Amino Acids in the Nutrition and Production of Sheep and Goats

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Abstract

In sheep and goats, amino acid nutrition is essential for the maintenance of health and productivity. In this review, we analysed literature, mostly from the past two decades, focusing on assessment of amino acid requirements, especially on the balance of amino acid profiles between ruminal microbial protein and animal production protein (foetal growth, body weight gain, milk and wool). Our aim was to identify amino acids that might limit genetic potential for production. We propose that much attention should be paid to amino acid nutrition of individuals with greater abilities to produce meat, milk or wool, or to nourish large litters. Moreover, research is warranted to identify interactions among amino acids, particularly these amino acids that can send positive and negative signals at the same time.

Keywords

Foetus · Growth · Meat · Wool · Milk · Immunity · Ovine · Caprine

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Abbreviations

BCAA	branched-chain amino acids
GIT	gastrointestinal tract
MP	metabolizable proteins
PDV	portal-drained viscera

5.1 Introduction

Management of amino acids in sheep nutrition and production has two major aims – achieving genetic potential for productivity and maintaining good animal health (Liu and Masters 2000, 2003). In an industrial context, these aims must also be achieved at an acceptable cost so the enterprise is profitable, because protein feeds and amino acid additives are usually expensive, and inefficiencies in the use of such feed components must be avoided.

The sheep industry supplies meat, milk and wool, and the rates of protein retention in these products is a dominant factor in productivity. Therefore, the management of amino acid nutrition for these productive processes aims at increasing, as much as possible, protein synthesis in the mammary gland and in the wool follicle, and protein retention in body weight gain –the difference between protein synthesis and breakdown (Wu 2018). In addition, because pregnancy is fundamental for flock propagation, the survival and growth of the foeto-placental units (the foetus

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must be neither too small nor too large), as well as associated growth and functional development of the uterus, are major targets of protein retention and therefore amino acid nutrition for ewe nutrition during gestation.

This review therefore focuses on amino acid nutrition during pregnancy and lactation, and during body weight gain and wool growth, in sheep. The relationships between these functions and the metabolism of the gastrointestinal tract (GIT) is also addressed because the GIT contains the largest immune biomass in the body. Most of the literature cited concerns sheep, but we also refer to some work on goats. To reflect the most recent advances, we primarily analysed literature published since 2000.

5.2 General Considerations in Amino Acid Nutrition

Dietary proteins are digested in the GIT and the resulting amino acids are absorbed into the body and transported to sites of protein synthesis to meet the requirements of the animal. In farm animals, the quality and quantity of dietary protein are usually referred to as 'profiles' (proportions of individual amino acids to the total amino acids or total protein) and as the amounts of essential amino acids entering the small intestine. In ruminants, in contrast to monogastric animals, dietary protein is degraded by rumen microorganisms to ammonia, amino acids and peptides that the microorganisms then use as nitrogen sources to support their own growth (Wu 2018). The host benefits from this process when the microorganisms flow into the small intestine and are digested. The amount of ruminal microbial protein that moves into the small intestine varies widely, with feed intake (as a proportion of body weight) and with the protein degradability of the dietary ingredients. Most dietary protein is degraded by the rumen microorganisms and used by those microbes as a source of nitrogen to synthesize their proteins, and this microbial protein enters the small intestine and is used by the host (Agricultural Research Council 1984), but some dietary protein can escape ruminal degradation. The amino acids contributed by this 'bypass protein' are very difficult to quantify, due to wide variation in by-pass rates, digestibility and amino acid composition of the various proteins (Ministry of Agriculture and Fisheries and Food Standing Committee on Tables of Feed Composition 1990). As a consequence, the amino acid profile of the ruminal microbial protein is used as an approximation of the profile available to the host, unless the dietary protein has been processed to enhance its rumen by-pass rate.

In farm animals, the primary purpose of the management of amino acid nutrition is to match the profile of amino acids, essential amino acids in particular, in the protein flowing into the small intestine [i.e., metabolizable proteins (MP) in ruminants)] with the amino acid profile of the products. This management is based on an assumption that the body does not need to modify the amino acid profile in MP in *do novo* protein synthesis because any such modification will lower the efficiency of utilization of dietary amino acids. To determine this supply-demand relationship, a basic strategy is to compare the amino acid profiles in MP with those in animal products. Table 5.1 lists the amino acid profiles of protein in rumen microbes, whole-body, wool, and milk for sheep, as well as milk for goats, and the uterine and umbilical uptakes of amino acids in ewes at 130 days of pregnancy. If the amount of an essential amino acid in ruminal microbial protein is much lower than the amount in the product protein, it is likely the dietary supply of this amino acid will not meet the demand of the body. As shown in Table 5.1, the pattern of most of the essential amino acids in ruminal microbial protein is similar to the patterns of body protein and milk protein, whereas the amounts of Ile and Val in microbial protein are much lower than the umbilical and uterine uptakes of these two amino acids in pregnant ewes. Among the traditionally classified dispensable amino acids, wool protein contains disproportionally high amounts of Cys (about five-fold), Arg, Pro and Ser (about two-fold), milk protein has higher proportions of Glu/Gln and Pro, and the uterus and foetus use a much

Table 5.1 Amino acid concentrations (g/kg protein) of microbial protein in the rumen, the whole-body of sheep (excluding wool) and wool protein

	Rumen microbial protein[a,b]	Whole-body protein[c]	Wool protein[d]	Goat milk[e]	Sheep milk[f]	Uterine uptake[g]	Umbilical uptake[g]
His	16–21	24	8–13	35	30	20	28
Ile	54–62	36	27–32	46	47	142	116
Leu	74–83	73	67–79	98	97	91	66
Lys	81–115	67	27–35	99	78	81	89
Met	16–25	18	4.4–6.3	22	27	12	30
Phe	49–57	39	25–36	50	42	38	55
Thr	52–66	49	54–66	40	43	46	54
Val	53–65	43	46–57	60	63	157	101
Ala	34–62	80	32–52	34	37	36	47
Arg	46–53	73	62–91	39	28	118	134
Asp[(h)]	112–129	86	55–66	72	84	0.1	−2
Cys	20–26	13	86–131	9	8	–	–
Glu[(i)]	127–141	132	111–142	200	217	97*	146*
Gly	49–65	96	46–86	16	18	−12	47
Pro	34–40	63	53–75	89	100	75	61
Ser	41–47	42	83–108	44	43	54	−27
Tyr	44–51	31	38–63	47	37	45	54

[a]Storm et al. (1983)
[b]Martin et al. (1996)
[c]MacRae et al. (1993). Calculated from the amino acid profiles of the carcass, gut, liver and skin and the corresponding protein contents in sheep by the authors. Wool protein is not included
[d]Reis (1979). Merino sheep wool
[e]Ceballos et al. (2009). Granadian goats
[f]Gerchev et al. (2005). Mean of amino acid concentrations for Tsigai and Karakachanska sheep
[g]Chung et al. (1998). Columbia-Rambouillet sheep, carrying a single fetus, pregnancy 130 days. *Including both glutamate and glutamine concentrations. Cysteine concentration was not reported in this paper. Amino acid profiles were calculated by the author based on the uterine and umbilical uptakes (g/kg fetus.d) of amino acids reported in the paper
[h]Asp = aspartate plus asparagine
[i]Glu = glutamate plus glutamine

higher proportion of Arg, compared with these amino acids in the ruminal microbial protein. The high content of these amino acids must endow special functions to proteins in the respective products – for example, Cys for disulphide bridges, Ser for hydrogen bonds, and Arg for salt bridges, in the structure of wool proteins (Popescu and Höcker 2007). The supply of these amino acids from the diet as well as from synthesis in the body, must be considered for the management of these biological processes. We will discuss some of these situations below.

A further consideration for understanding amino acid nutrition in animals is obligatory oxidation in the body after intestinal absorption – essential amino acids are either used for protein synthesis or disposed of by oxidation, including conversion to other amino acids, such as Met to Cys. The extent of oxidation therefore directly determines the utilisation efficiency of an amino acid in the body. This oxidation can be measured by using ^{13}C-labelled amino acid (Young and Borgonha 2000) and, for a given amino acid, the oxidation rate is defined as the amount oxidised as a proportion of the flux. Liu and Masters (2003) analysed published literature and calculated the oxidation rates of 0.18 for Cys, 0.03–0.04 for Leu, 0.15 for Lys, 0.16 for Met, 0.08–0.09 for Phe, and 0.04 for Thr. The sulfur-containing amino acids (Met and Cys) and Lys and have much higher oxidation rates than the others, suggesting lower efficiencies for protein synthesis and, therefore, higher dietary demands.

In essence, any essential amino acid oxidised in the body must be replenished from the diet, and the amount needed in the diet can be defined as the requirement of this amino acid for the corresponding physiological process. The requirements for the essential amino acids (Leu, Ile, Lys, Met+Cys, Phe, Tyr, Thr, and Try) for adult humans, based on measurements of their oxidation rates, is known as the Massachusetts Institute of Technology System that was proposed by Young and Borgonha (2000). For sheep, a comparable system has not been fully established – only Met and Cys requirements were reported by Liu and Masters (2000) and, for growing Kazakh lambs, the Met requirement has been estimated from Phe oxidation (Wei et al. 2017).

Another point worth noting is that amino acid nutrition is influenced by genetic potentials for productivity and dietary intake. At the same level of feed intake, animals achieving higher productivity certainly have a higher efficiency of utilization of dietary amino acids for protein deposition, compared to these with low productivity. For amino acids that are incorporated into specific products in particularly high proportions, such as wool protein and the gravid uterus, the same level of dietary intake of these amino acids may meet the demands of animals with low productivity, but could be inadequate for animals with high productivity. For example, Merino sheep of 55 kg live weight are fed 0.7 kg/day of a hay/barley/lupin diet containing 12% crude protein, providing 8% MP, an intake level that maintains body protein balance (i.e., no net protein deposition in the body) – the estimated Cys absorption would be 1.4 g/day, and after excluding obligatory oxidation, about 0.7 g/day Cys would be available for wool growth, equivalent to the amount needed for wool growth of 7 g/day (Liu and Masters 2000). This diet can thus meet the Cys demand of sheep growing up to 7 g wool per day, but not sheep growing more than 7 g wool per day.

5.3 Reproduction

In pregnant ewes, the primary purpose of amino acid nutrition is to support ovulation, fertilization, implantation, embryo development, and fetal growth through to birth (Wu 2018). This process begins with ovarian follicles and their oocytes going through a selection process regulated by an interplay of reproductive hormones, and some of the dominant follicle(s) eventually ovulate (Scaramuzzi et al. 2011). In sheep, the number of dominant follicles that ovulate, the ovulation rate, depends on the energy balance of the animal, and it seems unlikely that amino acid balance plays a role (Scaramuzzi et al. 2011). Fertilization leads to the formation of a zygote that moves into the uterus and simultaneously begins to develop into an embryo. Early embryos produce signals that lead to implantation and recognition of pregnancy about 2 weeks after fertilization. The process of embryogenesis involves consumption of nutrients, from internal reserves, oviduct fluid and uterine secretions. To create conditions for conceptus development, the glandular tissue of the uterus produces, or selectively transports from the bloodstream, a complex array of proteins and other molecules into the uterine lumen (Bazer et al. 2012). Most embryo deaths occur during this peri-implantation period so an optimal nutrient supply seems to be crucial to the success of implantation. The placenta begins to develop about 25 days after fertilization and it ensures adequate nutrition to support the growth and development of the foetus (Bazer et al. 2012).

Throughout the whole process, the nutritional status of the pregnant female is critical for the establishment and maintenance of pregnancy. The supply of amino acids plays a significant role in embryo development to the blastocyst stage. For example, studies with an *in vitro* mouse model have shown that non-essential and essential amino acids, and Gln, play opposite roles in the regulation of cleavage and blastocoel development (Lane and Gardner 1997; Van Winkle 2001). In cattle, the concentrations of both

essential and non-essential amino acids in the uterine fluid, 12–18 days post-estrus, are 2.1–3.9 fold greater than the concentrations in non-pregnant cattle (Groebner et al. 2011). In cultured ovine primary trophectoderm cells from day 15 conceptuses, concentrations of Arg, Leu, and glucose, but not Gln, seem to be critical for cell function, with Arg and Leu concentrations stimulating proliferation and migration of cells within the embryo (Kim et al. 2011). In ewes, the nutrient composition of uterine luminal fluid differs between days 3–16 of the cycle and days 10–16 of pregnancy. Similarly, in pregnant ewes, the amounts of glucose, Arg, Gln, Glu, Gly, Cys, Leu, Pro and glutathione in uterine fluid increase 3- to 23-fold between days 10 and 14 of pregnancy and remain high until day 16 (Gao et al. 2009). These observations suggest that pregnancy recognition is associated with transport of amino acids into the uterine lumen, with Arg, Leu, Val and Gln/Glu, being the most critical.

To distinguish the amino acids that are preferably used by the conceptus and associated tissues during pregnancy, Chung et al. (1998) measured the uptake of amino acids by the uterus and umbilical cord in pregnant ewes for 130 days. These observations can be compared with the amino acid profile of rumen microbial protein as a representative of dietary supply, and the amino acid profile in the whole-body protein of growing lambs (Table 5.1). It is clear that the uptakes of Arg, Ile and Val are 2–three-fold greater than their compositions in both rumen microbial protein and whole-body protein. It should be noted that any amino acid taken up can be used either for anabolism (protein deposit) or catabolism, and the amino acid profile in deposited protein would be similar to protein composition of tissue or body protein. There is no evidence that the amino acid profile of the whole-fetus differs from that of the whole-body of growing lambs. Therefore, a corollary is that these amino acids are not preferentially taken up specifically for protein deposition in the fetus, but for modulating metabolic processes in the gravid uterus, including uterine tissue as well as the fetus, the so-called "functioning amino acids" (Wu 2009). This concept is supported by the fact that the net uteroplacental uptake of Arg was about four-fold greater than the net fetal uptake in ewes at 129 days gestation (Thureen et al. 2002). The functions of Arg include: i) the synthesis of NO, an important molecule for regulating placental angiogenesis and uterine blood flow during gestation; ii) synthesis of polyamines, molecules that are essential for placental development and embryogenesis; iii) activation of the mTOR signaling pathway and regulation of hormone secretion, both thoroughly reviewed (e.g., Wu et al. 2016; Wu et al. 2014; Wu et al. 1999).

It is therefore not surprising that, in pregnant ewes, Arg supplementation has beneficial effects from the first trimester through to the birth of the foetus. In the early stages of embryogenesis, rumen-protected Arg saves weaker embryos from entering early degeneration by increasing the synthesis of NO and polyamines (Saevre et al. 2011). Parenteral administration of Arg between 100 and 121 days of gestation increases the birth weight of quadruplet lambs and improves post-natal survival (Lassala et al. 2011). The improvement in lamb survival with Arg supplementation, by feeding rumen-protected Arg or by intravenous Arg infusion, seems to be related to increased brown fat in the foetus, alleviation of slow fetal growth caused by poor maternal nutrition (restricted feeding), increased uteroplacental weight, increased birthweight, as shown in reviews and confirmed in a number of experiments, particularly in ewes that are under-fed or carrying multiple fetuses (Lassala et al. 2011; McCoard et al. 2013; Satterfield et al. 2012, 2013; Sun et al. 2018; Zhang et al. 2016; van der Linden et al. 2015). To maintain a high concentration of Arg in the maternal circulation, administration of citrulline was more effective than a direct supplement of Arg because of a longer half-life (Lassala et al. 2009). The beneficial effect continued on the post-natal growth of lambs up to about 2 months old when pregnant ewes had restricted feeding (60% of the nutrition requirement) but were supplemented with about 12 g/day rumen-protected Arg from day 54 gestation until parturition (Peine et al. 2018). Because it is now known that citrulline is not degraded by the ruminal

microbes of steers (Gilbreath et al. 2019, 2020a) and sheep (Gilbreath et al. 2020b), this amino acid (in an unprotected form) can be directly supplemented to the diets of sheep and goats to enhance their reproductive performance as suggested by these authors.

It is no surprise that there are few concerns about the supply of amino acids during early pregnancy, because so little biomass is involved in eggs, blastocysts, embryos, and early stage fetuses. The total requirement for amino acids, even those considered essential for pregnancy, would be negligible within the context of requirements for the whole body. On the other hand, evidence is accumulating for effects of periconception nutrition on the subsequent development of the embryo, the foetus, the newborn, with some effects persisting into adult life (e.g., Sen et al. 2016; Gardner et al. 2006). It is not clear whether such effects involve the supply of amino acids or energy. Moreover, some amino acids play regulatory roles that might be far more important than simply being building blocks for proteins. There are substantial changes in the reproductive endocrinology during the estrous cycle and pregnancy, and many of the hormones involved are controlled by neuronal activity in the brain, where several amino acids act as neurotransmitters (e.g., as Asp and Glu; Wu 2013) or as precursors for the synthesis of neurotransmitters (such as the large neutral amino acids, Try and Tyr; Growdom and Wurtman 1979). Indeed, in sheep, infusions of such amino acids have triggered the secretion of gonadotrophin and increased ovulation rate (Downing et al. 1995, 1996, 1997; Foster et al. 1989). One of the problems with these hypotheses is the concept that critical brain functions can be determined by normal variation in the supply of dietary amino acids, although extreme imbalances could be devastating.

In the last third of pregnancy, however, as the feto-placental units achieve significant mass and the uterus itself develops muscle and secretory tissue, protein deposition in the gravid uterus becomes significant and it becomes essential to meet this demand for amino acids. The processes that regulate metabolism prioritize the gravid uterus over other physiological processes, and preferentially allocate nutrients to it, deriving them from both nutritional sources and, where necessary, sacrificing maternal tissues. To ensure reproductive success, through the support of pregnancy and the subsequent lactation, it is essential to manage amino acid nutrition correctly. The literature mentioned above shows that supplementation of Arg can reduce the impairment of fetal growth in underfed ewes, but only partially. The effects of dietary restriction during early pregnancy on the subsequent performance of the offspring in sheep, described above, were long ago recognized for maternal undernutrition during late pregnancy (Everitt 1967). This phenomenon later became known as 'fetal programming' or 'developmental origins of health and disease (DOHAD)' in the context of human health. As in many other species, maternal undernutrition in sheep is now recognized as a factor that determines offspring performance in growth, reproduction and several aspects of homeostasis (Vinoles et al. 2014; Rhind et al. 2001; Bielli et al. 2002). Again, the role of amino acid nutrition in these phenomena is not clear, although Arg has been implicated (Sales et al. 2016). We can conclude that, because some amino acids have special physiological functions (Wu 2013), it is best to manage amino acid nutrition in a holistic fashion during pregnancy to ensure optimal life-time growth and development of offspring.

We also need to consider the role of amino acid nutrition as a determinant of the quality and quantity of colostrum and milk, an essential issue in neonatal survival and offspring growth (Banchero et al. 2015). The capacity of the mammary gland for milk synthesis depends largely on the number and efficiency of the mammary epithelial cells (Rezaei et al. 2016). The development of these cells begins in the embryo but most happens during puberty and pregnancy, when undernutrition can have profound effects subsequent milk yield and quality. We need to remember that the rates of milk synthesis and secretion are largely driven by the rate of lactose synthesis within the mammary epithelial cells, so limiting energy intake during pregnancy, even if

positive energy balance is maintained by *ad libitum* intake during lactation, will lead to reduced milk production at birth. Nevertheless, the supply of amino acids is important because they are involved in synthesis of milk proteins as well as the proliferation and function of the mammary cells. Interestingly, the amino acid profiles are very similar for milk and the rumen microbes (Table 5.1), so microbial protein can match the requirements for synthesis of milk protein without substantial modification. In other words, there does not seem to be any 'limiting' amino acids during lactation in sheep or goats. Even so, daily supplementation of multiparous Saanen dairy goats with rumen-protected Met increases milk yield (Flores et al. 2009). In another experiment with Karagouniko dairy ewes, dietary supplementation of fat plus rumen-protected Met from 2 weeks before lambing until the twelfth week of lactation increased milk yield by 37% during the first 7 weeks of lactation (Goulas et al. 2003). On the other hand, dietary supplementation with rumen-protected Met to Chios dairy ewes in last fifth pregnancy did not change milk yield. Similarly, in Danish Landrace × Saanen crossbred goats, milk yield was not affected by dietary supplementation of rumen-protected Met or Lys (Madsen et al. 2005). The same outcome has been reported for Blackface, Dorset and Comisana ewes (McCoard et al. 2016). However, the effects of Met or Lys supplementation on milk yield is likely to depend on the timing and size of the supplement. For example, the highest milk yield in these studies was less than 3 kg/day and the basal diets contained 14–18% crude protein. Mature dairy goats generally weigh 30–80 kg and daily milk yield varies among breeds, from 2.6 kg (Nigerian Dwarf) to 11.9 kg (Toggenburg) with the Alpine, Nubian, Oberhasli, and Saanen producing 7–10 kg (Park et al. 2007). Protein concentrations average at 35 g/L (Park et al. 2007). Therefore, amino acid nutrition might be an issue in dairy goats with very high milk production, but only if they are fed diets with limited crude protein.

5.4 Growing Sheep and Goats

Growth involves the accumulated outcomes of cell division and cell differentiation, and, in livestock production, is measured as growth rate and muscle gain. Critically, maternal nutrition during pregnancy influences fetal and post-natal growth, with under-nutrition seriously inhibiting the growth and development of skeletal muscle in the offspring (Wu 2018). The third trimester of pregnancy is particularly important for the proliferation of muscle cells and changes in amino acid supply might alter post-natal muscle growth (Greenwood et al. 2000). For example, nutrition-restricted ewes produce lambs with reduced body weight but, if they are provided with supplements of rumen-protected Arg during gestation, the outcome is restoration of neonatal birth weight, lamb weight at age 19 days, and brown fat reserves (Peine et al. 2018).

Overall, research on amino acid nutrition is scarce for growing lambs and kids compared to monogastric animals. It could be that growth rate is a smaller economic factor for ruminants under grazing conditions than for monogastric animals in intensive, in-door systems. Moreover, the great similarity between the amino acid profiles of whole-body protein and rumen microbial protein (Table 5.1) suggests that no particular amino acid (s) is deficient or preferably required for lamb growth. By contrast, in growing pigs, dietary supplementation of Lys is necessary for muscle protein accretion and thus muscle growth (Liao et al. 2015).

Numerous studies show that the branched-chain amino acids (BCAA; Ile, Leu, Val) have the unique ability to initiate signal transduction pathways that up-regulate translation, and therefore protein synthesis, in skeletal muscle (Kimball and Jefferson 2006; Yoshizawa 2004). However, the literature for the lamb is inconclusive. For example, van Nolte et al. (2008) fed Rambouillet wether lambs (35–46 kg body weight) a basal diet containing 14.3–15% crude protein, and then infused abomasally a mixture of

10 essential amino acids. They then removed individual essential amino acids from the infusion. Removal of Met and Thr reduced N retention (g/day) and the ratio of retained N to digested N, whereas removal of BCAA had no significant effect. On the other hand, Sang et al. (2010) found that dietary supplementation of 6-month old wether lambs (25 kg) with rumen-protected Leu (0.5, 1 or 2 g/day) for 15 days increased protein synthesis rate in *m. longissimus dorsi* and, in *m. biceps femoris*, protein synthesis was increased only at 1 g/day (Sang et al. 2010). In this experiment, the basal diet contained 11.6% crude protein. By contrast, intravenous infusion of Suffolk-cross wether lambs (32 kg; aged 8 months) with 1.3 g of a mixture of BCAA over 6 h did not change the protein synthesis rates in *vastus* muscle or *m. longissimus dorsi* (Wester et al. 2004). It appears that the effect of Leu supplementation on lamb growth varies with dietary protein level and the rate of Leu supplementation. We conclude that more work is required to resolve the issue of 'limiting' amino acids in growing lambs and kids.

5.5 Wool (Fibre) Growth

Amino acid and protein nutrition for fibre production was thoroughly reviewed a decade ago by Liu and Masters (2003) for sheep and by Galbraith (2000) for goats. Since then, there has been little new research.

The fibre is produced by follicles embedded 500–600 μm below the skin surface and, in Merino sheep, the biomass of the follicular tissue amounts to about 50 g, or 0.1% of live weight (Williams 1995). The fibre is composed almost entirely of protein and the net efficiency of dietary protein for wool growth is estimated to be 0.20–0.25 (Standing Committee on Agriculture 1990). This value is substantially lower than those for weight gain (0.59), pregnancy (0.85) and lactation (0.68) in sheep (Agricultural and Food Research Countil Technical Committee on Responses to Nutrients 1993). The low efficiency is mostly due to limits in the supply of Met+Cys from the diet, combined with the relatively low productivity (7–18 g/day) of protein retained in wool compared with values for 300 g/day body weight gain (about 45 g/day) and for 1 kg/day milk (about 50 g/day).

Wool protein contains about 10% Cys, and much higher proportions of Arg, Ser and Pro, compared with the ruminal microbial protein (Table 5.1). Most feed proteins contain at most 2% Met and 2–5% Cys (Ministry of Agriculture and Fisheries and Food Standing Committee on Tables of Feed Composition 1990). In the body, Cys can be synthesised from Met through the trans-sulphuration pathway (Finkelstein 1990) and the amount of Cys produced from Met is estimated to account for 5–22% of the Cys flux (Liu and Masters 2003). In addition, local synthesis of Cys in the skin and follicle provides substantial amounts for wool growth (Harris et al. 1997; Souri et al. 1998b). For these reasons, Cys + Met is usually considered to be the limiting amino acid for wool production.

Many studies have shown that supplementing Merino sheep with appropriate levels of Met (about 2–5 g/day) improves wool growth, but not during late pregnancy or early lactation (review: Liu and Masters 2003). In cashmere and Angora goats (Souri et al. 1998a), Met also improves fibre production. In cultured follicles, Met alone produces 80% of the response seen with Met +Cys, whereas the response to Cys alone varies – follicle growth and viability can be reduced while, with Met alone, follicle growth can reach 75% of that recorded with Met+Cys. Although the concentration of Met in wool protein is very low, it combines with Cys to play a major role, probably by initiating protein synthesis and cell division. By contrast, Cys provides a substrate for wool protein synthesis, as evidenced by the increases in expression of mRNA encoding a family of Cys-rich proteins (Fratini et al. 1994) and the synthesis of Cys-rich proteins (Harris et al. 1994).

Supplying more Met and Cys through dietary supplementation is not usually cost effective because of the high prices of the supplements and the price penalty paid because fibre diameter increases. On the other hand, the most effective way to improve feed efficiency and amino acid utilization for fibre growth in sheep and goats is probably genetic selection for high fibre growth rate. Within species, variation in fibre growth is

explained by variation in the proportion of active follicles and/or the efficiency of the follicles (fibre growth rate/follicle density). Thus, on the same plane of nutrition, sheep selected for high clean fleece weights grow more wool than sheep selected for low fleece weight, and wool growth rates are closely related to skin fractional protein synthesis rate and to skin total protein synthesis (Masters et al. 2000).

The rate of fibre production varies greatly across species. Merino sheep (53 kg body weight) produce about 4 kg greasy fleece (2.8 kg clean) per year (Mortimer et al. 2017). Angora goats (30–60 kg) can produce 1.5–4 kg mohair over 6 months and cashmere goats (30–70 kg) produce less than 1 kg of guard hair and cashmere per year (Lupton 2010). Angora rabbits (3.5–4.0 kg) can produce 1.2–1.4 kg clean wool per year at a net efficiency of 0.43 (Liu et al. 1992), and are obviously the most productive in terms of fibre produced per kg of body weight. These differences among species are associated with variation in net efficiencies of use of digested protein for fibre growth: 0.43 for Angora rabbit, 0.39 for Angora goat, and 0.20–0.25 for Merino sheep (review: Liu and Masters 2003). We do not have a value for cashmere production but it must be very low. There is a clear interaction between the genetic capacity for fibre growth and responses to dietary protein or amino acids, so we would not expect supplementation of low-productivity animals to greatly improve productivity or profitability. Experimental data supports this hypothesis: Angora goats show a substantial fibre response to supplementation with rumen-protected Met (62% vs 30%; Souri et al. 1998a, b) whereas, in cashmere goats, the fibre response is similar for a urea-based diet and a fish-meal diet (about 15% crude protein) providing similar levels of nitrogen (Galbraith, 2000), probably because the nutrient demand for fibre growth was already met by the urea. The variation among species therefore seems to be more dependent on genetic variation in follicle density and morphology than amino acid efficiency, so exploration of the molecular mechanisms that control follicle productivity is likely to be a more productive avenue towards improvements in fibre growth than dietary manipulation.

5.6 Amino Acid Nutrition for GIT Health and Nematode Infection

In sheep, the biomass of the gastrointestinal tract (GIT) is about 4–6% of body weight (Liu et al. 2005; MacRae et al. 1993) and, metabolically, GIT tissue has the highest turnover rate in the body (Lobley 1994) due to the renewal of desquamated enterocytes, and the production of secreted digestive enzymes, immune molecules and cells. Therefore, protein synthesis in the GIT accounts for 25–33% of whole-body protein synthesis (Lobley et al. 1994; Neutze et al. 1997) as well as 11%–23% of whole-body energy expenditure (McBride and Kelly 1990; Lobley 1994). There is no doubt that these high costs mean that relatively small proportions of amino acids and energy are available for anabolism of peripheral tissues, limiting body growth, the production of milk and wool, and fetal growth and development. This is an intrinsic aspect of mammalian biology. However, there is a wide range in protein turnover in the GIT in proportion to the whole body, suggesting considerable genetic variation that is likely to be closely associated with variation in whole-body efficiency of utilization of amino acids. To date, there has been little research into these issues in sheep and goats.

The use of amino acids by the GIT would be expected to have flow-on effects to the amino acid profile that is available to the peripheral tissues. In other words, does the GIT disproportionally incorporate specific amino acids into its proteins? This question may be partly answered by comparison of the amino acid profiles (g/kg total amino acids) of the GIT and the carcass, as done for sheep by, for example, MacRae et al. (1993). Using their data, we calculated the ratios of 18 amino acids in GIT to those in the carcass and found that 13 ratios fell into the range of 0.9–1.1 (i.e., close to unity). By contrast, the ratio was 1.6 for Cys, 1.2 for Met, Ser and Thr, and only 0.7 for 4-hydroxyproline. These ratios suggest that GIT proteins contain particularly

high concentrations of Cys, Met, Ser and Thr, so less of these amino acids would be available for other tissues. It should be noted that proteins secreted into the GIT lumen, such as digestive enzymes, some immunoglobulins and glycoproteins, are probably not included when GIT tissue is sampled for analysis. For example, in one study with sheep (Mukkur et al. 1985), goblet-cell mucin in the small intestine contained 94 g Cys, 243 g Thr, and 237 g Val per kg total amino acids (masses recalculated by the authors to remove ammonia). These values are over ten-fold higher than those in the sheep carcass (MacRae et al. 1993). We can see, therefore, how these proteins could affect the profile of amino acids in the GIT, but no quantitative data are available for the amounts of these proteins produced, so their impact on amino acid supply to other tissues is not known.

When not used in protein synthesis, some amino acids are oxidised in the GIT, and the oxidation rates could also alter the amounts and proportions that reach peripheral tissues. The small intestine of the sheep can catabolize Leu and Met, accounting for 26% and 10% respectively of the whole-body Leu and Met oxidation, whereas there seems to be no net catabolism of Lys and Phe (Lobley et al. 2003). Estimates of the magnitude of Leu oxidation in the GIT of sheep vary from 0–50% in the literature (Lobley et al. 2003; Yu et al. 2000), and we have not been able to find estimates for other essential amino acids in sheep and goats. This is perhaps no surprise because direct measurement of amino acid oxidation in the GIT requires surgical placement of catheters into specific positions in selected arteries and veins, as well as the small intestine, employment of isotope-labelled amino acids, and analysis of the end-product (mostly CO_2) of oxidation (Lobley et al. 2003; Yu et al. 2000). The techniques are complex, and therefore the data are rare. An alternative approach is to measure the sequestration of amino acids by the mesenteric-drained viscera (from the small intestine) and the portal-drained viscera (PDV, GIT plus spleen and pancreas; Lobley et al. 2003), as has been done in sheep (MacRae et al. 1997) and pigs (Fang et al. 2010). In sheep, the PDV recoveries of amino acids infused into the jejunum varied from 61% for His to 65% for Phe, 76% for Lys, 79% for Thr, 80% for Ile and Leu, and 83% for Val (MacRae et al. 1997). Therefore, 17–39%, depending on the amino acid, were used by the PDV. In pigs, the amount of Met used by the PDV accounted for 29–33% of dietary intake (Fang et al. 2010). It is worth noting that the sequestrated amino acid can be used either for protein synthesis or oxidation, and it is difficult to ascertain partitioning between these two processes. Yu et al. (2000) found about 14% of Leu sequestrated by the PDV in sheep was oxidized, with a slight increase to 15%–16% after infection with the helminth, *Trichostrongylus colubriformis*. Infection with *T. colubriformis* also leads to a considerable reduction in Met absorption (Liu et al. 2002).

As with the obligatory oxidation of amino acids in the liver, oxidation in the GIT is likely to serve a purpose. In the GIT, we can find all of the catabolic pathways for Met (Bauchart-Thevret et al. 2009; Liu and Masters 2003) through which S-adenosylmethionine (an important methyl donor), polyamines (spermidine and spermine), and Cys are derived. The aforementioned GIT proteins and secreted glycoproteins contain high proportions of Cys, and the GIT epithelium contains very high levels of glutathione (GSH), the synthesis of which demands Cys as a substrate (Wu et al. 2002). Cys synthesized from Met through the trans-sulphuration pathway (Finkelstein 1990) could be a significant source for GIT tissues, because the proportions of Cys are low in dietary and ruminal microbial proteins. The small intestine contains the highest levels of polyamines, spermine in particular, compared with other tissues (liver, lymph nodes, muscle, skin) in sheep (Liu et al. 2007), supporting its high turnover rate (Loest et al. 2002; Tabor and Tabor 1984). Met is catabolized through the aminopropylation pathway and provides the aminopropyl moiety for synthesis of spermidine and spermine. In rats, about 45% of spermidine and spermine are derived from *de novo* synthesis (White and Bardocz 1999). If this was also the case in sheep, synthesis of polyamines would certainly consume a considerable amount of Met. As for why Leu is oxidized in the GIT,

Lobley et al. (2003) speculated that the process might involve the interaction with signal cascades that regulate protein metabolism.

Among the dispensable amino acids, the GIT has specific and substantial demands for Gln. In the PDV of sheep, there is a net uptake of Gln when levels of protein are changed from slightly above maintenance (basal diet) to 3.8-fold maintenance by infusion of protein into the abomasum, whereas Glu was taken up at relatively low protein intakes (from maintenance to about 2.4-fold maintenance) but then released when the protein intake was more than three-fold maintenance (Freetly et al. 2010). Uptake of Gln by the PDV has also been observed in other studies with sheep (Foote and Freetly 2016; McNeil et al. 2016). The roles of Gln in GIT tissues have been thoroughly reviewed by Lobley et al. (2001) and it is clear that Gln is important for the provision of energy to rapidly growing cells. For example, it provides up to 30% of the energy needs of lymphocytes in cattle (Wu and Greene 1992). Intracellular Gln can either be deaminated to produced Glu plus ammonia, both of which are excreted out of cells, or partially oxidized to Asp, coupled with the formation of 9 ATP, far less than the 38 ATP produced from full oxidation of glucose (Rich 2003). However, Gln breakdown produces ATP at a much faster rate than oxidative phosphorylation (Aledo 2004), so it becomes an important energy source in cells with high proliferation rates, such as cancer cells and enterocytes (Aledo 2004). Gln is also a precursor that supplies half of the N required for synthesis of both purines and pyrimidines as well as aminosugars in all cell types (Calder and Newsholme 2002; Wu 2013).

In addition, the conversion of Gln to Glu (the glutaminolytic pathway) is more closely linked to cell proliferation than its intracellular concentration (Aledo 2004). High rates of conversion of Gln to Glu are seen in all lymphoid organs and cells, and Gln catabolism contributes more than a third of the energy requirement of immune cells (Duff and Daly 2002). The differentiation of B lymphocytes to plasma cells, and immunoglobulin synthesis, are Gln-dependent over the physiological range of Gln concentrations (Crawford and Cohen 1985). Since the GIT is structured to contain, or to be directly associated with, the highest biomass of immune components in the body, including the lymph nodes, Peyer's patch, immune cells, immunoglobulins and cytokines (Pastoret et al. 1998), one would expect an adequate supply of Gln to be essential for the maintenance of gut health. Indeed, in piglets weaned early (about 3 weeks of age), dietary supplementation with 1% Gln (on a fed basis) prevents jejunal atrophy, increases feed efficiency, and improves the immune responses to infection by *Escherichia coli* (Wu et al. 2011), suggesting that the Gln requirement is higher in gut-stressed animals. In calves weaned early (age 42 days), intravenous administration of Ala-Gln dipeptide at 1 g Gln per kg body weight (the total amount of Gln equivalent to 0.05% of the dietary intake of solid matter) increased blood $CD2^+$ and $CD4^+$ lymphocytes, serum IgA and IgG concentrations, and mucosal secretary IgA concentrations in jejunum and ileum, while decreasing the incidence of diarrhoea (Zhou et al. 2012). However, there is little information about the effect of dietary Gln supplementation on gut function in sheep or goats, probably because dietary Gln is destroyed by rumen microorganisms. To address this issue, we need a source of rumen by-pass Gln, although it is feasible that gut-stress is not as problematic in young sheep and goats as it is in weaning piglets.

A critical GIT health issue in grazing sheep and goats is infection by helminth nematodes. Severe infection depends on season/climate and management, but it causes chronic inflammation of GIT tissues, reduces feed intake and re-absorption of nutrients from the intestinal lumen, and can cause chronic diarrhoea, with the overall outcome being reduced productivity (Sykes and Coop 2001; Grencis et al. 2014; Williams 2011). Nematode infection also changes amino acid oxidation in the GIT. Yu et al. (2000) examined Leu metabolism in the GIT of lambs after infection with *T. colubriformis* larvae and found that, in the absence of detectable effects on whole-body leucine flux, there was a 24% increase in total GIT Leu sequestration and an increase from 22% to 41% in GIT Leu oxidation. These observations suggest that nematode

infection stimulates Leu oxidation in the GIT and reduces nutrient partitioning to the peripheral tissues. As mentioned above, infection of lambs with *T. colubriformis* reduces Met absorption into the peripheral tissues (Liu et al. 2002). Taken together, these observations suggest that nematode infection increases the consumption of some amino acids in the GIT, probably to support enhancement of metabolic processes for repair of damaged GIT tissue and to elicit immune responses.

The various pathophysiological responses to nematode infection include an increase in the secretion of mucus by the GIT (Theodoropoulos et al. 2001). The GIT is lined by a mucus layer that is continuously secreted and forms the first physical barrier that protects the GIT epithelium. The mucus has gel characteristics due to the presence of high molecular weight mucins (glycoprotein monomer or polymers), antibodies (immunoglobulin A in particular) and other molecules (Simpson et al. 2016; Theodoropoulos et al. 2001; Dharmani et al. 2009). An increase in the secretion of mucus is part of the initial nonspecific response to nematode infection, followed by activation of biosynthetic processes that involve changes in the chemical composition, and therefore structure, of the mucins; the final outcome depends on the nematode species and on the adaptation of the host to that species (Theodoropoulos et al. 2001; Menzies et al. 2010).

In the sheep small intestine, the mucin proteins contain high proportions of Cys and Pro and very high proportions of Thr, Ser and Val (Lien et al. 2001; Mukkur et al. 1985). The hydroxyl moiety in Ser, Thr and Pro services O-glycosylation, and the hydrosulphide moiety is used for forming disulphide bonds within the monomer and polymers, all of which are critical for resistance to proteolytic enzymes (Dharmani et al. 2009). In pigs, the mucin protein contributes 5–11% of the total endogenous protein in ileal digesta, depending on feed consumption, dietary protein and fibre concentration (Lien et al. 2001). The corollary is that the contribution in ruminant animals may be higher than for monogastrics due to the very high proportions of fibre in their diet. The extremely high proportions of Thr, Ser and Val in gastric and intestinal mucins means a high requirement for mucin synthesis and an increase in dietary demand, depending on the response in mucin secretion evoked by nematode infection. The problem is that there are no quantitative estimates of mucin secretion, even in healthy animals, so we have no way to assess the potential effects of these amino acids in the diet.

Investigation of the effects of supplementation with specific amino acids on epithelial barrier (mucin) and the immune responses of the GIT in ruminants is scarce. In sheep, we know that wool growth demands a high amount of SAA, particularly Cys, because they are deposited in wool, and that Cys is therefore drawn from the metabolic body pool, reducing the availability of Cys for, for example, immune competency. Indeed, in Romney sheep, the fecal worm egg counts are increased in animals selected for high fleece weight, and abomasal infusion of Cys (2 g/day) tended to increase peripheral eosinophil count, abomasal globular leukocyte count, and the immunoglobulin G response, yet no interaction between Cys supplementation and genotype was observed when selected and unselected animals were compared (Miller et al. 2000). Abomasal supplementation of 6-month old Suffolk cross lambs with both Cys (1 g/day) and Gln (5 g/day) for 12 weeks after infection with *T. colubriformis* led to an increase in nitrogen retention, along with reductions in circulating eosinophil count and peak faecal egg counts, but had no effect on final nematode counts (Hoskin et al. 2002). In lactating rats infected with *Nippostrongylus brasiliensis* (the adults reside in the small intestine), feeding the Met- and Leu-deficient diets (about 40% below the normal diet) increased the number of worm eggs in the colon, but had no effects on systemic immunoglobulin activity or the numbers of mast cells, goblet cells and eosinophils (Sakkas et al. 2013). This literature is limited, but suggests that

deficiency or supplementation of specific amino acids influences worm fecundity, as reflected in changes in fecal worm egg counts, but there is no conclusion with respect to influences on the immune responses to infection.

5.7 Concluding Remarks

Concomitant evolution of rumen microbes and their hosts over millions of years has resulted in great similarity in the amino acid profiles of microbial proteins and host whole-body protein. This similarity ensures a balance between the dominant supply of amino acids from the microbes and the host's needs for growth, survival and reproduction. However, the question is – has this balance been overridden by human interference? This question is most acute with the rapid progress in productivity driven by modern breeding practices, with changes that exceed by far the pace of evolution. The evidence is accumulating to support the view that the supply of amino acids to the host, from both rumen microbial protein and rumen by-pass protein, no longer meets the demands of high-performance animals that produce large amounts of meat, milk or wool, or need to nourish large litters. Without doubt, the pursuit of high productivity will continue, so research on amino acid nutrition needs to accelerate. Meanwhile, with regard to the processes, amino acids need to be seen as "two-way switches" that can send positive and negative signals at the same time, and thus play specific regulatory roles under specific conditions. For example, in cultured mouse mammary epithelial cells, Leu, Ile, and Val stimulated phosphorylation of ribosomal protein kinase beta-1 (S6K1), whereas Lys, His and Thr inhibited it (Prizant and Barash 2008). Similarly, in myogenic C2C12 cells, Leu and Gln have opposite regulatory effects on the phosphorylation of downstream effector S6K1 and eukaryotic translation initiation factor 4E binding protein in the mammalian target of rapamycin (mTOR) pathway (Deldicque et al. 2008). Leucine increases protein synthesis by stimulating the mTOR signaling pathway, and also enhances catabolism (Gannon and Vaughan 2016). In sheep, arginine affects both protein synthesis and proteolysis in cultured brown adipocyte precursor cells and, in those cells, the mTOR signalling pathway is promoted by an increase in the Arg concentration in maternal plasma (Ma et al. 2017). At the highest level of regulation, the brain, mTOR is deeply implicated in the control of energy homeostasis through the coordination of anabolic and catabolic processes focused on survival (Morentin et al. 2014). Therefore, much more attention must be paid to the interactions among amino acids in the regulation of biological processes.

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