

Amino acids in sheep production

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1. ABSTRACT

Increasing production efficiency with a high standard of animal welfare and respect for the environment is a goal of sheep farming systems. Substantial gains in productivity have been achieved through improved genetics, nutrition and management changes; however the survival and growth performance of multiple-born lambs still remains a problem. This is a significant production efficiency and animal well-being issue. There is a growing body of evidence that some amino acids have a role in regulating growth, reproduction and immunity through modulation of metabolic and cell signaling pathways. The purpose of this review is to provide an overview of what is currently known about the role of amino acids in sheep production and the potential for supplementation strategies to influence on-farm survival and growth of lambs.

2. INTRODUCTION

In some farming systems, genetic gain to improve prolificacy and carcass composition, improvements in crop production and better grazing management strategies have enhanced on-farm sheep performance. For example, the improvement in lambing percentage (number of live lambs per ewe mated) has been one of the key factors leading to increased productivity and profit for New Zealand sheep farmers. The New Zealand national lambing percentage has

increased over 26 percent in the last 40 years reaching an average of 124 percent (1). This gain coupled with the increase in market weight of lambs have both compensated for the 32 percent decrease in the national sheep stock numbers during the last 15 years, to maintain the productivity as measured by kg of meat produced (2). Lambing percentages of greater than 200 percent have been described in New Zealand (3, 4). Higher lambing percentages are associated with an increase in the proportion of twin- and triple-born lambs (5, 6), which have higher rates of mortality and reduced growth rates compared to singletons resulting from intrauterine growth restriction (IUGR) (7-9). In addition, IUGR can result in permanent negative effects on growth, feed efficiency, body composition and thus poor finishing, meat quality and long-term health, thereby decreasing farmer profits (10, 11). Addressing this constraint will reduce lamb mortality and improve on-farm productivity (e.g. more lambs finished at weaning or increased post-weaning growth to support hogget mating) and profit.

It is now well accepted that environmental signals play a key role in the ability of an animal to perform according to its genetic potential, with nutrients being one of the most important factors. Out of nutrients, the amino acids (AA) are not only the building blocks of proteins and other nitrogenous substances, glucose and fatty acids (12-15), but increasing evidence shows

that specific AA activate cell signaling ultimately influencing key metabolic pathways and important physiological functions (16-22). For example, in pigs, arginine and glutamine are crucial for intestinal growth, integrity and function and their supplementation can enhance embryonic survival, fetal growth, maternal milk production, neonatal immunity, and muscle and fat deposition without increasing food intake (23, 24). Importantly, research has demonstrated that animals have both metabolic and dietary needs for non-essential AA which were traditionally considered to be synthesized in sufficient quantities by the body to meet the needs of growth and optimal health, eliciting a rethink of the dietary requirements for all AA by livestock species (see (18) for review). Changes in dietary AA recommendations for pigs and chickens has already begun and is expected to deliver positive benefits through reduced protein content of diets, improved nutrient utilization, growth and production performance (see (18) for review). However, more research is required to understand what the AA requirements are for ruminants, and to identify the potential to address issues such as lamb survival, growth performance, feed efficiency and lifetime performance through specific AA supplementation. The effects of AA supplementation on sheep reproduction, postnatal growth and organ development is summarized in Table 1 and described in more detail in the subsequent sections. In addition, the physiological effects and potential biochemical mechanisms underlying AA supplementation in sheep is summarized in Table 2 and discussed in more detail in the following sections.

3. REPRODUCTIVE FUNCTIONS

It is well established that pre- and post-natal nutrition can influence reproductive functions of both males and female sheep (see (25) and (26) for reviews). For example, ewes underfed in the second half of gestation have offspring with fewer testicular Sertoli cells at birth (27) and onset of puberty in male lambs can be delayed following growth restriction *in utero* (28). However, there is a paucity of data on the effect of AA on fertility in sheep. In humans, a diet deficient in arginine decreases sperm counts by ~90 percent while the percentage of non-motile sperm increase by about tenfold (29), while in boars dietary supplementation of 1 percent L-arginine HCl increases sperm counts and sperm motility in boars (30). These observations highlight an important role for arginine in male fertility (see (31) and (32), for reviews). These effects may be mediated by increased synthesis of both nitric oxide (L-arginine is a NOS substrate) and polyamines which are essential to spermatogenesis and sperm viability. Using an *in vitro* approach, motility of sperm in rams has been shown to be dependent on nitric oxide action but that excess nitric oxide can be toxic and thereby reduce sperm motility (33). This indicates that there may be some potential benefits to ram fertility but experimentation *in vivo* has yet to be undertaken.

Nutrients also have a well-established effect on ovulation rate in ewes (34) but the role of AA to influence fertility has received little attention. Supplementation with branched-chain AA (BCAA) leucine, isoleucine and valine to ewes immediately before luteolysis increases plasma insulin concentrations and blood urea levels, which indicates increased supply of energy substrates to the follicles suggesting that BCAA may in part regulate the ovulation response to nutrient availability (35). Arginine supplementation to ewes has been reported to increase the number of corpora lutea and is associated with increased twinning rate (36). Supplementation of ewes with rumen-protected arginine may also increase ovarian blood flow (37). However, availability of tryptophan, tyrosine or a mixture of tyrosine and phenylalanine (dietary AA precursors for catecholaminergic and serotonergic neurotransmitters) has little effect on gonadotropin concentrations in sheep blood and therefore are unlikely to mediate an effect on ovulation rate (35). While these studies indicate that specific AA have the potential to influence reproductive physiology, more research is required to evaluate which AA may be important, the critical time windows for intervention and whether changes in endocrine and metabolic function translate into differential production performance (e.g. lambing percentage).

4. PLACENTAL DEVELOPMENT AND FUNCTION

The ovine placenta, like those of all other species, acts as an intermediary between the maternal and fetal vascular systems, to regulate gas exchange, waste elimination and nutrient transfer (38). In sheep, this regulated exchange begins during the initial stage of placental formation, at around 20 to 30 days of pregnancy, when the chorionic membrane attaches to the uterine wall and forms placentomes (39). Placentomes are highly vascular placental contact points with a fetal side (cotyledon) and a maternal side (caruncle), with their number generally established by 40 days of pregnancy (39). Each placentome can be classified based on their morphological type (type A to D) which may differ in their maternal-fetal exchange area (40), oxygen exchange efficiency (41) and glucose transport (42). Early studies have shown that the number of placentomes (43, 44), placentome type (45) and placental weight (44) can be influenced by modifying dam feed intake in early pregnancy. Other studies indicate that both placentome size and type, which has plasticity throughout pregnancy, may be important in regulating gas exchange, waste elimination and nutrient transfer (46).

The uptake, metabolism and transport of AA by the ovine placenta are critical to the survival, growth and development of the fetus (47). Amino acids are major fuels for fetal growth (48) and are essential precursors for many substances in the mammalian placenta and fetus including

Table 1. Summary of the effects of amino acid supplementation on sheep reproduction, postnatal growth and organ development in sheep

Target	Amino acid	Supplementation method	Period	Amount	Outcome	Ref.
Fetus	EAA-NEEA		d. 134 pregnancy for 12 h		↑ BCAA in plasma	73
	BCAA	Parenteral	d. 126 pregnancy		↑ Ammonia	73
	Arg	Parenteral	d. 100 to birth	345 mmol Arg-HCl/kg body weight 3 times daily	↑ Birth wt. of female twins	77
	Arg	Parenteral	d. 100 to 121	345 mmol Arg-HCl/kg body weight 3 times daily	↑ Birth wt. of quadruplets	76
	Arg	Parenteral	d. 60 to birth	155 µmol/kg body weight, three times daily	↑ Fetal growth and birth wt.	75
	Gln	I.V.	3 days per week in succession from gestational d. 109–132	100 mg/kg, three times daily	↓ Growth restriction, ↑ Gly, Arg, Asn in plasma	98
	EAA	I.V.	4 days during late gestation (d. 130)	Rate required to achieve a 25–50% increase in maternal BCAA concentrations	↓ Hypoxia, respiratory and metabolic acidosis	104
Lamb	Arg	I.V.		0.5. g/kg	↑ Somatotropin	87
	Arg	I.V.		0.2.5-0.5. mg/kg	↑ Somatotropin	88
	Arg	Abomasal infusion	7 days	0.5. g ARG-HCl/kg BW 0.4. g/kg ORN.HCl	↑ Somatotropin	92
	Arg-Orn	Post-ruminal		Arg-HCl (0.5.g or 0.7.5 g/day/kg body weight) and OrnHCl (0.4.2 g/day/kg body weight)	↑ Somatotropin and IGF-1	94
	Arg	Oral	Birth to weaning	500 mg Arginine-HCL/day/kg body weight	↑ Growth in first 3 weeks	95
Mammary gland	Met, Lys	Oral			Gains in BW and N balance were increased in lambs nursing ewes fed protected amino acids	119
	RP-methionine or RP-lysine		8 to 23 weeks post-partum	Methionine 0.2.5 g; lysine 0.7.5 g/100 g DM	Changes milk fat composition	159
	RP-methionine	Rumen protected met+fat	2 weeks pre-partum to 13 weeks post-partum	5 g/kg of feed	↑ Milk yield, daily fat and protein yield in the first 7 weeks of lactation	160
	Arg	I.V.	d. 100 to parturition	345 µmol/kg bodyweight	Transient increase in milk protein yield and the absolute concentration of some milk free AA ↓ milk somatic cell counts	154
Muscle	Mixed AA	Parenteral	d. 130 gestation	2 h	Up-regulation of S6K	140
	Arg, Lys, His, Thr, Met, Cys	I.V.		10 days	↑ Rate of initiation of mRNA translation	146

(Contd...)

Table 1. (Continued)

Target	Amino acid	Supplementation method	Period	Amount	Outcome	Ref.
Ovary	BCAA		5 days immediately before luteolysis		Regulates ovulation	35
	Arg	Diet	15 days	0.5. g kg (-1) of body weight	↑ Corpus luteum number and twinning	36
	Arg	Diet	d. 8 to d 13 of the estrous cycle	360 mg/kg BW	↑ Blood flow	37
Placenta	Arg	Parenteral	d. 60 to birth	155 μmol/kg body weight, three times daily	↑ NOS	75
Fat	Arg				↑ Hypertrophy of brown adipose tissue	200, 201
	Arg	Parenteral	d. 100 to birth	345 mmol Arg-HCl/kg body weight 3 times daily	↑ Brown adipose tissue	77
Wool	Met, Lys	Abomasal infusion		Methionine and lysine were infused at a daily rate of 2,46 and 5 g per sheep	↑ Wool growth rate	120

Table 2. Physiological effect and potential biochemical mechanisms underlying amino acid supplementation in sheep

Amino acid	Physiological effect	Potential biochemical mechanism	References
Arginine	Fertility	Increased nitric oxide production	33, 36
	Conceptus development	mTOR pathway activation	84
	Placental function and morphology	Increased thermogenic gene expression	75
	Fetal growth and development		75-77
	Neonatal growth		95
	Prolificacy		36
	Thermogenesis		77, 200, 201-204
	Milk yield and composition		154
	Immune function		182
Lysine, methionine	Milk yield and composition	Increased plasma low density lipoprotein synthesis	159, 160
Leucine, alpha-ketoisocaproate	Immune function	Modulation of T-cell type and number	180, 181

proteins, neurotransmitters and polyamines (49, 50). Placental transport is a major mechanism responsible for fetal AA homeostasis (51-53). Similar to observations in humans (54), growth-restricted ovine fetuses have reduced plasma concentrations of AA, and changes in the circulating concentrations of AA in the fetus mimic that of the dam (55, 56), highlighting the importance of placental AA transport across the placental barrier. Most AAs are delivered to the ovine fetus in greater amounts than required for net rate of accretion (57, 58), in an energy-dependent process (59, 60), resulting in fetal AA concentrations being higher than maternal AA concentrations (61). The relationship between maternal and fetal flux of AA has previously been reviewed (62). Briefly, fetal AA uptake

depends on the maternal AA concentration, and is mediated by AA transporters (63). There is no fetal uptake of maternal glutamate, aspartate and serine and these AA are produced by fetal tissue (57, 64-67). The fetal liver produces glutamate, which is taken up by the placenta, to produce glutamine and which is then returned to the fetus (68). Maternal serine is transformed in the placenta into fetal glycine some of which is delivered into the fetal circulation (66, 69) resulting in no transport of serine from the mother to the fetus. Instead, serine is produced mainly by the fetal liver (65).

Developmental changes occur in maternal and fetal plasma AA concentrations during pregnancy in

sheep that are mediated by the placenta. For example, between 40 and 140 days of pregnancy, changes in the concentrations of all AA, except for proline and tyrosine are observed in maternal plasma, while changes in the concentrations of AA, except for alanine, asparagine, isoleucine, leucine, phenylalanine, tryptophan and valine, are observed in fetal plasma (55). Factors other than stage of pregnancy may influence maternal and fetal AA profiles. For example, maternal nutrient status can influence both maternal and fetal plasma AA concentrations (70-72). In the pregnant ewe, prolonged maternal infusion of a mix of essential AA (EAA) and non-essential AA has been shown to only increase branched-chain AA (BCAA) and phenylalanine concentration in the fetus (73). In contrast, maternal hypo-aminoacidemia induced a reduction in fetal plasma EAA concentrations (74). Further, IUGR resulting from increased litter-size in sheep (9) is associated with changes in the transport of specific AA such as leucine across the placenta (59) and twin fetuses at 140 days of pregnancy have lower concentrations of histidine and glutamine and tend to have lower arginine and leucine concentrations in plasma compared to singletons (46) indicating altered placental nutrient transfer.

Fetal and maternal plasma AA concentration can also be influenced by maternal breed (61). Selection for ewes adapted to harsh environmental and limited nutrition conditions has been shown to provide the advantage of being able to maintain AA availability to the fetus during maternal nutrient restriction by altering placental efficiency (72) which may be mediated by changes in placentome morphology (45). However, it is yet to identify any potential role AAs may play in regulating these changes. Some studies hint at the potential use of arginine to modify placentome vascularity in early to mid-pregnancy (56, 75). Arginine is used by the placenta to produce nitric oxide, a key promoter of angiogenesis which is necessary to increase blood flow, and thus nutrient transfer, to and from the placenta (39). In sheep, placentome nitric oxide synthesis peaks on day 60 of pregnancy, and then again at day 120 (56). Parenteral administration of arginine between day 60 and birth prevents fetal growth restriction in nutrient restricted sheep by potentially increasing placental nitric oxide production (75).

There are a greater number of studies investigating AA nutrition from mid-pregnancy (day 100) to parturition, because this is the time period when fetal growth restriction is hypothesized to occur (76). Thus, the focus, like the earlier stages of pregnancy, has been on maternal-fetal effects and nutrient exchange rather than potential placental changes (morphology, gene expression, protein abundance, vascularity and cell turnover). In addition, only a small sub-set of the AA nutrition studies conducted during mid-pregnancy to parturition hypothesize what could be happening in the placenta (73, 76). For example, short term parenteral

administration of ewes (pregnancy day 126) with BCAAs increases fetal and maternal ammonia concentrations which the researchers suggest occurs because the placenta transaminates the extra BCAAs to provide glutamate for the production of progesterone or for purine biosynthesis (73). The parenteral administration of arginine to ewes carrying multiple fetuses has been shown to enhance the birth weight of female twins (pregnancy day 100 to birth; (77)) and quadruplets (pregnancy day 100 to 121; (76)), potentially through modification of placental vascularity and/or blood flow. Research has shown long-term treatment with sildenafil citrate increases fetal AA availability by increasing placental vascularity, leading to greater fetal growth (71). We have identified that improved growth of female twin fetuses and increased brown adipose tissue stores in all lambs in response to arginine administration (77) is associated with increased placental weight and increased proportion of type B everted placentomes (van der Linden, Sciascia, Sales, Oliver, McCoard unpublished observations). Placentome eversion indicates enhanced placental nutrient transport (41) highlighting the potential benefits of using AA to modify placental function and thus lamb birth weight.

It is clear that to date, there have been no specific studies investigating the role AA play in regulating ovine placental development and/or function during early to mid-pregnancy (day 30 to 100), and only a few published studies from mid-pregnancy (day 100) to parturition. This is not surprising as ovine nutrition research during pregnancy focuses on maintaining maternal reserves and nutrient delivery to the developing fetus rather than directly modifying placental function to enhance nutrient delivery. Considering the critical role the placenta plays in maintaining nutrient homeostasis in both the fetus and the pregnant dam, more research in this area is needed. Amino acids are not just nutrients, they have been shown to act as signalling molecules in a number of tissues in sheep and other species, to regulate pathways essential to growth and survival (19), and thus provide potential future research directions in modifying placental function to support fetal development.

5. EMBRYONIC, FETAL AND POSTNATAL GROWTH

Amino acids play an essential role in the development and growth of the conceptus (reviewed by (21)) as illustrated by the ability of increasing AA availability to the fetus to influence fetal growth (70, 78, 79). Long-term treatment with sildenafil citrate also increases the availability of AA in the conceptus by enhancing blood flow leading to greater fetal growth (71). Amino acids are required by the fetus for protein synthesis and oxidative metabolism. This is particularly important in sheep where approximately 25 percent of the fetal oxidative metabolic requirements in late gestation are derived from AA, which

is substantially higher than humans (10 percent) and cattle (15 percent) (80). Maternal nutrient status (70, 71) and breed (61) can affect fetal AA homeostasis.

During conceptus (embryo and associated membranes and fluids) development in the sheep, there are substantial changes in the fetal: maternal plasma AA ratios and the AA concentrations in both amniotic and allantoic fluid with substantial increases in the concentrations of alanine, citrulline, and glutamine in allantoic fluid between 30 and 60 days gestation (55). Both amniotic and allantoic fluid are a source of nutrition for the developing fetus (55, 81). Amino acid transport into the uterine lumen and uptake by the conceptus is mediated by coordinated changes in the expression of AA transporters (82, 83). Using a translational knock-down of the arginine transporter SLC7A1 to generate an *in vivo* nitrous oxide-deficient ovine conceptus, led to retarded conceptus growth during the peri-implantation period (pregnancy day 1 to 30) was associated with reduced levels of arginine, citrulline, ornithine, glutamine, glutamate and polyamines in the conceptus and ornithine and polyamines in uterine fluid (84). That study illustrated the importance of nitric oxide (regulator of angiogenesis and vasodilation) for conceptus growth, that nitrous oxide synthase-3 is the key enzyme for conceptus nitric oxide production and that this protein regulates arginine availability for polyamine synthesis in the conceptus, which are essential for the development and survival.

The AA that has received the most attention in the literature in terms of embryonic, fetal and postnatal growth in sheep, and other species, is arginine. During early to mid-gestation, arginine is abundant in ovine uterine fluid (82) and allantoic fluid (55). In nutritionally restricted singleton-bearing ewes (i.e. 50 percent NRC recommendation), parenteral administration of arginine in doses of 155 $\mu\text{mol/kg}$ body weight, three times daily from 60 days of pregnancy to parturition, ameliorates fetal growth restriction and increases birth weight (75). Similar increases in birth weight were observed in quadruplets when their non-restricted-fed dams were supplemented with 345 mmol Arg-HCl/kg body weight 3 times daily from day 100 to 121 of pregnancy (76). Using a similar treatment regime and extending supplementation to term (147 days) resulted in increased birth weight of female twin-born offspring but not of males (77). The potential for maternal arginine supplementation to increase the birth weight of female twin born lambs contrasts with the results from the study by Lassala *et al.* (2011) where twin-born lamb birth weight was unaffected. This difference is likely due to the period of supplementation ending immediately prior to parturition compared to 125 days gestation in the Lassala *et al.* (2011) study. Fetal growth in twin compared to single-born lambs diverges from approximately 115 days of gestation (85) highlighting the importance of nutrient supply to the twin-born sheep fetus in the last 30 days of pregnancy. This concept of

critical developmental time windows, and the potential for increasing fetal nutrient requirements in multiples to influence their response to AA supplementation ewes, was also highlighted by Satterfield *et al.* (86).

Arginine and ornithine can stimulate the production of somatotropin when administered via pulse intravenous infusions (87, 88). However, when administered into the abomasum via continuous infusion, arginine fails to affect endogenous somatotropin levels in lactating goats and cows (89-91) but increases somatotropin levels when given to growing lambs and beef heifers (92, 93). Davenport *et al.* (94) subsequently demonstrated that increasing post-ruminal supply of arginine HCl (0.5 g or 0.75 g/day/kg body weight) and ornithine HCl (0.42 g/day/kg body weight) can increase circulating somatotropin and IGF-1 concentrations but improved growth performance was not observed. These observations are consistent with our recent research which indicates that while supplementation of artificially reared neonatal lambs with milk fortified with 500 mg Arginine-HCL/day/kg body weight can increase lamb growth in the first 3 weeks of life, there was little evidence of an effect of arginine supplementation thereafter (95). These observations suggest that there are critical early developmental time windows where supplementation with arginine may be able to influence performance.

Glutamine is the most abundant free AA in colostrum and maternal milk (96) and in the body (97). Glutamine can influence immune function, growth performance and intestinal integrity (21) and is involved in the synthesis of other AA including ornithine, citrulline, arginine and proline, protein synthesis and has been used in humans as a supplement for low birth weight infants. Using an alcohol-induced fetal growth restriction model, glutamine supplementation (pregnancy day 109-132) has been shown to ameliorate alcohol-induced fetal growth restriction and the fetal bioavailability of glutamine and glutamine-related AAs (e.g. glycine, arginine and asparagine) (98). Intravenous administration of L-alanyl-L-glutamine dipeptide improves weight gain, intestinal integrity and aspects of immune function in early-weaned calves (99) but 1 percent L-glutamine supplementation of milk replacer does not alleviate growth depression in calves fed soy protein concentrate (100). Glutamine is also important as a fuel for intestinal enterocytes in sheep (101) as in other mammals (102, 103). While there is no evidence supporting the potential for glutamine to influence specific physiological functions in sheep, studies from other species indicate significant potential, especially for improving the integrity of the gut of the neonate, immune function and associated growth performance.

These studies highlight the potential for specific AA or combinations of AA to influence embryonic and fetal growth which may influence subsequent

survival and postnatal growth of lambs. It is important to acknowledge that despite the potential positive outcomes, adverse effects have also been reported. For example, short term (4 day) intravenous supplementation of pregnant sheep (pregnancy day 131) with a high protein diet enriched with a combination of essential AA at the end of gestation can lead to fetal hypoxia and respiratory and metabolic acidosis (104). Maternal AA supplementation has been reported to result in AA imbalances (73, 104, 105) which may explain some these effects. It is also important to highlight that the fetal response to AA supplementation may differ between normally growing and growth restricted fetuses. Growth restricted fetuses have differential substrate metabolic rates and hormone concentrations (106-108) and altered mRNA levels of key regulatory genes (109-111) which may influence their response to AA supplementation. These observations highlight the need for further research into the functional roles of AA, dose-response relationships and critical time windows for intervention on embryonic and fetal growth. Much of the current knowledge is based on component studies focused on understanding the roles for AA (or combinations of AA) in the regulation of tissue development, growth and function as outlined in the subsequent sections.

6. ORGAN DEVELOPMENT AND FUNCTIONS

6.1. Gastrointestinal Tract

For the first few weeks of life the diet of pre-ruminant lambs consists primarily of maternal milk and the gastrointestinal tract (GIT) functions in a similar manner to the GIT of monogastric species (112). Due to a range of factors, increasing prolificacy (twin and triplet births), immaturity of the neonatal immune system and intensive indoor-lambing practices, this time period is one of the most vulnerable for the establishment of pathogenic GIT infections (113). Secretory diarrhoea is the most common disease and is caused by infections with enteropathogenic *Escherichia coli*, *Clostridium perfringens*, rotavirus, coronavirus, and cryptosporidia, which can result in growth delays (and culling) and even death (113). To date, no studies have been conducted to investigate the potential of AA, or other non-antibiotic interventions (pre-, pro and syn-biotics), in protecting the pre-ruminant ovine GIT from pathogenic infection. Access to colostrum by the neonate and adequate intake of maternal milk have been viewed as cheaper less intensive alternatives to the use of supplementary nutrition. However, as prolificacy and the use of artificial rearing systems increase neonates are reared on milk replacers that do not contain the protective factors found in maternal colostrum and milk (114). Studies in piglets and other animals indicate that supplemental leucine, glutamine and/or arginine may improve GIT development and help protect against infection, or support the maintenance of normal GIT function during infection by maintaining tight junction integrity and reducing the inflammatory response (21).

As the pre-ruminant lamb transitions to a solid diet the rumen begins to grow and by adulthood, the ovine rumen can make up to 80 percent of the GIT volume (115). Almost all consumed diet enters the rumen, with the metabolites being passed onto the small and large intestine for utilization by the sheep (115). Thus, some nutritional interventions are targeted to this transition period to improve rumen development and function and subsequently enhance ovine health and performance (115). Creep-feeding systems result in better rumen development compared to sole maternal feeding as a solid diet triggers rumen growth, stimulates function and enhances the growth of rumen-associated-microbial communities (115). However, whilst a solid diet aids rumen development Harrison *et al.* (116) report that diet source appears to have very little impact on the proportions of AAs in digesta passed onto the ovine duodenum and ileum (116). Yet, there are major shifts in the pattern of AAs being delivered to the liver and peripheral tissues of the sheep, suggesting a functional change in how the GIT absorbs and or metabolizes AA (117). Potentially limiting AA for growth of the ruminant lamb have been identified in a study by Storm and Orskov (118), who observed that only methionine, lysine, arginine and histidine reduced microbial nitrogen retention when omitted from the diets of 2-month old Suffolk x (Finnish Landrace x Dorset Horn) castrated male lambs. However, supplementation with non-rumen protected AA show no consistent effect on sheep performance as they are generally metabolized by the rumen microbes before they can reach the small intestine. These studies also do not investigate the effect on GIT development or function. Rumen protected AAs have been used to bypass the rumen fermentation and assess the effect on the development and function of ovine mammary (119) and wool growth (120), but to date no work has been conducted to assess the effect on the GIT.

6.2. Skeletal muscle

Ovine skeletal muscle accounts for 25 to 30 percent of body mass at birth (121) and up of 70 percent of carcass weight at slaughter (122). The main components of muscle are the fibres whose number and size are the main determinants of ovine muscle mass. The number of muscle fibres is established during prenatal myogenesis (123, 124), and myogenesis is complete prior to birth (85, 125). Muscle continues to grow as a result of fibre hypertrophy (126). Skeletal muscle has lower priority for nutrients compared to other tissues such as brain, heart and liver during fetal development resulting in muscle being more vulnerable to nutrient deficiency (127). In a maternal *ad libitum* fed state, the fetal hindlimb takes up most of the AA (128). During maternal fasting, several AA, including glutamine and alanine are released by the fetal hindlimb (129), while the uptake of BCAA increases (130, 131). Glutamine (132) and alanine (133) released from the hindlimb can potentially generate glucose in the fetal liver.

The effect of restricted maternal nutrition on fetal and muscle growth depends not only on its level but also on the timing (134). Severe maternal nutritional restriction (i.e. 50 percent of total requirements) during early pregnancy (less than 80 days pregnancy) is associated with a decrease in the number of secondary fibres, affecting fetal muscle growth (127, 134, 135), with postnatal carryover effects (127). Restricted fetal nutrition in late pregnancy (greater than 100 days) resulting from twin-induced placental insufficiency can reduce muscle weight (134, 136) and is associated with reduced muscle protein synthesis (137) and fibre density (135). In the latter stages of pregnancy, skeletal muscle growth increases rapidly (138), and the fetus responds to infusion of specific (e.g. arginine) or a mix of AA by increasing protein synthesis (79). This response during fetal life appears to be associated with the activation of mTOR signaling in skeletal muscle (139, 140). A study by Sciascia *et al.* (141) has shown that the *semiteminosus* muscle of late-pregnancy twins have lower abundance of mTOR signaling proteins and rRNA compared to singletons. Zhu *et al.* (142) has also shown that maternal nutrient restriction from day 28 to 78 of pregnancy results in a reduction of the active forms of mTOR (Ser2448) and ribosomal protein S6 (Ser235/236), signaling factors associated with the regulation of protein synthesis. The reduced activation of mTOR and RPS6 was associated with a lower ratio of secondary to primary muscle fibres, which can negatively impact muscle development. In contrast, maternal over-nutrition (1.5 times NRC total requirements), negatively impacts the activation of mTOR and reduces cell density of fetal muscle (143). The results from these studies highlight the potential role of mTOR in regulating fetal muscle development by linking its role as a nutrient sensor to the nutrient status of the fetal environment.

Intracellular concentrations of AA can regulate mTOR signaling (144, 145), however, the mechanisms through which AA modulate the activation of mTOR are not fully understood. There is limited literature examining the association of AA supplementation and mTOR activation in sheep. A study by Brown *et al.* (140) showed that parenteral administration of a mix of AA into the fetus resulted in the insulin dependent up-regulation of S6K in the fetal *biceps femoris* skeletal muscle. Other studies in sheep have shown that ewe lambs (5 to 8 months old) infused with a mixture of 6 AA (arginine, lysine, histidine, threonine, methionine, and cysteine) in the ratio found in bovine milk resulted in improved rate of initiation of mRNA translation (146). A recent study by Sales *et al.* (147) has shown that free AA concentration varies in the fetal muscle at term, according to maternal size, nutrition and number of conceptus. The *semiteminosus* muscles of twin fetuses have reduced concentrations of leucine, threonine and valine and higher concentrations of methionine, ornithine, lysine and serine (136). In addition, increased *semiteminosus* muscle weight was

positively correlated ($r = 0.7$) with arginine concentration suggesting that arginine may act as a limiting nutritional and/or signaling AA for twin fetal muscle growth (147). Parenteral arginine supplementation of pregnant ewes from day 100 to birth increases birth weight of female twin-born lambs, potentially through an increase in muscle growth mediated by increased abundance of mTOR (95). Similarly, arginine supplementation during the first 3 weeks of life increases the efficiency of body growth, resulting in heavier muscles at weaning but only in females (95). Studies in other species indicate it may play an important role in regulating muscle mass (148, 149), however the potential role for arginine to be an activator of mTOR signaling in ovine skeletal muscle remains to be established.

6.3. Mammary gland

The ovine mammary gland goes through five distinct stages of development, *in utero*, pre-pubertal, pubertal, pregnancy, lactation and involution, and studies show that altered planes of nutrition throughout development can impact future milk production (150, 151). A high plane of dam nutrition (high: straw *ad libitum* +1.0 kg concentrates vs. low: straw *ad libitum*) from d 70 – d 140 of pregnancy increases milk yield, in early lactation (150). A low plane of nutrition from d 98 - parturition negatively impacts ewe milk yield and composition (152). A low plane of dam nutrition from d 21 – d 140 of pregnancy has been shown to reduce fetal mammary gland size at d 140 but increase first-lactation milk production of their offspring (153). The authors suggest that improved first-lactation performance was the result of inherited epigenetic changes, however a subsequent study by Sciascia *et al.* (154) showed that the increased first-lactation milk production was linked to changes in the abundance of fetal mammary gland mTOR signaling proteins. Interestingly, the mTOR pathway can be activated by AA sufficiency or specific AA, suggesting AA nutrition may play a role in modulating future lactation potential *in utero* (145).

Studies of ovine mammary AA requirements have tended to focus on the lactation period. This is no surprise as this is the period where ewes produce milk for their young and the direct effects of nutritional intervention can be immediately measured. The biological imperative to produce milk is enormous, placing increasing metabolic and physiological demands on the ewe. For example, the protein mass of the ovine mammary gland increases 100 fold during lactation. Increased mammary gland protein mass and milk secretion is initially achieved through the mobilization of body reserves and then by increased feed intake (155). Studies investigating mammary gland AA requirements during lactation identified three AA believed to be critical or limiting in supporting peak milk production, lysine, methionine and leucine (156). The “limiting AA” theory states that as milk production by the ovine mammary gland increases the dam must increase

the supply of substrates. It was hypothesized as the mammary gland approaches peak lactation a specific AA (first-limiting) or set of AA (co-limiting) becomes limiting as a direct substrate for milk protein synthesis or is used in the Krebs cycle for the production of other AAs, thus preventing milk production from increasing further (156). Lysine is taken up in excess of requirements for milk protein synthesis and is limiting in corn based diets used widely in the US dairy industry, whilst methionine is an EAA that cannot be adequately supplied by rumen microbial protein synthesis (157). It is currently unknown if AA are limiting in the diet of pastoral grazed sheep.

Original AA studies focused on using the three potentially limiting AA as nutritional supplements to complement for dietary inadequacy, or to increase milk production by increasing their availability to the lactating gland (119, 158). One of the first published studies to investigate the potential effect of AA supplementation on ovine lactation was conducted by Lynch *et al.* (119). The authors showed no significant effect of supplemental rumen protected (RP) methionine and lysine (methionine 0.11 g; lysine 0.13 g/100 g DM) on milk yield, milk solids, crude protein, ammonia or AA in black-faced ewes' (5 days to 8 weeks post-partum) fed a low or moderate crude protein diet (119). A later study by Baldwin *et al.* (158) utilizing Dorset ewes fed (5 days post-partum for 6 weeks) chopped alfalfa hay supplemented with RP-methionine (0.10 g/100 g DM) also showed no effect on milk yield or composition. Work by Sevi *et al.* (159) showed that the addition of RP-methionine or RP-lysine (methionine 0.25 g; lysine 0.75 g/100g DM) to the diet of lactating ewes' (8 to 23 weeks post-partum) may not influence milk yield, but changes fat composition. The authors used Comisana ewes' and showed dietary RP-methionine or RP-lysine supplementation increased the ratio of long- to short-chain fatty acids, a reduction in the potential health benefits of ovine milk. However this was offset by a modest increase in the ratio of unsaturated to saturated fatty acids. Sevi *et al.* (159) provided a potential explanation as to why the availability of additional methionine and lysine did not increase milk production. They discussed that the observations from their study suggest that increased methionine and lysine availability was being preferentially used for plasma low density lipoprotein synthesis, resulting in changes in the saturated fatty acid profiles of milk from supplemented ewes. To date, this theory has not been directly tested however a study by Goulas *et al.* (160) may provide some indirect insight. Goulas *et al.* (160) assessed the effect that supplemental RP-methionine (5 g/kg of feed) would have on milk production in twin-bearing Karagouniko ewes (2 weeks pre-partum to 13 weeks post-partum) fed a diet enriched with animal fat. Ewes fed the RP-methionine + animal fat diet had significantly increased milk yield, daily fat and protein yield in the first 7 weeks of lactation. After weaning (wk 8 to 13), no significant differences in milk production were

observed (160). Several possible reasons exist as to why Goulas *et al.* observed increased milk production whereas previous authors did not, breed differences, intervention time-point/frame, basal diet, protein quality and the addition of animal fat to the diet. The inconsistent results obtained from the various supplementation studies suggests that these AA are not limiting AA, or there is another concept being overlooked.

Another concept being considered is the use of AA as signaling molecules to activate pathways that increase lactation potential (157, 161, 162). The limiting AA hypothesis focused on AA as a nutrition source, but it is recognized that they play a much wider role beyond that of substrates for mammary gland protein synthesis (157). Arginine is extracted in the greatest quantities relative to milk protein output and is a precursor to nitric oxide (NO) production which regulates the local nutrient environment of the mammary gland through alteration of the capillary blood supply (157). Arginine administration (0.1 g/kg of bodyweight) to late-pregnant Holstein cows, from 7-days pre-partum to parturition, was shown to increase milk yield (163), whilst unpublished observations by Sciascia *et al.* (154) has shown arginine administration (345 μ mol/kg bodyweight) from day 100 to parturition in Romney ewes had no effect on milk yield. However, Sciascia *et al.* (154) were able to observe a transient increase in milk protein yield and the absolute concentration of some milk free AA. No conclusions on the use of arginine to improve lactation potential can be drawn from these two studies, but each was able to elicit a positive effect on mammary function and thus, provides the basis for future research using arginine or one of the precursors (citrulline (164) or N-carbonyl-glutamate (165)) known to elevate ruminant plasma arginine levels.

6.4. Immune system

The ovine immune system is one of the most characterized due to its use as a model of immune system physiology (166). It is comprised of two defensive systems, the innate and adaptive, each with distinct yet not mutually exclusive functions. The innate system is the first line of defense and begins with physical barriers such as the skin and mucous layers, and a host of antimicrobial factors. When the physical barriers are breached, cytokines produced by monocytes-macrophages and other non-immunological cells, such as fibroblasts and endothelial cells, regulate the next step. The cytokines can directly act against invading pathogens or indirectly by activating downstream immune-modulatory mechanisms that trigger the inflammatory response and activate natural killer cells and macrophages. The adaptive defense system is activated after antigens from the invading pathogen are processed and recognized. Once the antigen is recognized, the adaptive defense system makes immune cells specifically designed to attack that antigen and "remembers" the antigen to protect against future infections.

Dam and neonatal nutrition are important for the development and maintenance of a competent immune system (167, 168). Neonates are born with an immature immune system that cannot adequately protect them against invading pathogens, and protection is almost solely derived from the colostral antibodies (169). Then, as the neonate transitions from a liquid diet to a solid diet the rumen and associated microbial communities change, which can lead to increased susceptibility to pathogenic infection (170). Additionally, sheep may not acquire immunity to gastrointestinal tract parasites until 8 to 24 months of age (168). During late pregnancy and lactation, dams are believed to be at greater risk of adverse health effects from pathogenic infection due to changes in hormonal status that somehow lead to a depression in the immunological mechanisms that protect against pathogenic infection (168). Any infection during the pregnancy can lead to abortion (171), reduced weight of the dam and fetus (172) or lowered milk quality (173), however the exact mechanisms behind this depression are unknown.

Protein nutrition has been investigated extensively in sheep as observations from studies show that the source of dietary protein or level of metabolizable protein can decrease the susceptibility of sheep to nematode infection (172, 174). Van Houtert *et al.* (172) used fish meal as a source of RP-protein (and beneficial fatty acids) to supplement the diet of 3-month old Merino wethers experimentally infected with the nematode *Trichostrongylus colubriformis*. Supplemented wethers had increased circulating levels of neutrophils, intestinal sheep mast cells and lowered fecal eggs counts, and no loss in daily weight gain. Bricarello *et al.* (174) investigated the effect of diets with moderate (75 g) and high (129 g) metabolizable protein per kg of dry matter would have in Ile de France and Santa Ines lambs experimentally infected with *Haemonchus contortus*. Whilst both breeds were able to resist the pathophysiological effects on the higher protein diet, reduced *Haemonchus contortus* was only observed in Santa Ines lambs, indicating breed specific differences in the role increased protein supply plays in resistance to nematode infection. How dietary protein elicits a protective response to nematode infection is still debated but the prominent theory is that nematodes interfere with protein metabolism, resulting in reduced metabolite production by rumen bacteria (175) and/or AA uptake by the host (175) which influences the ability of the immune system to respond to nematode infection (176). Like many other tissues and aspects of ovine development and function, the role of AA in regulating and supporting immune is poorly understood. Studies investigating protein deficiency may shed some light on the potential role AA play in sheep. Diets deficient in protein result in reduced plasma availability of most AA including glutamine, arginine, tryptophan, methionine and cysteine (14) which have well established roles in modulating immune function (14, 177). Also, AA

supplementation studies in other species indicate that the arginine-family (178) and BCAA (14) can enhance immune function by stimulating the cyto-toxic activity of cells involved in the innate immune response and activate cytokine production.

A study by Kuhlman *et al.* (179) illustrated that in mixed-breed ram lambs infected with *Brucella abortus* antigen and porcine red blood cells, ruminally protected alpha-ketoisocaproate (RP-KIC) enhanced, RP-leucine depressed, and RP-isovalerate had no effect, on the immune response. In a follow-up study, Nissen *et al.* (180) were able to show that the opposing effects of RP-KIC and RP-leucine were due to how they differentially modulated the immune response. Lymphocyte blastogenic responsiveness to phytohemagglutinin-P and pokeweed mitogen was increased with KIC, whilst leucine responsiveness was decreased. This was a direct result of the increased percentage of circulating T4 cells in KIC fed-lambs and lowered percentage of circulating T19 cells in leucine-fed lambs (180). The same research group was also able to show that dietary KIC supplementation can compensate significantly for lymphocyte suppression and decreased ratio of T4 to T8 cells caused by treatment of lambs with adrenocorticotrophic hormone (181). Recently, Sciascia *et al.* (154) showed treatment of Romney ewes from day 100 to parturition with arginine reduced milk somatic cell counts, which are primarily cells from the innate defense system tasked with protecting the gland during infection (182). How arginine supplementation leads to the reduction in somatic cell counts is still unclear, but it could be linked to its role in nitrous oxide or free radical production by immune effector cells (178, 183) but further research in sheep is still required. In addition, the mechanisms by which KIC and arginine affect immune function do not seem to overlap, suggesting treatment with both may enhance two independent functions of the immune defense system in sheep.

6.5. Adipose tissue

White adipose tissue (WAT) is the site of storage of excess energy in the form of triacylglycerols and it is formed when nutrients are consumed in excess of requirements. In periods of nutrient insufficiency, WAT undergoes lipolysis to provide non-esterified fatty acids for use by skeletal muscle and other organs. Strategies to reduce excess fat accretion are important for meat production from livestock species (21). Prolonged changes in maternal feed intake alter fetal WAT development (184). White adipose tissue mass in non-pregnant mammals can be decreased with dietary arginine supplementation including rats (30, 185, 186), humans (187) and pigs (188, 189). The mechanism mediating these effects are thought to involve decreased *de novo* synthesis of glucose and triacylglycerides and increased glucose and long-chain fatty acid oxidation (32, 186). Arginine supplementation also

increase lipolysis and inhibit lipogenesis by modulating key enzymes involved in fat metabolism and anti-oxidative response (190). Interestingly, dietary supplementation of adult rats with arginine decreases WAT with an associated increase in brown adipose tissue (BAT; see below) but the mechanisms responsible remain to be elucidated (30, 186). While the effects of arginine supplementation on WAT and the associated mechanisms are well established in humans and some animal species (191), little is known about the effects of AA on WAT in sheep. In sheep, there are well established gender differences in adiposity in early postnatal life (192). Both gender and twinning can also influence carcass fatness with twins having reduced carcass fatness compared to singles and twin females having more carcass fat compared to their male counterparts (193). Similar, but smaller differences were also reported by Afolayan *et al.* (194) likely resulting from differential production systems and genetics. The potential for AA supplementation to modify fat deposition in sheep has important implications for meat animals driven by increased demand for lean product of consistent quality with some processors imposing significant penalties when carcasses fail to meet specifications. This is important for producers due to rising costs of growing or purchasing high quality feed.

Brown adipose tissue is a specialized fat store that when metabolized by the newborn lamb, assists the lamb to adapt to the cold challenge of the extra-uterine environment and to avoid hypothermia (195). Hypothermia is a major cause of on-farm lamb losses in the first few days of life (196). There is a rapid increase in the mass of BAT from 70 to 120 days gestation followed by a decline in deposition to term (147 days; (197)), at which stage BAT represents around 80 percent of all adipose tissue in the newborn lamb. Although BAT only accounts for 2 percent of birth weight, 50 percent of the heat generated in newborn lambs comes from BAT metabolism for non-shivering thermogenesis (198, 199). Both the mass and metabolic activity of BAT is important for thermoregulation in the first few days of life, and thus modulation of these factors has the potential to improve neonatal thermogenesis and survival.

Maternal L-arginine supplementation during mid to late gestation increases BAT stores of fetuses carried by underfed (200), diet-induced obese (201) or well-fed ewes (77). The effect of maternal L-arginine supplementation on BAT mass of the late-gestation fetus is associated with an increase in adipocyte hypertrophy but not hyperplasia, and expression of the thermogenic genes UCP-1 and PRDM16, with elevated cortisol potentially regulating the expression of UCP-1 (202). This observed increase in BAT mass and metabolic activity was associated with an increase in the rectal temperatures of the lambs within 2 hours of birth, consistent with increased metabolic activity (202). Rapid up-regulation of genes such as UCP-1 around birth (203)

generates heat through uncoupling of ATP synthesis from the oxidative process (204) mediating in part the development of BAT and onset of BAT thermogenesis. This process is intricately coordinated with a series of endocrine changes (205, 206). These results illustrate the potential for maternal arginine supplementation to enhance thermoregulatory ability in the neonate but studies to evaluate the potential of such approaches to improve lamb survival on-farm have yet to be undertaken.

7. SUPPLEMENTATION STRATEGIES – CONSTRAINTS AND OPPORTUNITIES

Unprotected AAs can be added directly to the diets of pre-ruminant lambs to balance for nutritional deficiencies or as a nutraceutical to improve performance; however at the onset of rumen development microbial proteases and deaminases rapidly degrade AAs, which are soluble in the rumen liquid phase (207). An approach used by researchers to prevent rumen degradation of AAs is to administer AAs parentally, (75-77), or by-passing the rumen by direct administration into the abomasum (120, 208). Both methods do not require complex or expensive encapsulation to protect the AA and researchers are able to assess the direct effect of an AA or groups of AAs on ovine performance. Parenteral administration has the added advantages of permitting researchers to control how much AA enters circulation and where, the last point is important for *in utero* studies where AA can be infused directly into the fetal circulation, bypassing metabolism by the placenta. However, the primary limitation with parenteral and direct abomasal infusions is that they are impractical for use in production systems, therefore AAs that are resistant to degradation by the rumen have been developed.

The most widely-applied methods are the use of encapsulated AAs or AA analogues, which have been used to assess the effect of supplemental AA on ovine lactation (160), wool growth (120) and neonatal growth (119). Protected AAs are less susceptible to rumen degradation but are still available for intestinal absorption. There are several potential advantages to using rumen protected AA (RPAA) in ovine diets: A small amount of RPAA, containing a single or group of AAs, can be used to replace traditional supplemental protein feeds that were used to compensate for dietary AA inadequacies, making the supplementation more specific and reducing costs for industry and research. This leads to increased flexibility in the formulation of diets and enables components like energy density and fat content to be further optimised for different stages of growth or lactation. Ideally, RPAA should be stable when pelleted and when incorporated into silage-based total mixed rations in which the pH can be as low as 3.6. (within the pH range of the abomasum where they would be degraded). Also, RPAA should be certified as safe by the relevant regulatory authorities. One of the first

studies in ruminants to develop RPAAAs was conducted by Amos and Evans (209), who encapsulated methionine and lysine in polymerised urea and formaldehyde. Whilst Amos and Evans (209) were able to increase abomasal availability, plasma levels did not increase. In addition, the AA content of each product was quite low, RP-lysine had between 29.9. and 31.2. percent and RP-methionine 38.0.4 percent. Another method for the production of polymer encapsulated RPAA was patented by Dannelly (210). Dannelly (210) combined methionine or lysine with zein, non-water-soluble polymers or fatty acids and optional plasticizing agents such as triacetin or propylene glycol (210). Wright and Loerch (211) used this method to successfully increase plasma methionine concentrations, but no effect was observed on dry matter intake, fibre and nitrogen digestibility's and nitrogen retention in Dorset-sired crossbred wether lambs. Polymer formulations have been improved over time and there are now commercial products available for ruminants such as Smartamine M® (70 percent methionine) and ML® (15 percent methionine, 50 percent lysine). Another RPAA product, Mepron M85® is coated with compounds that are not pH-sensitive, which are resistant to ruminal, but not intestinal digestion. To date, only Smartamine M® has been utilized in sheep with mixed results (160, 212).

The complexity, costs of development and application of encapsulated AAs has led researchers to investigate the use of AA analogues as substitutes. Like encapsulated AAs, AA analogues are resistant to degradation by the rumen, however, unlike encapsulated AAs the free AA is not released and absorbed by the abomasum. Analogues of AA are absorbed intact by the abomasum and processed by distal tissues to yield the required free AA (213). Methionine and lysine have been the focus of many ovine studies and several AA analogues have been tested for their ability to increase plasma concentrations and regulate ovine phenotypes (213, 214). Methoxinine (O-methyl-DL-homoserine) has been used to study wool composition (214), 2-hydroxy-4-methylthiobutanoic acid (HMTBA) has been used for methionine absorption studies in Suffolk cross lambs (213), and, maleyl-methionine and d-2-methyl-maleyl methionine have been utilized to assess the effect of methionine on nitrogen retention and wool growth in Merino weathers (120). A commercial methionine analogue is available under the brand name Megalac-Plus®, which contains 13 g of methionine hydroxy analog with 450 g of Megalac® (calcium salts of long chain fatty acids), and others include Alimet® and Rhodimet®. To date, these have not been used in ovine studies. Hydroxymethyl lysine (HML) a synthetic lysine analogue has been tested in Suffolk ewes and shown to linearly increase plasma lysine concentrations (215). Analogues of leucine have also been utilized to study their effect on ovine immune function (180). Alpha-ketoisocaproate was shown to enhance immune function whilst isovalerate depressed immune function (180).

Improving the growth performance of lambs has the potential for eco-efficiency gains. For example, in hard hill country extensive sheep farms in New Zealand, there have been significant productivity gains through increased meat production in the last 10-15 years (181). Such gains include a 47 percent increase in saleable product per hectare, 21 percent reduction in nitrate leaching per kg of saleable product and a reduction of 40% in greenhouse gas emissions per kg of saleable product. While these gains in eco-efficiency were not observed on easy hill finishing operations, these observations highlight the potential to reduce the environmental impact of sheep farming through increased animal performance. Therefore, the ability to improve animal performance through AA supplementation offers the tantalizing opportunity to deliver eco-efficiency gains, however such gains remain to be investigated. With the increased focus on sustainable farming practices and reducing the impact on the environment, new approaches to improve animal performance coupled with eco-efficiency gains are becoming increasingly important for the livestock sector.

8. SUMMARY AND PERSPECTIVE

Traditional ovine studies have provided a wealth of data on the transport and metabolism of AA by several key organs. However, the application of this knowledge has been limited by the theory that AAs are solely a limiting component in the diet. Amino acids have been shown to participate in a wide range of functional roles beyond their use as a dietary precursor required for the synthesis of protein, glucose and other cellular metabolites, roles that may have the potential to improve ovine growth and health. Recent studies indicate that AAs and their analogues can act as pharmacological agents to improve production outcomes in sheep. Particular focus has been paid to arginine and BCAA, whose administration has been shown to improve reproductive function, muscle growth, mammary gland development, immune function and fetal development and survival. Although a number of biochemical and molecular mechanisms have been proposed to explain roles for arginine and BCAA in improving ovine growth and health, direct experimental evidence is needed to support these propositions. There is a significant gap in our knowledge about the potential use of AAs as pharmacological agents to improve production and health in sheep. This gap provides current and future researchers with an opportunity to develop new knowledge about AA biochemistry and physiology to aid in the design of nutritional interventions to help improve ovine production performance.

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