowledge may be especially important to completely understanding ruminant nutritional physiology. The ruminants' need to metabolize relatively large loads of ammonia and produce amino acid derived blood glucose, as a result of forestomach microbial fermentation, makes this animal unique to the nonruminant.

Previous research from this lab has demonstrated EAAC1 and GLT-1 to be the only system X^-_{AG} transporters expressed by small intestinal epithelium, liver, and kidney of mature sheep and cattle (Howell et al., 2001) and of fully ruminanting wether lambs (Howell et al., 2003). Furthermore, Gissendanner (2003a) demonstrated the extent of EAAC1, GLT-1, and GS changes in skeletal muscle and adipose tissues of Angus steers from backgrounding through finishing animals. On the other hand, expression of EAAC1 and GLT-1 by epithelial tissue did not change (Gissendanner et al., 2003b). However, the expression of glutamate system X^-_{AG} transporters and metabolizing enzymes has not been reported in skeletal muscle or adipose tissue of sheep and no information could be found about the potential effects of genetic type on glutamate transporters and metabolizing enzyme expression.

In the present study, growing PP and percentage White Dorper lambs (½ D and $^{15}/_{16}$ D) were used to determine if increasing percentage White Dorper genetics had an effect on growth rate, carcass characteristics, content of system X^-_{AG} transporters, and activity of enzymes involved in glutamate metabolism. Lambs of the same sex and age were fed the same diet and exposed to the same environmental elements in order to minimize non treatment effects. The model for this study was intended to be representative of growing lambs in a normal production setting, with the only treatment effect being genetic type. In order to achieve this goal, all lambs were managed as part of a larger flock and were fed the same diet and exposed to the same management practices from birth to the slaughter.

Animal Weights and Rates of Gain

Initial weights were higher ($P < 0.1$) for ½ D lambs at the start of this trial, ranging from 38.1 ($^{15}/_{16}$ D) to 45.2 kg (½ D). To minimize non treatment effects, theoretically, similar initial body weights would be desirable. Birth weights for ½ D and

$^{15}/_{16}$ D lambs did not differ from PP lambs (4.8 kg). However, ½ D lambs (5.2 kg) weighed more ($P < 0.05$) than the $^{15}/_{16}$ D lambs (4.4 kg) (Appendix Table 1). From birth, the ½ D lambs seemed to excel in growth rate over the PP and $^{15}/_{16}$ D lambs. Based on this information, differences in initial body weight are a reflection of the growth patterns of lambs of different genetic types.

Weights of the PP and $^{15}/_{16}$ D lambs were similar on both d 21 and 35. However, ½ D lambs weighed more ($P < 0.05$) than $^{15}/_{16}$ D lambs on both weigh dates (Table 4.3). Daily gains were not different among genetic types for either d 0 to 21 or 21 to 35. The weight advantage of the ½ D lambs continued through d 35. Day 35 weights were the last common weights recorded for all lambs. Thereafter, lambs were slaughtered as they reached the targeted slaughter weight of 50.0 kg. Slaughter weights (49.2 to 50.8 kg) therefore, did not differ among genetic types. From d 0 to slaughter, ½ D lambs had slower ($P <0.01$) rates of gain than PP and $^{15}/_{16}$ D lambs. This pattern of growth contrasts rates of gain prior to the start of this trial (birth to d 0; Appendix Table 1) when ½ D lambs excelled in growth. These differences in the growth patterns were attributed to compensatory gain of PP and $^{15}/_{16}$ D lambs after 153 DOA (d 0 to slaughter). Overall, the number of days required to reach the targeted slaughter weight did not differ among genetic types.

Plasma Glutamate, Glutamine, and Ammonia Concentrations

Plasma glutamate, glutamine, and ammonia levels in PP, ½ D, and $^{15}/_{16}$ D lambs on d 21 and 35 are shown in Table 4.4. There were no lamb genetic type effects on any of these variables on d 21. Day 35 plasma glutamate, glutamine, and ammonia levels were not affected by genetic types. Based on the data from two sampling dates, it was concluded that similar amounts of glutamate and glutamine were available for tissue uptake by all lambs, regardless of genetic type.

Table 4.3. Weights and rates of gain of Polypay (PP), ½ White Dorper ½ Polypay (½ D), and $^{15}/_{16}$ White Dorper $^{1}/_{16}$ Polypay ($^{15}/_{16}$ D) lambs from d 0 to slaughter[1]

	Genetic Type			
	PP	1/2 D	15/16 D	SEM
Day 0 to 21, 21 d				
Wt d 0, kg[2]	39.7[a]	45.2[b]	38.1[a]	5.2
Wt d 21, kg[3]	46.4[a, b]	51.1[a]	43.6[b]	5.8
Total gain, kg	6.7	5.9	5.5	1.2
ADG, kg/d	0.32	0.28	0.26	0.06
Day 21 to 35, 14 d				
Wt d 35, kg[3]	48.0[a, b]	52.7[a]	45.3[b]	5.9
Total gain, kg	1.6	1.6	1.7	1.6
ADG, kg/d	0.11	0.11	0.12	0.12
Day 0 to 35, 35 d				
Total gain, kg[3]	8.3	7.5	7.2	2.1
ADG, kg/d[2]	0.24	0.21	0.21	0.06
Day 0 to slaughter				
No. days	65.2	53.5	64.0	15.6
Slaughter wt, kg	50.8	51.2	49.2	3.7
Total gain, kg[4]	11.1[a]	6.0[b]	11.1[a]	3.2
Total ADG, kg/d[4]	0.17[a]	0.11[b]	0.18[a]	0.04

[1] Values are least squares means and pooled SEM.
[2] Least squares means within a row with different superscripts differ ($P < 0.1$).
[3] Least squares means within a row with different superscripts differ ($P < 0.05$).
[4] Least squares means within a row with different superscripts differ ($P < 0.01$).

Table 4.4. Levels of glutamate, glutamine, and ammonia in blood plasma of Polypay (PP), ½ White Dorper ½ Polypay (½ D), and $^{15}/_{16}$ White Dorper $^{1}/_{16}$ Polypay ($^{15}/_{16}$ D) lambs[1]

	Genetic Type			
	PP	1/2 D	15/16 D	SEM
Glutamate, µmol/L				
d 21	66.8	67.5	68.1	0.01
d 35	97.7	100.7	92.8	0.02
Glutamine, µmol/L				
d 21	218.2	213.3	220.3	0.03
d 35	244.8	229.0	226.8	0.03
Ammonia, µmol/L				
d 21	85.6	114.3	109.8	0.05
d 35	90.5	101.4	94.7	0.03

[1] Values are least squares means and pooled SEM.

Carcass Measurements

Hot carcass weights (HCW) were similar for PP, ½ D, and $^{15}/_{16}$ D lambs (Table 4.5) ranging from 25.2 (PP) to 26.1 kg (½ D). However, dressing percentages (DP) were higher ($P < 0.1$) for $^{15}/_{16}$ D (51.47) than PP (49.62) lambs. Digestive tract weight variation (fill) can contribute to variation in DP. Although these weights were not recorded in this study, all lambs were treated the same from birth to slaughter. The assumption was that fill variation was nonsignificant. The only organ weights taken were liver and kidney (Table 4.5). These were not affected by lamb genetic type, therefore it was assumed that organ weights did not contribute to differences in DP. It is well established that wool length variation can significantly affect DP. Also, replacing wool with hair as percentage White Dorper genetics increases could affect DP. Because slaughter weights and HCW were not different among genetic types, it is assumed that neither fill, organ weight, nor pelt weight affected DP in this study. Instead, the higher DP may be an indication of more lean muscle mass in the $^{15}/_{16}$ D than in the PP lamb carcasses.

Backfat, measured at the 12th and 13th rib junction, was not statistically different among genetic types (Table 4.5). Measurement of BWT (measured approximately 13 cm off the midline) is an indicator of BCTRC (Staab et al., 2000). However, based on the anatomical location where BWT is measured, fat can be a significant contributor to this thickness. The body wall of the ½ D lambs was thicker ($P < 0.05$) than either the PP or $^{15}/_{16}$ D lambs, indicating more external fat had been deposited. This could be due to the fact that the White Dorper breed is an earlier maturing breed than the PP. In contrast, the increase in external fat is not noted in the $^{15}/_{16}$ D lambs, which seems contradictory to this statement. Comparisons of fat deposition in hair vs. wool sheep breeds have shown hair breeds of lambs tend to deposit more fat internally whereas external deposition (backfat) tends to be more extensive in wool breeds (Boyd, 1983; Shelton, 1983; Horton and Burgher, 1992). This difference in fat deposition may have also played a role in the differences observed in DP among lambs. If the $^{15}/_{16}$ D lambs deposited more fat around kidney, pelvic, and heart areas, this KPH fat would not have been removed before HCW were recorded and could account in part for the differences found for DP.

Table 4.5. Carcass measurements and tissue sizes of Polypay (PP), ½ White Dorper ½ Polypay (½ D), and $^{15}/_{16}$ White Dorper $^{1}/_{16}$ Polypay ($^{15}/_{16}$ D) lambs[1]

	Genetic Type			
	PP	1/2 D	15/16 D	SEM
HCW, kg	25.2	26.1	25.3	4.2
DP, %[2]	49.62[a]	51.05[a,b]	51.47[b]	1.73
CCW, kg	23.8	25.1	23.8	4.4
12th rib fat, cm/22.7 kg CCW	0.35	0.42	0.32	0.15
Body wall thickness, cm/22.7 kg CCW[3]	0.69[a]	1.08[b]	0.78[a]	0.24
Loineye area, sq cm/22.7 kg CCW[2]	11.9[a]	14.6[b]	17.0[c]	2.13
Yield grade	1.82	2.23	1.73	0.63
BCTRC, %[3]	48.4[a]	48.8[a]	50.3[b]	1.0
Loin wt, % CCW[4]	8.8[a]	10.3[b]	10.5[b]	0.7
Rack wt, % CCW[3]	8.0[a]	8.4[a]	9.2[b]	0.6
Leg wt, % CCW	29.1	30.0	30.1	2.1
Boneless leg wt, % CCW[2]	17.8[a]	17.8[a]	19.0[b]	1.1
Shoulder wt, % CCW[2]	22.0[a]	21.0[a, b]	20.9[b]	1.0
Boneless shoulder wt, % CCW	11.3	11.6	11.6	0.7
Trim wt, % CCW[3]	9.3[a]	10.6[a]	13.7[b]	2.1
Liver wt, g/ 22.7 CCW	863.6	826.2	846.6	123.1
Kidney wt, g/22.7 CCW	60.5	58.8	59.4	7.7

[1] Values are least squares means and pooled SEM.
[2] Least squares means within a row with different superscripts differ ($P < 0.1$).
[3] Least squares means within a row with different superscripts differ ($P < 0.05$).
[4] Least squares means within a row with different superscripts differ ($P < 0.01$).

Muscle (lean) tissue contributes to live animal and carcass conformation. In turn, lambs that are heavier muscled tend to dress higher. Loineye area/ 22.7 kg CCW was larger in 15/16 D lamb carcasses than PP and ½ D lamb carcasses. Yield grade was numerically lower (1.73) in 15/16 D lambs. Both of these measures are indicators of carcass lean tissue. The percentage BCTRC was higher ($P < 0.05$) for 15/16 D (50.3 %) lambs than in PP (48.4 %) or ½ D (48.8 %) lambs. This, plus LEA and yield grade, point to the 15/16 D lambs having a larger amount muscle.

The loins from ½ D and 15/16 D weighed more ($P < 0.01$) than those from PP carcasses. The racks of 15/16 D were heavier ($P < 0.05$) than racks of either PP or ½ D carcasses. Both of these wholesale cuts are indicators of muscle quantity and shape throughout the carcass.

Leg weights (bone in), measured as a percentage of CCW, were similar among genetic types. However, boneless leg weights were heavier ($P < 0.1$) in 15/16 D (19.0 %), than PP (17.8 %) or ½ D (17.8 %) lambs. This finding indicates the 15/16 D lambs have more lean muscle tissue in the leg and implies legs of PP and ½ D lambs tend to contain more bone than the 15/16 D lambs. Shoulders (bone in) from PP lambs weighed more ($P < 0.1$) than White Dorper cross lambs. But, boneless shoulder weights did not differ among genetic types, indicating shoulders from PP lambs contained more bone mass than either ½ D or 15/16 D lambs.

Overall, an anlysis of the high-priced wholesale cut data indicates the 15/16 D lambs produce carcasses with more lean tissue than either PP or ½ D lambs. Substantiating evidence for this finding is a higher DP, numerically smaller amount of backfat, thinner body wall, larger LEA, and numerically lower yield grade. However, trim fat from the 15/16 D carcasses was highest ($P < 0.05$). It was concluded that 15/16 D lambs produced carcasses that have more lean muscle tissue mass and more fat (internal), but less bone than PP and ½ D lambs.

Tissue Expression of System X^-_{AG} Transporters and Glutamate Metabolizing Enzymes

A paramount goal of this study was to determine if growth and carcass characteristics were associated with changes in glutamate transporter and/or metabolizing enzyme expression, as indicated by changes in protein and mRNA content of liver,

kidney, LD muscle, and Sub Q fat. Immunoblot analyses for GS, GDH, ALT, EAAC1, and GLT-1 are shown for liver, kidney, LD muscle, and Sub Q fat in Figure 4.3, with corresponding densitometric data shown in Table 4.6.

The content of liver and kidney GS, GDH, EAAC1, and GLT-1 did not differ among genetic types. However, ALT content in the liver of ½ D and $^{15}/_{16}$ D lambs was 52 and 54 % less ($P < 0.01$) than PP lambs. Also, kidney ALT (high band) was 23 and 34% less ($P < 0.1$) in ½ D and $^{15}/_{16}$ D lambs, respectively, than PP lambs. A decreased expression of ALT in the liver and kidney tissues indicates a higher capacity for ALT enzyme activity. This implies alanine and α-ketoglutarate production may be lowered in the cytoplasm of liver and kidney cells of ½ D and $^{15}/_{16}$ D lambs, whereas pyruvate and glutamate production may be decreased in the mitochondria of these same cells (Figure 2.6). A result of this decreased capacity in percentage White Dorper lambs may be less pyruvate leaving the mitochondria causing less to enter the TCA cycle and less usable energy production. Furthermore, glutamate flux through these tissues may be decreased in the ½ D and $^{15}/_{16}$ D lambs.

The GS and GDH contents of LD muscle were below detection. Following the outlined protocol for this trial, 50 µg of LD muscle tissue homogenate were used to determine the content of glutamate transporters and enzymes. Because GS and GDH contents were below detection, a second gel was run using 75 µg of LD muscle homogenate. GS and GDH content were still nondetectable. Even though the LD muscle was devoid of detectable amounts of GS protein, mRNA for GS and GDH were detected in the LD muscle of all lambs (Table 4.7). In fact, GS mRNA expression was 59% higher ($P < 0.05$) in ½ D than PP lambs. A lack of protein expression, with detectable mRNA expression, implies translation was prevented or down regulated for the GS and GDH proteins. The ALT protein expression in LD muscle, as in liver and kidney tissue, was higher ($P < 0.01$) in PP than in ½ D and $^{15}/_{16}$ D lambs. Expression was 32 and 72 % less, in ½ D and $^{15}/_{16}$ D lambs, respectively, than in PP lambs (Table 4.6). The mRNA expression of ALT was 44 % higher ($P < 0.1$) in ½ D than PP lambs. Again, the relative amounts of protein expression and mRNA expression in LD muscle do not correspond with one another, indicating regulation of translation.

Figure 4.3. Immunoblot analysis of liver, kidney, longissimus dorsi muscle, and subcutaneous fat tissues for glutamine synthetase (GS), glutamate dehydrogenase (GDH), alanine transaminase (ALT), EAAC1, and GLT-1 in Polypay (PP), ½ White Dorper ½ Polypay (½ D), and $^{15}/_{16}$ White Dorper $^{1}/_{16}$ Polypay ($^{15}/_{16}$ D) lambs[1, 2, 3]

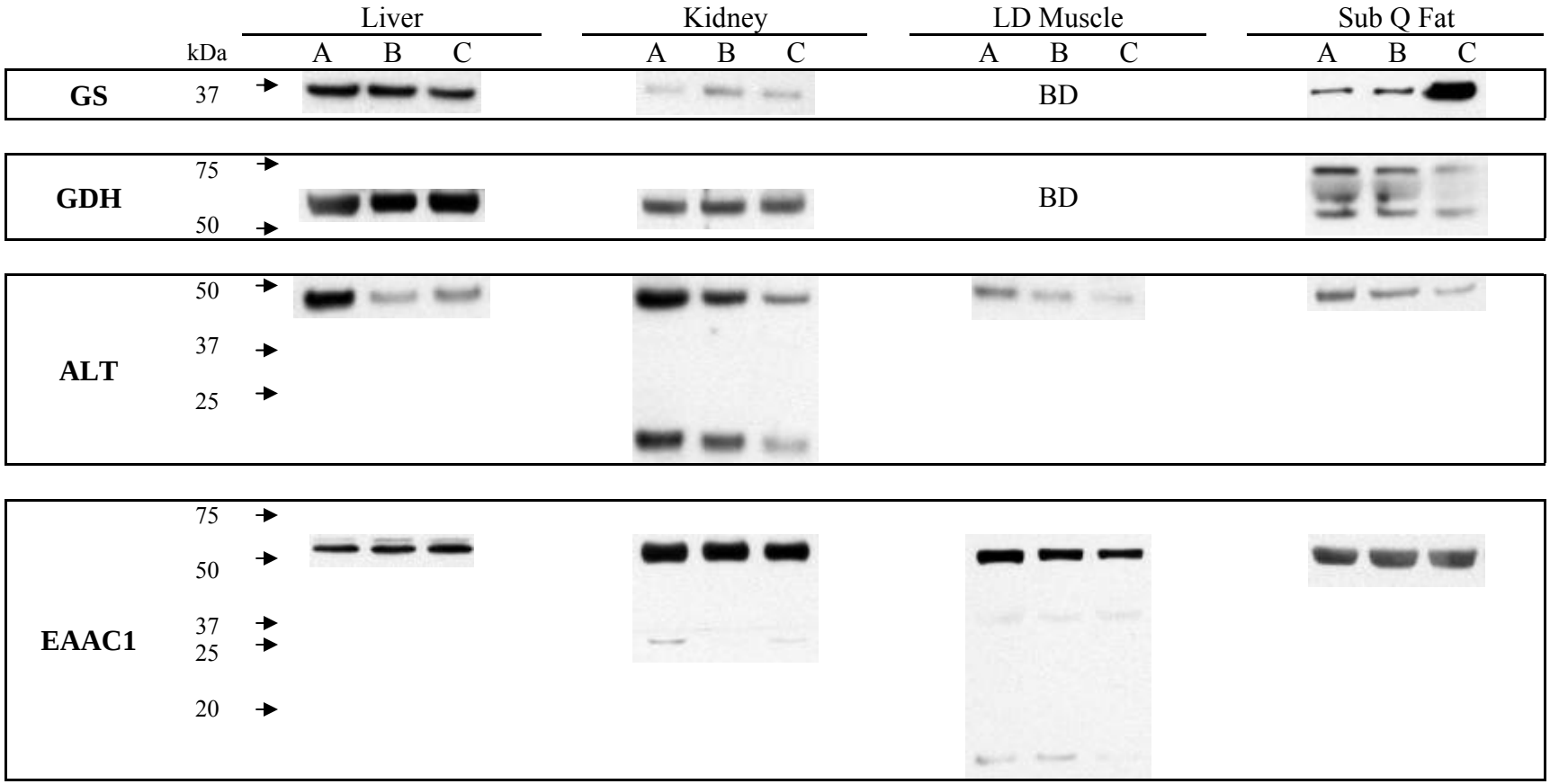
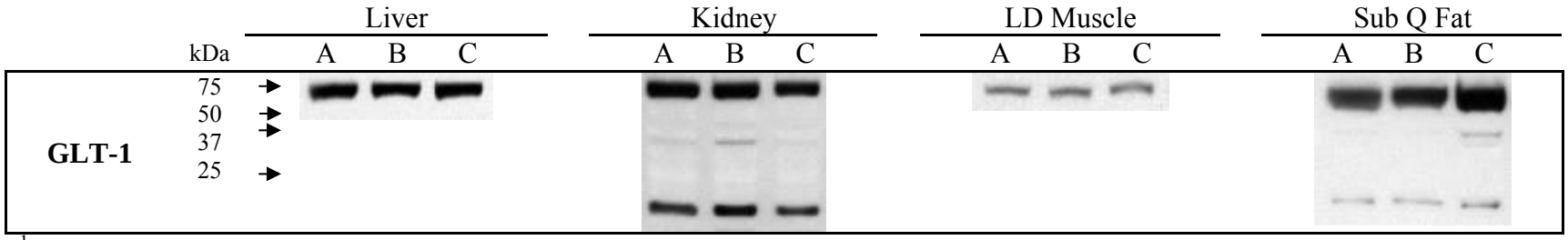


Figure 4.3. (continued)



[1] Autoradiograph data for each tissue is from one of six lambs analyzed per treatment group.
[2] A=PP, B=1/2 D, C=15/16 D.
[3] BD=Below detection.

Table 4.6. Comparison of GS, GDH, ALT, EAAC1, and GLT-1 protein content of liver, kidney, longissimus dorsi muscle, and subcutaneous fat tissue in Polypay (PP), ½ White Dorper ½ Polypay (½ D), and $^{15}/_{16}$ White Dorper $^{1}/_{16}$ Polypay ($^{15}/_{16}$ D) lambs[1, 2]

	Genetic Type			
	PP	1/2 D	15/16 D	SEM
Liver				
GS	1.00	1.05	1.01	0.57
GDH	1.00	1.08	0.98	0.22
ALT[5]	1.00[a]	0.48[b]	0.46[b]	0.25
EAAC1	1.00	1.02	1.01	0.18
GLT-1	1.00	0.92	1.03	0.20
Kidney				
GS	1.00	1.17	0.88	0.69
GDH	1.00	0.99	0.86	0.32
ALT (high band)[3]	1.00[a]	0.77[b]	0.66[b]	0.23
ALT (low band)	1.00	0.88	0.60	0.41
EAAC1 (high band)	1.00	0.48	0.55	0.53
EAAC1 (low band)	1.00	0.61	0.93	0.81
GLT-1 (high band)	1.00	1.18	1.28	0.42
GLT-1 (low band)	1.00	1.09	1.18	0.43
Longissimus dorsi muscle				
GS	BD	BD	BD	
GDH	BD	BD	BD	
ALT[5]	1.00[a]	0.68[b]	0.28[c]	0.18
EAAC1 (high band)	1.00	1.02	0.91	0.17
EAAC1 (low band)[4]	1.00[a]	0.50[a, b]	0.11[b]	0.74
GLT-1	1.00	1.15	0.98	0.27
Subcutaneous fat				
GS[3]	1.00[a]	0.83[a]	1.99[b]	0.94
GDH (high band)[3]	1.00[a]	0.61[b]	0.54[b]	0.35
GDH (low band)	1.00	0.80	0.96	0.56
ALT[4]	1.00[a]	0.65[b]	0.49[b]	0.29
EAAC1	1.00	1.03	0.99	0.13
GLT-1 (high band)[3]	1.00[a]	1.25[b]	1.23[b]	0.23
GLT-1 (low band)	1.00	1.27	1.43	0.67

[1] Values (arbitrary densitometric units) are least squares means and pooled SEM's. Liver, kidney, longissimus dorsi muscle, and subcutaneous fat tissue were collected and GS, GDH, ALT, EAAC1, and GLT-1 quantified by densitometric evaluation of all immunoreactive species identified by immunoblot analysis (Figure 4.3).
[2] BD=below detection.
[3] Least squares means within a row with different superscripts differ ($P < 0.1$).
[4] Least squares means within a row with different superscripts differ ($P < 0.05$).
[5] Least squares means within a row with different superscripts differ ($P < 0.01$).

The PP lambs also had 89 % higher ($P < 0.05$) expression of EAAC1 (low band) transporter in LD muscle tissue than $^{15}/_{16}$ D lambs, while PP and ½ D lambs had similar EAAC1 transporter expression. Thus, the capacity to concentrate glutamate into the LD muscle cells was lower in $^{15}/_{16}$ D lambs, indicating the need for glutamate in LD muscle cells may be lower in these lambs. Also, as in the liver and kidney, ALT enzyme capacity was lower in ½ D and $^{15}/_{16}$ D lambs, suggesting a decreased glutamate flux and less pyruvate feeding into the TCA cycle for usable energy production. In theory, more glutamate may be entering LD muscle tissue in PP than $^{15}/_{16}$ D lambs and more glutamate may be converted to pyruvate by ALT in PP lambs than in ½ D or $^{15}/_{16}$ D lambs. However, based on the carcass measurments, PP lambs contained less lean tissue than ½ D and $^{15}/_{16}$ D lambs, which may indicate they used more energy from glutamate. They produced less LD muscle tissue than $^{15}/_{16}$ D lambs. This suggests the $^{15}/_{16}$ D lambs may be more efficient users of glutamate as an energy source for lean tissue or LD muscle of these lambs has a lower glutamate requirement than that of PP lambs.

At the same time, GLT-1 transporter content of Sub Q fat tissue was lower ($P < 0.1$) in PP lambs than ½ D and $^{15}/_{16}$ D lambs (Table 4.6). GLT-1 (high band) was increased by 25 and 23 % ($P < 0.1$) in ½ D and $^{15}/_{16}$ D lambs, respectively, compared with PP lambs. While PP lambs have a higher capacity to transport glutamate into LD-muscle tissue, they have a decreased capacity to transport glutamate into Sub Q fat tissue. Furthermore, GS content of Sub Q fat from $^{15}/_{16}$ D lambs was 99 and 116 % higher ($P < 0.1$) than PP and ½ D lambs. An increase in GS suggests an increased capacity to convert glutamate to glutamine (Figure 2.4). On the other hand, GDH (high band) content of Sub Q fat was 39 and 46 % less ($P < 0.1$) in ½ D and $^{15}/_{16}$ D than PP lambs. Increased GDH in the percentage White Doper lambs Sub Q fat suggests a higher capacity to produce glutamate (Figure 2.7). ALT content was lower in ½ D and $^{15}/_{16}$ D lambs than PP lambs, just as in all other tissues evaluated.

The LD muscle of PP lambs had a higher capacity to concentrate glutamate (EAAC1) than lambs with White Dorper genes. Furthermore, the LD of PP lambs had the capacity to produce more pyruvate (ALT) which can be converted to usable energy in the TCA cycle, while the capacity to concentrate glutamate in Sub Q fat (GLT-1) was decreased.

Table 4.7. Comparison of GS, GDH, and ALT mRNA content in longissimus dorsi and subcutaneous fat tissue in Polypay (PP), ½ White Dorper ½ Polypay (½ D), and $^{15}/_{16}$ White Dorper $^{1}/_{16}$ Polypay ($^{15}/_{16}$ D) lambs[1]

	Genetic Type			
	PP	½ D	15/16 D	SEM
Longissimus dorsi muscle				
GS[3]	0.75[a]	1.84[b]	1.52[a, b]	0.83
GDH	0.91	1.51	1.61	0.75
ALT[2]	0.14[a]	0.25[b]	0.19[a, b]	0.11
18S	25.8	24.1	23.4	2.4
Subcutaneous fat				
GS	4.59	3.72	4.10	1.57
GDH	4.02	3.05	3.10	1.52
ALT	0.13	0.15	0.09	0.06
18S	25.4	25.3	25.7	0.7

[1] Values (arbitrary units) are least squares means and pooled SEM.
[2] Least squares means within a row with different superscripts differ ($P < 0.1$).
[3] Least squares means within a row with different superscripts differ ($P < 0.05$).

Concurrently, glutamate in fat was metabolized to α-ketoglutarate and pyruvate from reactions with GDH and ALT (Figures 2.6 and 2.7). Theoretically, the α-ketoglutarate produced by the reaction with GDH, can feed into the ALT reaction with alanine and further increase the production of pyruvate and ultimately usable energy. These data indicate, PP lambs had the capacity to utilize more glutamate in both LD muscle, whereas the percentage White Dorper lambs had a higher capacity to utilize glutamate in Sub Q fat.

Conclusions

Based on the data of this study, $^{15}/_{16}$ D lambs may be more efficient producers of carcass lean than the PP or ½ D lambs. Genetic type does have an effect on system X^-_{AG} glutamate transporter and metabolizing enzyme expression which was reflected in carcass composition. Given the economic importance of carcass quality to producers, an analysis of the merits of different genetic types should be conducted by the producer if maximum lean tissue production is to be attained.

CHAPTER V

Summary

In the first experiment, a two-phase digestion/N metabolism trial was conducted with 18 growing wether lambs of three genetic types: PP, ½ D, and ¾ D. During phase 1, a HR diet was fed to lambs and total feces and urine were collected to determine digestibility coefficients and N retention values. For Phase 2, lambs were transitioned to a HC diet and the same measurements were taken. Differences in digestibility where not observed when the HR diet was fed. However, DM and N digestibility was higher in ½ D lambs than PP and ¾ D lambs when the HC diet was consumed. N retention was greatest in ½ D regardless of the diet consumed. These data indicate ½ D lambs tend to be more efficient at digesting and retaining N than either the PP or ¾ D lambs.

In the second experiment, 18 wether lambs of three genetic types (PP, ½ D, and $^{15}/_{16}$ D) were managed as part of a larger flock and the effect of genetic type on the expression of glutamate transporters and metabolizing enzymes was determined. Lambs were slaughtered as they reached a targeted slaughter weight of 50.0 kg. Lamb carcasses were evaluated for muscle, fat, and bone development and tissue samples were collected from the liver, kidney, LD muscle, and Sub Q fat. All tissue samples were analyzed for the protein and mRNA content of GS, GDH, ALT, EAAC1, and GLT-1. The $^{15}/_{16}$ D produced the most lean tissue while having the lowest capacity to utilize glutamate derived energy. Therefore, these lambs appeared to be more efficient than the PP or ½ D lambs. The PP lambs were the least efficient, producing the lightest muscled carcasses, but having the highest glutamate uptake and utilization.

Results of the two experiments reported in this thesis indicate genetic type can have an affect on N metabolism in growing lambs and these effects are likely a result of the differential expression of glutamate transporters and associated metabolizing enzymes.

CHAPTER VI

Implications

Given the importance of N and glutamate metabolism to support whole body tissue maintenance and growth, knowledge regarding the regulation of these processes can aid in the production of high quality animal products. The research conducted for this thesis has demonstrated that animals with different genetic makeup can have differential efficiencies of N and glutamate metabolism. These differences can affect whole animal production and economic benefits to producers.

APPENDIX

Appendix Table 1. Pre-trial weights and rates of gain of Polypay (PP), ½ White Dorper ½ Polypay (½ D), and $^{15}/_{16}$ White Dorper $^{1}/_{16}$ Polypay ($^{15}/_{16}$ D) lambs from birth to the start of trial[1]

	Genetic Type			
	PP	1/2 D	15/16 D	SEM
Birth to weaning, 70 d				
Birth wt, kg[3]	4.8[a, b]	5.2[a]	4.4[b]	0.7
Weaning wt, kg	23.7	29.4	24.0	6.9
Total gain, kg	18.9	24.2	19.6	6.3
ADG, kg/d	0.28	0.32	0.29	0.08
Weaning to Initial (d 0), 83 d				
Initial wt (d 0), kg[2]	39.7[a]	45.2[b]	38.1[a]	5.2
Total gain, kg	16.0	15.8	14.1	3.1
ADG, kg/d	0.19	0.19	0.17	0.04
Birth to Initial (d 0), 153 d				
Total gain, kg[2]	34.9[a]	40.0[b]	33.7[a]	4.7
ADG, kg/d[2]	0.23[a, b]	0.25[a]	0.22[a]	0.03

[1] Values are least squares means and pooled SEM.
[2] Least squares means within a row with different superscripts differ ($P < 0.1$).
[3] Least squares means within a row with different superscripts differ ($P < 0.05$).

Appendix Table 2. Carcass measurements and tissue sizes of Polypay (PP), ½ White Dorper ½ Polypay (½ D), and 15/16 White Dorper 1/16 Polypay (15/16 D) lambs[1]

	Genetic Type			
	PP	1/2 D	15/16 D	SEM
HCW, kg	25.2	26.1	25.3	4.2
DP, %[2]	49.62[a]	51.05[a,b]	51.47[b]	1.73
CCW, kg	23.8	25.1	23.8	4.4
12th rib fat, cm	.36	.46	.33	0.06
Body wall thickness, cm[3]	.71[c]	1.19[d]	.81[c]	0.09
Loineye area, sq cm[3]	12.5[c]	16.2[d]	17.7[d]	0.4
Yield grade	1.82	2.23	1.73	0.63
Leg wt, kg	6.9	7.5	7.2	2.0
Boneless leg wt, kg	4.2	4.5	4.5	0.8
Loin wt, kg[3]	2.1[c]	2.6[d]	2.5[d]	0.6
Rack wt, kg[3]	1.9[c]	2.1[c,d]	2.2[d]	0.5
Shoulder wt, kg	5.2	5.3	5.0	1.2
Boneless shoulder wt, kg	2.7	2.9	2.8	0.7
Trim wt, kg[3]	2.2[c]	2.7[d]	3.2[d]	1.0
Liver wt, g	897.4	911.2	875.2	102.5
Kidney wt, g	63.0	64.8	62.2	8.6

[1] Values are least squares means and pooled SEM.
[2] Least squares means within a row without a common superscript differ ($P < 0.1$).
[3] Least squares means within a row without a common superscript differ ($P < 0.05$).

Appendix Figure 1. List of abbreviations

½ D	½ White Dorper ½ Polypay
¾ D	¾ White Dorper ¼ Polypay
$^{15}/_{16}$ D	$^{15}/_{16}$ White Dorper $^{1}/_{16}$ Polypay
α-KG	alpha-ketoglutarate
AA	amino acid
ADF	acid detergent fiber
ADG	average daily gain
ADP	adenosine diphosphate
ALT	alanine transaminase
ATP	adenosine triphosphate
BCTRC	boneless closely trimmed retail cuts
BD	below detection
BW	body weight
BWT	body wall thickness
CCW	cold carcass weight
CP	crude protein
DFN	daily fecal nitrogen
DM	dry matter
DNI	daily nitrogen intake
DNR	daily nitrogen retained
dNTP	deoxyribonucleoside triphosphate
DOA	days of age
DP	dressing percentage
DTT	dithiothreitol
DUN	daily urinary nitrogen
EAAC1	excitatory amino acid carrier 1
EAAT4	excitatory amino acid transporter 1
EAAT5	excitatory amino acid transporter 5
EDTA	ethylenediaminetetraacetic acid
GDH	glutamate dehydrogenase
GLAST1	glutamate and aspartate transporter 1
GLT-1	glutamate transporter 1
GluH	glutamate with an attached H^+
GS	glutamine synthetase
HC	high concentrate
HCW	hot carcass weight
HEPES	*N*-(2-hydroxyethyl)piperazine-*N'*-2-ethanesulfonic acid
HR	high roughage
HRP or HRPO	horseradish peroxidase
Ig-G	immunoglobulin G
LD	longissimus dorsi
LEA	loineye area
N	nitrogen
NAD^+ or NADH	nicotinamide adenine dinucleotide

Appendix Figure 1. (continued)

ND	nitrogen digestibility
NDF	neutral detergent fiber
NPN	nonprotein nitrogen
OM	organic matter
PCR	polymerase chain reaction
PP	Polypay
Rl-T	real time
ADL	acid detergent lignin
RT	reverse transcriptase
SEB	solution extraction buffer
SEM	standard error means
SLC1	solute carrier family 1
Sub Q	subcutaneous
Wt	weight

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