Wool quality and rumen-protected Lysine in merino ewes during late pregnancy and early lactation

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Declaration

This thesis to the best of my knowledge and belief is original and does not contain any work which has been previously published, except where acknowledged within text.

This thesis does not contain material which has been submitted for similar academic awards at this, or any other university.

Rohan Michael Rawson Leach

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Abstract

Pregnancy and lactation have a significant influence on wool production, both in quality and quantity. This is due mainly to the increase in nutritional demands, with high quality feed required to satisfy ewe demands during these peak periods. The highest period of demand occurs in the last three weeks of pregnancy and the first 3 weeks of lactation, coinciding with peak milk production (Lloyd, 1963).

A ewe's nutritional requirements vary throughout the year and breeding season peaking during late pregnancy and early lactation. Protein and energy are in higher demand due to additional requirements from the foetus and milk production (Masters *et al.* 1993). Of the two processes, lactation is by far the most demanding with the ewe's nutrient requirements increase dramatically following parturition, particularly for those nursing twin lambs.

Nutritional requirements have a profound effect on a sheep's ability to grow wool with rate of wool growth being linearly related to feed intake (Allden, 1979). In a grazing system this is harder to assess as it is not known what exactly the livestock are eating in terms of quantity or metabolic composition. Nutritional values of a pasture can vary due to botanical composition, plant age and total dry matter (DM). To compound the problem, variations between years and seasonal fluctuations influence pasture quality and quantity (Finlayson *et al*. 1995).

The majority of wool protein is composed of the sulphur amino acid cysteine, and therefore the first limiting amino acid in sheep. The other sulphur containing amino acid, methionine, can also influence cysteine levels through metabolism to cysteine (Reis 1988). Methionine is the preferred supplement because it is an essential amino acid with important metabolic functions as well as being a source of cysteine (Staples *et al.* 1993). As such, many studies have used methionine supplements and obtained significant increases in wool growth rates (Langlands 1970; Wright 1971; Cottle 1988; Stephenson *et al.* 1991; Pickering and Reis 1993; Staples *et al.* 1993; Mata *et al.* 1995).

Wethers on diets deficient in lysine grew less wool comparative to other protein supplements such as casein, whole egg protein, egg albumen and wheat gluten (Reis and Colebrook, 1972). This is supported by Reis and Tunks (1978) who state that addition of lysine to the diet of wethers significantly increased all aspects of wool growth.

Rumen microbes degrading amino acids have meant that early attempts at supplementary feeding of amino acids resulted in few gains in wool production. This is due to the deamination of the acids in the rumen during the fermentation process of microbes (Chulupa 1976). Presently amino acids can be fed in forms which bypass the rumen and reach the abomasum, where they are absorbed and used for protein synthesis (Lycnh *et al.* 1991; Staples *et al.* 1993).

LysiPEARL is a commercial form of rumen protected lysine, with applications in the dairy industry. It has a high bioavailability with the acidic pH of the abomasum enabling absorption of lysine by ruminants (Elwakeel *et al.* 2012). Movaliya *et al.* (2013) inspected the health effects rumen protected lysine had on Jaffrabadi buffaloes. Lysine had no significant effect on haematological and biochemical parameters except blood urea nitrogen suggesting it could be used for improvement in digestibility of nutrients, intake of dietary crude protein and positive nitrogen balance. Another study using LysiPEARL on steers measured muscle growth and indicates that muscle hypertrophy is increased with the addition of encapsulated lysine and methionine (Hosford *et al.* 2013).

The data from this experiment supports the null hypothesis that there is no difference in reproducing ewes fed rumen-protected lysine in any of the measured categories.

There is an interaction between lambing status and treatment during lactation on the wool growth rate of ewes. The trend (P=0.074) indicates that supplementation with rumen bypass lysine increases wool growth rates in non-reproducing ewes, but not in lactating ewes compared to dry. This outcome was expected as supplementing lysine into diets has increased fibre length in non-reproducing sheep and goats (Reis and Colebrook, 1972; Reis and Tunks 1978; Sahlu and Fernandez 1992) however this has not been observed in reproducing ewes (Stewart *et al.,* 1993; Masters *et al.*, 1993). This supports our results of increased fibre length in dry ewes.

Literature Review

Pregnancy and lactation have a significant influence on wool production, both in quality and quantity. This is due mainly to the increase in nutritional demands, with high quality feed required to satisfy ewe demands during these peak periods. The highest period of demand occurs in the last three weeks of pregnancy and the first 3 weeks of lactation, coinciding with peak milk production (Lloyd, 1963).

However, seasonality of pasture production means that feed quality is often extremely variable. This creates poor quality wool as nutrients are partitioned away from wool production towards those more essential for animal maintenance. For high wool quality and fibre strength, management of wool sheep must involve a consistent plane of reasonable nutrition throughout the year (Allden, 1979).

The importance of crude protein and metabolisable energy on wool growth rates has been well documented. These nutrients must be present at the right levels and ratios to optimise wool growth (Black et al. 1973). However another option may be the addition of specific amino acids directly into the diet. Reis and Schinickel, (1963) found that abomasal infusion of methionine substantially increases wool growth in wethers. Methionine has by far been the most studied amino acid due to its high requirement for wool growth however it is believed that lysine may be the amino acid which is the next most limiting.

Allden, (1979) showed that wool growth is linearly related to feed intake however when intakes are limited, producers may be faced with having to supplement livestock. Supplementation of amino acids has until recently, not been achievable in field situations due to breakdown of amino acids in the rumen (Chulupa, 1976). However, bypass protein or rumen protected supplements are now commercially available and have shown effective results across animal industries, particularly the dairy and fibre industries.

1)Pregnancy and Lactation: Effect on Wool

1.1) Wool growth and quality in relation to pregnancy and lactation

Wool growth rate and wool characteristics are determined by genetic, hormonal, climatic, health and production factors. Wool growth rate in particular is affected by nutrient availability in the form of feed intake, diet composition and pre- and post-natal factors such as under-nutrition (Kempton, 1979).

Pregnancy and lactation are stressful periods in the life of a sheep with wool growth rates negatively affected as a result (Masters *et al.*, 1993). The largest reduction in growth occurs in the last trimester of pregnancy and during the first several weeks of lactation also known as the periparturient period. This difference is even more pronounced in twin bearing ewes (Oddy and Annison, 1979). During pregnancy and lactation wool growth can be suppressed by 20-60% (Corbett, 1979). This translates to a reduction of 10-15% in fleece weight per annum for a pregnant ewe as opposed to a non-pregnant ewe (Oddy and Annison, 1979). However lamb production in a wool ewe enterprise can account for up to 50% of total enterprise profit and cannot be forgone (Warn *et al.*, 2006).

The suppression of wool growth in pregnant and lactating ewes is believed to be a result of competition for nutrients between metabolic processes (Oddy and Annison, 1979). Absorbed nutrients must be shared between the wool follicles, maternal tissues, the growing foetus and milk production with wool receiving very low percentages of this share (McNeil *et al*, 1997). When nutrients become scarce, they are allocated to the bodily function deemed most important in a biological development called nutrient partitioning. Those processes concerned with the maintenance of animal life, such as resting metabolism and thermoregulation, receive the largest amount of nutrients. Functions involved with reproduction receive lesser amounts. Wool production is not an essential process in terms of animal biology and as a result, is allotted far lower nutrient quantities than other functions (Houdijk *et al.*, 2001).

Pregnancy and lactation influence various wool characteristics such as reducing fibre diameter, staple strength, fibre length and clean fleece weight (Masters *et al.*, 1993). As

these characteristics are only affected in the short term (i.e. the duration of pregnancy and lactation), variations are obtained along the wool staple. These variations ultimately affect the staple strength and therefore the processing quality of the raw wool reducing the price of the wool clip. Variations in wool commonly result in a reduction in staple strength. This reduction is due to changes in the physical properties of the wool such as a reduction in the minimum fibre diameter (Orwin *et al.* 1980) or an increase in the rate of change of fibre diameter (Hansford and Kennedy 1990).

1.2) Nutrition and its role in pregnancy and lactation

Deficits in nutrition can lead to health problems in pregnant animals. Ewes are especially susceptible to hypoglycaemia during late pregnancy (Bergman, 1973), which leads to reductions in uterine and foetal uptake of glucose, resulting in depressed foetal growth and possible death of ewe and foetus. To avoid these health and management problems, producers need to maintain high energy and protein intakes during reproductive stages. There is massive utilisation of amino acids and protein stored in muscle and other tissues by the ewe in order to meet nutrition deficits. However, it has been seen that increasing protein levels in diet has produced increased levels of protein deposition in foetal, placental and uterine tissues (McNeil *et al.*, 1997). This gives promising signs of what may be measureable in terms of fleece growth and characteristics.

The rumen environment and diet are the two most important factors when considering nutrient supply in sheep. In the rumen microbial degradation occurs; carbohydrates are broken down to volatile fatty acids (VFA) and proteins are hydrolysed and the amino acids degraded to produce mainly VFAs and ammonia. VFAs are the main energy yielding substrates for sheep (Faichney and Black 1979) contributing an estimated 70-80% towards animals energy requirements (Annison and Armstrong 1970). These enable growth of the rumen microbes which are later digested in the omasum and abomasum. They are absorbed along the digestive tract (Weston and Hogan 1968) and are used to synthesis products that can be used by the sheep for growth, maintenance and fattening (Annison and Armstrong 1970).

A ewe's nutritional requirements vary throughout the year and breeding season peaking during late pregnancy and early lactation (see Table 1). Protein and energy are in higher

demand due to additional requirements from the foetus and milk production (Masters *et al.* 1993). Of the two processes, lactation is by far the most demanding with the ewe's nutrient requirements increase dramatically following parturition, particularly for those nursing twin lambs. The energy requirement of ewes suckling twins is almost three times higher than during the last trimester of pregnancy (Horton *et al.,* 1992).

Table 1: Merino ewe requirements of metabolisable energy (ME) in megajoules per day and crude protein (CP) in grams per day at different reproductive stages (Reis, 1979; Masters *et al.,* 1993; Hatfield *et al.*, 1995; McNeil *et al.*, 1997)

Reproductive Stage	MJ ME/day$_{(A)}$	CP g/day	CP (g/day): ME (MJ/day) ratio
Pregnancy			
Day 91-106	7.80		
Day 107-120	8.65	148 - 225$_{(B)}$	17.2
Day 121-134	9.60	148 - 225$_{(B)}$	21
Day 135-birth	10.60	148 - 225$_{(B)}$	26.0
Lactation	15.70	349-442$_{(C)}$	22.2 – 28.2
Non-reproducing	7.08	120-150$_{(D)}$	16.9 – 21.2

A: Information from Masters *et al*. 1993
B: Information from McNeil *et al*. 1997
C: Hatfield *et al.* 1995
D: Reis, 1979

1.3.1) Nutrition and pregnancy

Energy requirements for pregnancy increase with progress of gestation and foetus number (Rattray *et al*., 1974). Metabolic adaptations in the animal can help mitigate nutrient deficits during pregnancy and lactation. Rates of whole-body glucose production (mainly hepatic gluconeogenesis) in pregnant ewes generally exceed those of non-pregnant ewes (Steel and Leng, 1973).

While glucose production is higher in pregnant ewes, hepatic amino acid catabolism is reduced and muscle proteolysis is increased. As the pregnant ewe is not always able to meet the glucose demands of the growing foetus it therefore, must utilise energy reserves during times of under-nutrition (Morgante, 2004). Good body condition and fat scores of 3 (scale of 1-5) are important in ewes, as fat mobilisation serves to spare this maternal utilisation of glucose and amino acids (McNeil *et al.*, 1997). Mobilisation of fat reserves is indicated by

elevated plasma concentrations of non-esterified fatty acids (NEFA) and β-hydroxybutyrate (β-OHB) (Robinson *et al*., 2002). These are especially helpful in grazing situations where β-OHB concentrations greater than 1.0 mmol/L are deemed high and indicate fat mobilisation and inadequate feeding (Morgante 2004). However this ability is not unlimited and cannot be sustained for very long with ewes on a consistently poor plane of nutrition. Lower lamb birth weights and reduced birth rates are common in these situations (McNeil *et al*, 1997).

McNeil *et al.* (1997), found that with increasing protein in the diet, the higher the nitrogen retention in the gravid uterus and maternal tissues. In this experiment ewes were fed one of three diets consisting of low protein (LP), medium protein (MP) and high protein levels (HP) with crude protein levels of 79, 116, 157 g/kg respectively. This can be seen in Figure 1 with HP the only diet to return a positive value for N accretion in the carcase.

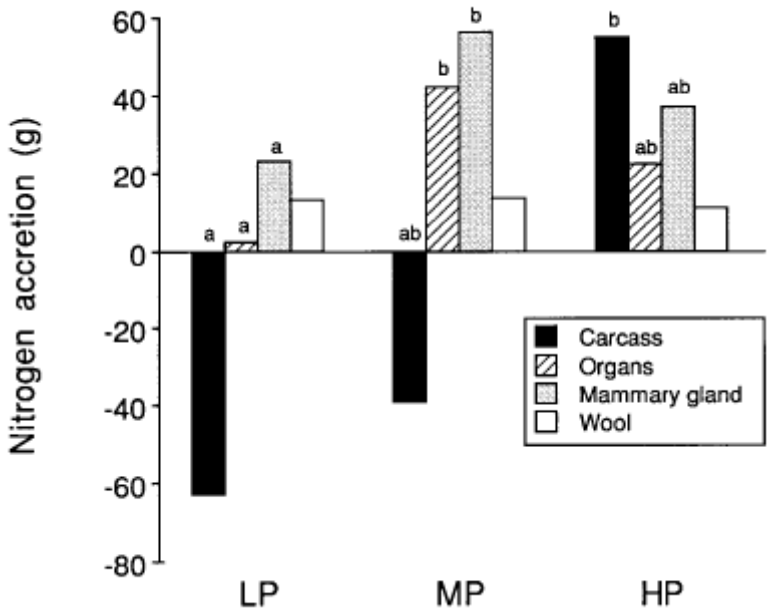

Figure 1: Nitrogen accretion between d 110 and 140 of pregnancy in maternal tissue components of ewes fed low (LP), medium (MP), or high protein (HP) diet during late pregnancy. Within tissue components, means lacking a common letter are different (P<0.05) (McNeil et al, 1997).

1.3.2) Nutrition and lactation

Lactation is by far the more nutritionally demanding metabolic state when compared to pregnancy (Horton *et al.*, 1992). The physiology of lactation can be considered a two-stage process. The first stage involves mammary differentiation and limited synthesis and

secretion of pre-colostrum for some weeks before birth; the second involves the onset of increased milk secretion just before parturition and extends for several days postpartum. The second stage is the more important period as limited specific nutrient demands are observed in the first stage having little influence on productivity (Fleet *et al*., 1975; Tucker, 1985).

Mobilisation of high levels of fatty acids from adipose tissue during and after parturition is the metabolic hallmark of the transition from pregnancy to lactation. Body fat loss is observed at a high rate for the first few days and weeks immediately following parturition (Dunshea *et al*., 1988). This is where it is important for ewes to be in good condition, as body fat loss is almost unavoidable. As with pregnancy (Bell, 1993), lactation causes an increase in voluntary feed intake. However rumen capacity is often reached and in cases where pasture quality is suboptimal, bodyweight loss is seen. Carbohydrate metabolism in the early post-parturient ruminant is dominated by the mammary requirement for glucose, mostly for lactose synthesis (Paterson and Linzell, 1974). As the demand for glucose far exceeds what digestion can provide, ruminants are able to mobilise protein stored in skeletal muscle for catabolism into glucose.

Milk production of the lactating ewe typically peaks in the second or third week of lactation at about 8-9 kg/week in merinos (Lloyd, 1963). Thereafter, production decreases steadily to approach one third of the peak daily amount or less by approximately week 12 of lactation (Treacher, 1983). Nutritional needs correspond to this production curve (Figure 2) with higher nutritional demands occurring during this first 2-3 weeks. After day 42 of lactation Hatfield *et al.* (1995) showed that CP intake has no effect on bodyweight with nutritional requirements having diminished to a non-limiting level. Twin bearing ewes produce up 28% more milk than single bearing ewes and as such have higher nutritional requirements (Hatfield *et al.*, 1995).

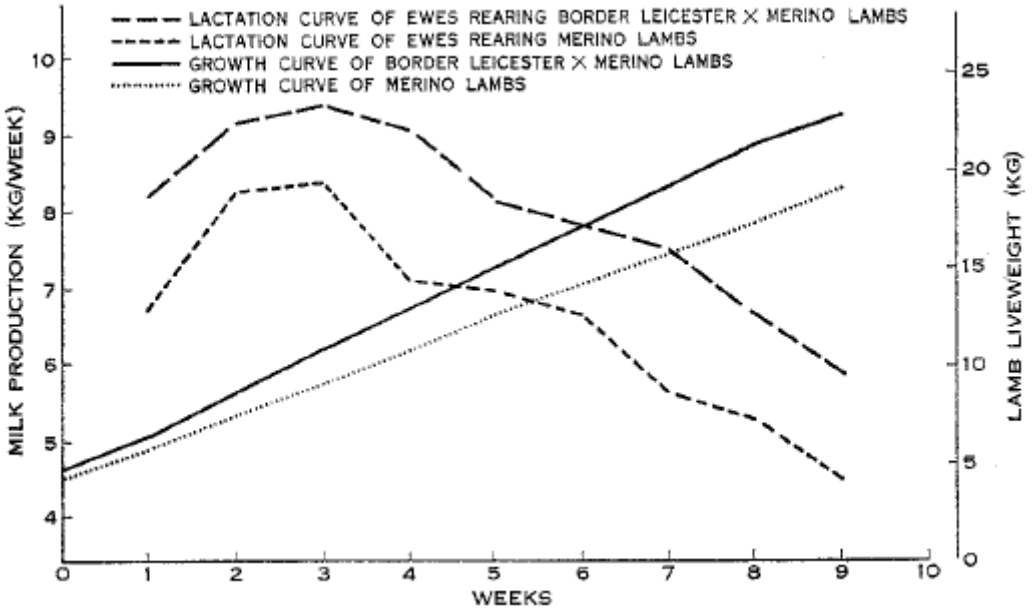

Figure 2: Comparison of milk production of ewes rearing Border Leicester x Merino lambs with that of ewes rearing Merino lambs (Lloyd, 1963)

1.3) Pregnancy, lactation and amino acids

Carbon and nitrogen are two key elements required for foetal growth and they are supplied mainly by glucose (and its intermediates, such as lactate) and amino acids (Steel and Leng, 1973; Wilson *et al.*, 1983). Direct measurement of foetal oxidation given by Bell (1995) states that in well fed ewes, glucose and acetate acquired from the dam, account for no more than 70% of the energy used for foetal respiration. This means that 30-40% of the substrates required for foetal maintenance come from amino acids.

Wool growth of sheep is often limited by a lack of absorbed sulphur containing amino acids (Pickering and Reis, 1993). Cysteine is foremost among these limiting amino acids composing the majority of wool protein fibres. Cysteine for wool growth can be obtained directly or through metabolism of methionine to cysteine via the transulphuration pathway (Staples *et al.* 1993). Wool production has been increased by injecting L-cysteine subcutaneously and by infusing DL-methionine or L-cysteine directly into the abomasum (Langlands, 1970). The same conclusions may not apply to the reproducing sheep with multiple studies finding no effect on wool growth in pregnant and lactating ewes when sulphur amino acids are added to animal diet (Williams *et al.*, 1978; Masters *et al.*, 1993).

However some studies have observed significant increases in wool growth and characteristics (Staples *et al.,* 1993). Reproducing animals are synthesising many non-wool proteins for use in the graved uterus and other maternal tissues. They are also using body reserves found in fat cell and, in particular, skeletal muscle to buffer deficiencies in nutrition (Morgante, 2004).

In previous work (Masters *et al.*, 1993), it was concluded that methionine and cysteine amino acid levels in the blood remain unchanged in reproducing ewes throughout late pregnancy and lactation. However, this was an observational study with no treatments (other than pregnant or non-pregnant) being allocated. Further work (Stewart *et al*. 1993) confirmed that amino acid concentration in the blood was present at sustained levels throughout late pregnancy in treatments injected with methionine via the abomasum. This indicates that increased protein deposition is not occurring as methionine levels become non-limiting.

While increases in wool growth have not been achieved with amino acid supplement in pregnant and lactating ewes, it has in many cases had effects. A significant difference was observed in protein deposition in lactating ewes injected with methionine. Levels of lysine, valine, alanine and serine in the blood decreased, demonstrating an increased level of protein deposition resulting in faster growth rates in the ewes (Stewart *et al*, 1993). Ewes given sulphur amino acids have a higher percentage of sulphur in their wool (Williams *et al.*, 1978). This is because the availability of sulphur amino acids to the wool follicle is increased allowing synthesis of high sulphur proteins. However increases in the sulphur content of wool are not invariably associated with increased rates of wool production (Reis, 1967; Reis and Tunks, 1974).

1.4) Supplementation during pregnancy and lactation

Current feeding standards for pregnant sheep are based on the requirements for growth of the gravid uterus and wool (McNeil *et al*, 1997). A high level of nutrient mobilisation occurs in pregnant and lactating ewes regardless of diet quality. Even with diets supplied to meet nutritional requirements, the lactating ewe may not be able to keep up with the high demand required (Oddy and Annison, 1979).

Large variations in required metabolisable energy (ME) for reproducing ewes are seen in the literature, ranging from 60 kcal/ day to 160 kcal/ day. These figures are in addition to maintenance ME requirements (Rattray *et al.*, 1974). However it is agreed that ME requirements steadily rise as gestation continues, peaking during lactation. As can be seen in Table 1, ME requirements for a lactating ewe can be more than double that of a dry ewe with rates at 15.7 MJ required per day (Masters *et al.*, 1993). According to McNeil *et al* (1997), reproducing ewes require at least 2.7 Mcal/ day.

High rates of wool growth require 120-150g protein absorbed from the intestines per day (Reis, 1979). In non-reproducing sheep around 12g of clean dry wool is produced per 100 g of absorbed amino acids (Cottle, 1998). In reproducing ewes high wool growth rates require around 220g of crude protein (CP) to be ingested (McNeil *et al*, 1997). As seen in table 1, Hatfield *et al.*, (1995) recommends much higher rates of protein intake in sheep during lactation. When given *ad libitum* access to feed, ewes will exceed these levels of CP intake, ingesting up to 550g CP/day (Hatfield *et al.*, 1995).

Raising the proportion of grain in the diet can be limited by the occurrence of digestive and metabolic disorders such as bloat, rumen acidosis, reduced fibre digestibility and low-fat milk on low-fibre diets (Horton *et al*, 1992). This is perhaps where a protein protected supplement may be of use. On the other hand, excess of supplemental methionine has an adverse effect on feed intake (Satter et al., 1975) which could occur due to amino acid imbalance.

2)Nutrition and Wool Quality and Growth

2.1) Environmental limitations

Nutritional requirements have a profound effect on a sheep's ability to grow wool with rate of wool growth being linearly related to feed intake (Allden, 1979). In a grazing system this is harder to asses as it is not known what exactly the livestock are eating in terms of quantity or metabolic composition. Nutritional values of a pasture can vary due to botanical composition, plant age and total dry matter (DM). To compound the problem, variations between years and seasonal fluctuations influence pasture quality and quantity (Finlayson *et al*. 1995).

Pasture quantity or feed availability is very important when considering wool growth. In wethers grazing seasonally variable pastures Birrell (1992) found a relationship between feed intake, pasture quality and clean wool production. Wool growth rates varied from 4 g/day to 23 g/day depending on time of year. Growth rates were higher in spring/ summer and lower in winter. This was due mainly to available feed and hourly rates of feed intake being higher in spring and summer. This close relationship between pasture availability and wool production is supported by (Schlink *et al.* 1999) that found that seasonal fluctuation in wool growth was similar to seasonal variation in pasture crude protein and digestible DM/ha. While the majority of variance in wool growth and staple strength is explained by differences in seasonal diet composition, further research found bodyweight is in fact the main cause of differences in wool growth rates (Allden 1979). This highlights the variability in wool growth and the myriad contributing factors which can influence growth rates.

Differences in pasture quality are also extremely important to wool growth rates. A balance of ME and CP must be achieved for wool to grow at optimum levels (Black *et al*. 1973). The effect of protein on wool growth is well noted (Reis *et al.*, 1992), however if ME is limiting to wool growth rates an increase in CP intake will not result in increased growth (Walker and Norton, 1971). The optimum ratio depends on the specific pattern of amino acids absorbed, the efficiency of utilization of the absorbed energy and the rate of change in body weight. This relationship indicates that in mature wethers, the optimum CP (g)/ ME (MJ) ratio for wool growth varies with digestible energy intake. For optimum wool growth this CP/ ME

ratio is around 12 (Walker and Norton, 1971; Black *et al*. 1973). Without plant bioassays this is difficult to ascertain these nutrient levels in grazing situations and so field supplementation should be given at this ratio. As can be seen in Table 1, this ratio when calculated from data from different sources (Masters *et al.,* 1993; McNeil *et al.,* 1997; Hatfield *et al*., 1995; Reis, 1979) can be quite variable and exceeds the ratio of 12.

Many studies have been undertaken to determine pasture effects on wool growth (Reed 1972; Donnelly *et al*. 1983; Kenny and Reed 1984). However factors such as stocking rate, soil type and climate all influence the pasture composition and quality. Therefore a general rule cannot be applied as to which specific pasture enables the greatest wool production. However as seen in Donnelly *et al.* (1983), legumes as a monoculture or as part of a grass pasture give the highest wool growth rates. They found that over a period of three years, Lucerne grew more wool per sheep then phalaris at lower stocking rates but that phalaris grew more wool than Lucerne at higher stocking rates. This measurement between legumes and perennial grasses has been repeated often, with general consensus being that legumes are more digestible and result in higher wool growth (Kenny and Reed 1984; Reed 1972). The higher protein content in legumes, such as Lucerne and Persian clover, results in much higher wool growth when compared to perennial grasses, 13-14 g/day compared to 9 g/day respectively. This amounted to a yearly average up to 700 g/ head higher (Kenny and Reed 1984). This is due to the far higher protein contents observed in lucerne (24.1% or greater) when compared to pasture grasses such as fescue (14.7%) or phalaris (13%) (Radcliffe and Cochrane, 1970). Legume species also have higher digestibility (Radcliffe and Cochrane, 1970) and as such higher rates of flow of digesta through the rumen are observed in ruminants eating legumes such as clover or lucerne (Allden, 1979).

2.2) Response of wool growth to sulphur containing amino acids

The majority of wool protein is composed of the sulphur amino acid cysteine, and therefore the first limiting amino acid in sheep. The other sulphur containing amino acid, methionine, can also influence cysteine levels through metabolism to cysteine (Reis 1988). Methionine is the preferred supplement because it is an essential amino acid with important metabolic functions as well as being a source of cysteine (Staples *et al.* 1993). As such, many studies have used methionine supplements and obtained significant increases in wool growth rates

(Langlands 1970; Wright 1971; Cottle 1988; Stephenson *et al.* 1991; Pickering and Reis 1993; Staples *et al.* 1993; Mata *et al.* 1995) however the majority of these were performed on non-reproducing animals.

Indeed, it is suggested by some researchers that sulphur containing amino acids are non-limiting in pregnant ewes having minimal effect on increasing wool growth rates (Williams *et al.* 1978; Masters *et al.* 1993 and Stewart *et al*. 1993). Only marginal responses were reported in areas such as increased wool sulphur content (Williams *et al.* 1978) or increased weight gain (Stewart *et al*. 1993). A study by Baldwin *et al.* 1993 also showed no response in Dorset ewes and lambs fed methionine supplement in either weight gain, milk yield or wool growth. However Stewart *et al.,* (1993) did observe increased rates of protein deposition in ewes fed Met indicating that during lactation and the peak of nutritional demand, supplementation may have an effect on wool growth. Staples *et al.,* (1993) also showed supplementing Met to have significant effects on ewes rearing twin and single lambs with increases of 11.4% and 13.7% respectively. Fibre length, staple strength and total fleece volume were all shown to be increased in the treatment group.

2.3) Response of wool growth to Lysine and other amino acids

Study on the other amino acids effect on wool growth is far less substantial. Reis (1970) supplemented various amino acids (glycine, glutamic acid, arginine, lysine, and threonine) into wethers consuming roughage-based diets with no significant stimulation on wool growth rates or characteristics. However work by Reis and Colebrook (1972) with gelatine and zein found that amino acids other than the S-amino acids are important for wool growth. Lysine deficiency increases staple length while causing decreased fibre diameter (Reis and Tunks 1978) and was corrected by the addition of lysine to the diet (Reis and Tunks 1976). Wethers on diets deficient in Lys grew less wool comparative to other protein supplements such as casein, whole egg protein, egg albumen and wheat gluten (Reis and Colebrook, 1972). This is supported by Reis and Tunks (1978) who state that addition of lysine to the diet of wethers significantly increased all aspects of wool growth.

Stewart *et al.,* (1993) showed no increase in wool growth with ewes injected with a mixture of valine, arginine, lysine and threonine. In fact a slight reduction in growth was observed being put down to amino acid imbalance or to endocrine effects. In Angora goats, addition

of lysine to the diet has resulted in decreased yield of mohair fibre. However an increase in fibre length is also seen in high lysine diets (Sahlu and Fernandez 1992).

Reis and Colebrook (1972) account for the decreased yield and increase in staple length in diets low in Lys, through the changing protein compositions in wool or hair fibres. Altering the chemical composition of the fibres contributes to different growth rates and leads to reduced staple strength (Reis, 1979). Reis and Tunks (1978) showed that a complete mixture of essential amino acids infused into the abomasum stimulated wool growth. Therefore amino acids must be present in the right compositions to ensure maximum production.

The importance of methionine and lysine in wool growth is in their ability to be directed away from the fibre follicle (wool) and be used in the synthesis of inner root sheath proteins. As the wool fibre is not rich in either Met or Lys, the importance of these amino acids for fibre growth is not necessarily due to a proportionally high requirement of these amino acids for use as substrates in the synthesis of wool protein, but in their production of other proteins (Reis and Tunks, 1978).

2.4) Response of wool growth to protein

Over the years zein and casein have been used in numerous studies due to their unusual amino acid compositions (Reis and Schinckel, 1962; Reis and Colebrook, 1972; Black *et al*., 1973; Reis and Tunks, 1976). Zein has a high proportion of leucine, a trace of tryptophan, and no lysine while casein is low in leucine. Zein has been shown to reduce wool fibre diameter and produce an area of weakness in wool fibres corresponding to when it was fed in the diet (Reis and Colebrook, 1972). Researchers have attributed this to the low levels of Lys altering the chemical composition of the wool fibre (Reis and Colebrook 1972; Reis, 1979; Stewart *et al.*, 1993). Casein infusion on the other hand has been shown to consistently increase yields in non-bred sheep when given post-ruminally (Reis and Schinckel, 1962; Reis and Colebrook, 1972). This is due to the high levels of amino acids present and in forms readily available for animal uptake (Reis and Colebrook, 1972).

3)Amino Acids

3.1) Supplementation of protein and amino acids

Supplementing sheep during pregnancy and lactation for reproductive and wool growth performance has been common practice for many years amongst sheep graziers. In grazing animals the most limiting dietary component is protein (Kenny and Reed 1984). Through research the importance of protein on wool growth was recognised, with higher protein supplements giving increased wool growth (Marston 1955). High protein supplements, such as Lucerne chaff and legume grain, have shown to increase wool growth when compared to lower protein supplements (Dove and Robards 1974). However it is important to note that wool growth in fine wool sheep is less responsive to nutrition and supplementary feeding then in medium and broad wool flocks (Lee and Williams 1994).

Investigation into optimal rates of protein intake was undertaken in merinos for optimal wool growth. Wethers and both bred and non-bred ewes were studied with various emphasis placed on protein as a determining factor in wool growth (Ferguson 1959; Reis and Schinckel 1962, Reis and Schinckel 1964; Hogan and Weston 1967 and Colebrook *et al.* 1968). Colebrook *et al.* (1968) showed a generally linear increase in wool growth with increasing crude protein intake in wethers (up to 250 g/day). However, Ferguson (1959) failed to show that different levels of crude protein in the diet increased wool growth rates. Instead, increasing overall feed intake was the determining factor in increasing wool growth. Hogan and Weston (1968) used diets from the experiments of Ferguson (1959) with different levels of crude protein and found that very similar amounts of protein N were leaving the rumen. This highlighted the role that the rumen environment plays in degrading protein. This is a result of degradation of the protein by the rumen microbes (McDonald 1950; Chalmers and Synge 1954).

Table 2: Amino acids supplied to abomasum in reproducing sheep. Note increases with progression of reproductive state (Stewart *et al.*, 1993).

Day of Pregnancy or Lactation	Amino acid available to abomasum (g/day)				
	Met	Lys	Arg	Val	Thr
128-134	2.9	6.7	2.9	4.8	3.8
135-Birth	3.2	7.6	3.2	5.4	4.3
Birth-21	5	11.7	5	8.3	6.7

As with more general nutrients like protein and energy, amino acid requirements also increase during pregnancy and lactation. Daily doses of methionine of 2.5 g/kg of feed resulted in a response in wool growth in non-reproducing sheep (Reis and Schinckel 1963) with obvious increases in reproducing sheep (Stewart *et al.*, 1993) as can be seen in Table 2. This is due to increasing competition and partitioning of amino acids as well as increasing feed intakes (Black et al. 1973).

3.2) Rumen digestion of amino acids

There has been considerable work done on Methionine and Cysteine supplementation. However, rumen microbes degrading amino acids have meant that early attempts at supplementary feeding of amino acids resulted in few gains in wool production. This is due to the deamination of the acids in the rumen during the fermentation process of microbes (Chulupa 1976). Presently amino acids can be fed in forms which bypass the rumen and reach the abomasum, where they are absorbed and used for protein synthesis (Lycnh *et al.* 1991; Staples *et al.* 1993). Rumen microbes ferment and break down amino acids in a process called deamination. This deamination of amino acids into urea is nutritionally wasteful process with often more urea created than can be utilised by rumen bacteria (Annison 1956). Coupled with this is the increased energy requirement to synthesis this urea N back into usable amino acids once it has been absorbed in the abomasum (Kajikawa *et al.* 2007).

In a study by Chulupa, (1976) it was found that different amino acids have different rates of breakdown. Arginine and Threonine are rapidly degraded, while Lysine, Phenylalanine, Leucine and Isoleucine are degraded at an intermediate rate. Valine and Methionine are

degraded more slowly and were considered the only real options for amino acid supplementation. The experiment was designed to inundate the rumen with high levels of Met. The rumen microbes would be unable to degrade it totally and some would escape fermentation, passing further down the intestinal tract intact and undigested to be absorbed in the abomasum.

This fairly simple line of thinking was replaced with attempts to develop forms of amino acids that were resistant to rumen degradation (Ferguson 1975; Wheeler et al. 1979; Ayoade *et al.* 1982; Stephenson et al. 1990; Staples *et al.* 1993). Evidence was presented that where methionine or methionine hydroxy analogue (MHA) were given as the methyl or ethyl esters, plasma methionine increased rapidly (Ferguson 1975). The amino acid was encapsulated in copolymers which are hard and impermeable at the pH found in the rumen, but disintegrate at the lower pH of the abomasum (Wheeler *et al.* 1979). Ayoade et al. (1982) studied various methionine compounds and had some success with ethyl ester of Met (MEE) with a rapid rise in plasma methionine concentration on adding MEE to the rumen. Stephenson *et al*. (1990) used a methionine analogue (butanoic acid) which shares a very similar structure to DL-Met, except the amino group has been replaced by a hydroxyl group. Treatment resulted in increased Met blood plasma levels. Also showing positive signs was coating Met with a copolymer (2-vinylpyridine/ styrene) (Staples *et al.* 1993).

3.3) Administration of amino acid supplements

It was first observed by Marston (1935) that wool growth was elevated in response to subcutaneous injections of cysteine. Stephenson *et al*. (1990) replicated the subcutaneous injection method to use as a control treatment on merino wethers tested against oral supplementation. It increased wool growth far more than the negative control and oral administration. However few other researchers have used the technique due to discomfort caused to animals (Downes *et al.*, 1966).

Studies indicate extensive degradation of DL-methionine in the rumen and poor utilisation for wool growth (Reis and Tunks, 1978). However, direct abomasal supplements given to sheep produce substantial increases in the rate of wool growth (Reis and Schinckel 1963). It can therefore be said that the level of dietary protein is not as important to wool growth as to the amount of protein reaching the abomasum. It is with this knowledge that the vast

majority of studies in the field have employed this method of administration, either through abomasal fistula or direct injection of supplement into the abomasum (Reis and Schinckel 1961; Reis 1970; Williams *et al.* 1978; Pickering and Reis 1993). Coating amino acids for rumen protection is the most recent method of administration for feed supplementation.

It is through the administration of dietary amino acids where the most gains for producers can be made. This is the only practical method of supplying unrestrained grazing sheep with amino acids to increase wool growth and benefit wool characteristics. Numerous methods have been utilised to incorporate rumen protected amino acids (Stephenson *et al*. 1990). These included adding a water-miscible liquid supplement (Alimet) to drinking water daily, mixing in a molasses lick solution and administered as a daily drench. All methods proved to be feasible options however the addition of supplement in water or in a lick solution is more practical.

The frequency of dosing is also an area for consideration, especially when applied to producer situations. The majority of experiments have used continuous infusion or daily dosing of supplement. This is not practical in a field situation where feeding may occur two or three times per week. Some researchers have found similar responses when Met is given as an infrequent large dose or as a frequent small dose (Robards 1971; Wheeler *et al.* 1979) while others have recorded greater responses in more regular applications.

3.4) Commercial amino acid supplements

A number of different commercial products have also been used over the years, with varying degrees of success. Alimet was used by Stephenson *et al*. (1990) in sheep to increase wool growth and is a methionine hydroxy analogue. Smartamine is another product which has been extensively used in the dairy industry and is a copolymer coated methionine and lysine (Misciattelli *et al.* 2003; Socha *et al.* 2005). It has been shown to increased yield of energy-corrected milk, milk true protein, and milk fat and tended to decrease concentrations of plasma glucose in dairy cows (Socha *et al.* 2005). Addition of rumen-protected Met to protein meal improved average daily growth rates in weaner steers (Klemesrud *et al.* 2000).

Smartamine has been used in both wool and meat sheep with varying degrees of success (Staples *et al.* 1993; Weise *et al.* 2003). It was surmised by Weise *et al.* (2003) that production gains for meat sheep were unlikely with the addition of rumen protected Met in sheep fed high quality diets. This suggests that production gains for the addition of amino acids will be in situations where diet and protein are limiting. This is supported by Staples *et al.* (1993) who obtained significant increases in wool growth in non-reproducing sheep and increased staple strength in breeding ewes.

LysiPEARL is a commercial form of rumen protected lysine, with applications in the dairy industry. It has a high bioavailability with the acidic pH of the abomasum enabling absorption of lysine by ruminants (Elwakeel *et al.* 2012). Movaliya *et al.* (2013) inspected the health effects rumen protected lysine had on Jaffrabadi buffaloes. Lysine had no significant effect on haematological and biochemical parameters except blood urea nitrogen suggesting it could be used for improvement in digestibility of nutrients, intake of dietary crude protein and positive nitrogen balance. Another study using LysiPEARL on steers measured muscle growth and indicates that muscle hypertrophy is increased with the addition of encapsulated lysine and methionine (Hosford *et al.* 2013).

3.5) Future research required

As LysiPEARL has been used in the dairy industry further research should be focused on the milk production of ewes. This would involve milk composition analysis as well as total milk yield. The interaction between lamb growth rates from supplemented and non-supplemented ewes could also be investigated.

The vast majority of research on the effect of specific amino acids on wool quality has been through direct abomasal injections. This is due to degradation of supplement in the rumen limiting abomasal nutrient uptake. Future work on wool production needs to apply recent advances in technology associated with rumen protected amino acids.

Weaner lambs are also another class of animal susceptible to gastrointestinal worm infestation. As protein has already shown to be effective in reducing worm populations, specific amino acids could be tested; namely methionine and lysine

4) Materials and Method

4.1) Introduction

Wool growth is suppressed during pregnancy and lactation with yield reduced by as much as 60% (Corbett 1979). Wool characteristics like fibre diameter and staple strength are also reduced, which can cause difficulties in processing, resulting in a price reduction (Orwin *et al.* 1980). It is therefore important to maintain sheep nutrition at a constant plane to avoid reductions in staple strength and ensure a consistent fibre.

However as wool production is usually undertaken in grazing situations this can be extremely difficult. Seasonality of pasture, in particular native pasture, means that metabolic quality of fodder fluctuates (Finlayson *et al*. 1995). In cases of variable pasture quality and reproducing ewes, supplementary feeding must therefore be carried out to adjust intake levels of crude protein and metabolisable energy.

Wool is largely dependent on protein levels of feed and by extension, the amino acids present in feed. Methionine and the effect it has on wool growth have been studied extensively. It has been shown to benefits to wool growth and properties in both reproducing and non-reproducing sheep (Staples *et al.* 1993). Comparatively Lysine has had far fewer wool growth studies. In studies done on angora goats increases in fibre length are seen in high lysine diets (Sahlu and Fernandez 1992). This is supported in work done on sheep by Reis and Colebrook (1972) who found an increase in staple length.

However these experiments involved the addition of lysine via injections of supplements directly into the post-ruminal digestive tract. No work has been done on the addition of lysine into unrestrained grazing sheep or reproducing sheep.

This experiment aims to investigate the effect of an amino acid rumen bypass supplement (LysiPEARL) on various wool quality characteristics of pregnant and lactating ewes.

H_0: There is no difference in wool fibre diameter, wool growth rate, faecal egg count and liveweight gain in reproducing ewes fed rumen bypass lysine and reproducing ewes not feed lysine.

H_A: There is a difference in at least one measured feature (wool fibre diameter, wool growth rate, faecal egg count and liveweight gain) in reproducing ewes fed rumen bypass lysine and reproducing ewes not feed lysine.

4.2) Experimental design

The experiment was designed as a 2 x 2 factorial, with dry and pregnant/lactating ewes and supplementation with lysine and nil supplementation. Animals were stratified across treatments on the basis of fleece weight and bodyweight 4 weeks prior lambing for dry and pregnant ewe averages. Groups were also based on pregnancy status of individual ewes with even numbers of each status in each group.

4.3) Animals and Diet

A research flock of merino ewes were artificially inseminated on 22nd and 29th April (day 0) and supplementary fed for the next four months to maintain a condition score of 3. They were then scanned on 17th July to determine pregnancy status. The study used 33 mature Merino ewes from the UNE Kirby farm, aged from 3-5 years. These were split into dry ewes (n=14), single bearing ewes (n=12) and twin bearing ewes (n=7). All ewes' were shorn on the 29th July with 12 months wool with fleece weights recorded as a untreated covariate measurement. At shearing, ewes were treated for internal and external parasites dosed according to weight. Rametin Lev combo drench and Avenge pour-on with 13mL and 60mL used respectively. The experimental treatment began on 4th September and continued for 47 days. Mid lambing was on 23rd September with a spread of 11 days around this date. Animals were sampled for worm egg counts (WEC), wool length (mm), wool micron (µm), blood plasma levels and bodyweight (kg).

All sheep were fed supplementary rations of cereal based sheep and cattle pellets (Rumevite©) for several weeks leading up to the experiment. This was delivered on the ground with approximately 260 g fed per head 3 times per week. During the treatment period sheep were fed 300g of the pellets three times per week (Monday, Wednesday, Friday). The pellet nutritional information is contained in Table 2. The treatment group received a coating of 5% ration weight of molasses and water mixture (about 3:1 respectively) to use as an adhesive for supplement to stick to.

7 grams of rumen protected Lysine was then sprinkled on top of the piled pelleted ration so that a total of 21 g was ingested weekly. Lysine was in the form of LysiPEARL which is protected from the rumen through a copolymer coating. As this was the first time this commercial product was supplemented to sheep the rate was calculated by the supplier as a g/kg liveweight and was considered the maximum dosage rate. Information supplied by the suppler indicates that 54% will be absorbed directly, resulting in abomasal intake of 3.78g per ration (see Appendix). Any sheep that did not eat their entire ration were recorded.

Table 3: Feed analysis report of Rumevite© sheep pellets.

Test	Result
Dry Matter (%)	89.8
Moisture (%)	10.2
Crude Protein (% of dry matter)	14.6
Acid Detergent Fibre (% of dry matter)	12.0
Neutral Detergent Fibre (% of dry matter)	18.4
Digestibility (DMD) (% of dry matter)	80.3
Digestibility (DOMD) (Calculated) (% of dry matter)	79.3
Est. Metabolisable Energy (Calculated) (MJ/kg DM)	11.7
Ash (% of dry matter)	12.3

Ewes were run on a 5 ha paddock for the duration of the treatment. Pasture was mostly native and unimproved. On each day of feeding, sheep were moved to sheep yards and fed in individual pens to ensure ration was being eaten and to remove variation between dominant and shy feeders. The paddock was relatively clean from worm infestation, with the paddock being spelled for several months prior to the experiment. Larval differentiation procedures were then carried out at the onset of the trial to determine worm burdens. Ewes were also periodically weighed to assess condition and lamb status. These periods were at key management or reproductive intervals and were as follows: 29/7/2013 – shearing, 9/9/2013 – late pregnancy, 30/9/2013 – mid lambing and 21/10/2013 3rd week of lactation.

Figure 3: Sheep in individual pens, receiving supplement ration

4.4) Measurement of worm egg counts (WEC's)

A bulk worm egg count was undertaken around 28 days after application of Rametin Lev combo drench (day 132) to assess the presence of worms in the flock. This involved the collection of 7-10 fresh individual faecal samples of more than 30 g. The sample was then homogenised with the aid of spatulas and modified pestles. This homogenised sample was then reduced until 30 g was left. The sample was then diluted at a 5:1 ratio of deionised water to faeces and again thoroughly mixed with the use of drill equipment. A Whitlock Universal (4 x 0.5mL) egg counting well was used to count the samples with 5 egg counting bays used to provide replication. 600 microliters of saturated salt solution was injected into each well and a sieve was placed into the faecal sample and 150 µL drawn up and injected into each well. Eggs were counted under a microscope with the total in all 5 wells then averaged. This figure is then multiplied by 60 to give an eggs/g.

Once worm presence was established, larval cultures were examined to differentiate between nematode species. Leftover homogenised faeces were mixed with equal parts vermiculite in a jar which provided a growth medium for the eggs. A small measure of water was added to the mixture to moisten and then placed in a growth oven at 24^{o}C for 5 days. More water was added to moisten on day 5 and left for another 2 days. Sample was then placed under deionised water, with a petri dish placed on top. The jar and petri dish were then inverted with adult worms falling to the bottom. These worms were then drawn up with a pipette and added to a centrifuge tube along with water solution. Left for several days to settle to the bottom of centrifuge tubes, supernatant was then removed from tube, leaving worm mass at bottom untouched. A single drop of worm mixture was then placed on a microscope slide and then examined under a microscope.

During the treatment period individual WEC's were undertaken weekly. Individual WEC's required removing fresh samples from the lower bowels of each sheep. 2-2.6 g of each sample was then diluted with deionised water. Samples were then homogenised with the use of a drill. 600 µL of saturated salt solution was then added to the worm egg counting wells, in the same process outlined in bulk worm egg counting. Tubes containing faecal samples were inverted before placing sieves to ensure even mixture and suspension of worm eggs. 150 µL of sample was then pipetted out and injected into the wells containing salt solution. Eggs were counted under a microscope with one well count used for each sample.

4.5) Measurement of wool production

Wool growth rate was estimated by the dyebanding technique of Wheeler *et al.* (1979). Dye was applied to a 10cm strip on the left hand, mid side of the sheep with the use of a syringe and modified 21 gauge needle. Dye was applied 3 weeks prior to parturition, at parturition and again 3 weeks into lactation to determine the wool growth of both the period of late pregnancy and early lactation. Dyeband was then removed with hand clippers two weeks after the final dyeband was placed.

Once removed, samples were measured with a ruler in millimetres. Staples were straightened with uneven staples and fibres removed from the sample. Whole staple length, as well as both dyebanded sections was measured 5 times with results averaged. Each

dyeband section was then cut with dressmaker scissors and placed in zip-lock plastic bags with 2 samples obtained; one for pregnancy and one for lactation. A measure of 1.5 mm was then added to the whole staple length as this is the amount removed by clippers (Williams and Chapman 1966). These samples were then sent to New England Fibre Testing Pty Ltd for micron testing. This involved the use of a mini-corer taking small snippets from the samples and an OGDA machine measuring the fibre diameter under a microscope.

Co-variant measurement of wool was carried out at shearing prior to the trial period. Ewes had 2 dyebands applied, one in mid-March and the second, 70 days later with the same technique outlined above. Dyebands were then removed prior to shearing. Shearing took place 4 weeks after the final dyeband was applied and fleece weights (FW) measured. Wool staples were then trimmed to only include the dyebanded section of growth (DB). All other clipped portions of the staple were classed as Remainder (R). The below equation was then used to obtain wool growth (WGR) in grams per day. This was then used as a co-variant when measuring trial growth. Micron was also measured for these samples.

$$\left(DB_{(g)} + R_{(g)} + FW_{(g)}\right) \times \left\{\left(\frac{DB_{(g)}}{DB_{(g)}+R_{(g)}}\right) \div length\ of\ DB\ period_{(days)}\right\} = WGR_{g/d}$$

4.6) Measurement of blood plasma

Blood samples were taken three times throughout the experiment and once before to provide a baseline. The base sample was one week prior to treatment at day 132 of parturition. A following sample was taken at day 146 to provide a late pregnancy measure. The third sample was taken at day 158 to provide data for mid lambing period and a final bleeding sample 3 weeks following mid lambing to measure lactation period.

Samples were taken via jugular venepuncture using 18 gauge needles and 10mL heparin vacutainers. Samples were centrifuged at 3,500 rpm for 15 minutes at 4^{o}C. Plasma supernatant was frozen at -20^{o}C for analysis. However, due to limited funds analysis was not conducted at time of publication.

4.7) Analysis of data

All data was then analysed using JMP 11 statistical data analyser. Co-variants were included in both fibre diameter and wool growth rate analysis. As worm egg counts have a skewed

distribution (Dash *et al.,* 1988) data was transformed using $\sqrt[3]{WEC}$ = transformed WEC's (TWEC) and then back-transformed to eggs per gram data with $TWEC^3$.

Twin bearing ewes were not analysed separately to single bearing ewes due to lamb mortality and late lambing reducing twin bearing ewe numbers to a level where statistical error was expected (n=2). Due to lamb mortality, pregnant ewes not raising a lamb and uneven lamb birth dates, data was omitted from analysis according to relevance and time period. Ewes that lambed late had wool data shifted from lactation period to pregnancy period and had no data for lactation period with the same for bodyweight data. Pregnant ewes that did not raise a lamb during treatment had lactation data removed. Any ewe that had data modified was removed from WEC analysis.

5) Results

5.1) Wool growth rates

Treatment with lysine failed to have a significant effect on wool growth rates (mm/d) during the periods of both pregnancy and lactation in ewes (P=0.576 and P=0.487 respectively). The effect of the independent variables lysine supplement and reproductive status on the change in fibre length (mm) of the ewes is displayed in Table 3.

There was a significant difference in the length of wool grown by pregnant and dry ewes (P=0.002 for pregnancy and P=0.004 for lactation).

Interactions between treatment and lamb status during the lactation period were trending towards significance (P=0.074). This trend was not observed in the interactions during the pregnancy period (P=0.865).

The covariate of the pre-experimental wool growth rate was highly significant for both pregnancy and lactation (P=0.039 and P=0.007 respectively).

Table 4: The wool growth (millimetres per day) of reproducing (W) and dry ewes (D) with a lysine rumen-bypass supplement either given (S) or withheld (N). Values are least square mean (LSM) with standard errors (s.e) and p-value also given.

	Period							
	Pregnancy				Lactation			
Treatment	Level	LSM	s.e	P value	Level	LSM	s.e	P value
	N	0.194	0.009	0.576	N	0.206	0.011	0.487
	S	0.187	0.008		S	0.216	0.009	
Lambing status	Level	LSM	s.e	P value	Level	LSM	s.e	P value
	D	0.214*	0.009	0.002	D	0.235*	0.010	0.004
	W	0.167*	0.008		W	0.186*	0.010	
Interaction	Level	LSM	s.e	P value	Level	LSM	s.e	P value
	N,D	0.217	0.013	0.865	N,D	0.217**	0.014	0.074
	N,W	0.172	0.013		N,W	0.195	0.016	
	S,D	0.212	0.013		S,D	0.253**	0.014	
	S,W	0.163	0.011		S,W	0.178	0.012	

* = Significant difference (P<0.05)

** = Trending towards significance (P<0.01)

5.2) Fibre diameter

The effect of the independent variables lysine supplement and reproductive status on the change in fibre diameter (μm) of the ewes is displayed in Table 3.

The results for fibre diameter of animals over the trial period indicate that lysine treatment had no effect on the fibre diameter grown for either pregnancy or lactation periods (P=0.28 and P=0.61 respectively).

In contrast, reproductive status had a significant effect with ewes rearing lambs having a lower fibre diameter then those that did not raise a lamb for both pregnancy and lactation (P<0.0001 for both periods).

The covariate of the pre-experimental fibre diameter was highly significant for both pregnancy and lactation (P=0.0016 and P=0.0067 respectively).

Table 5: The fibre diameter (μm) of reproducing (W) and dry ewes (D) with a lysine rumen-protected supplement either given (S) or withheld (N). Values are least square mean (LSM) with standard errors (s.e) and p-value also given.

	Period						
	Pregnancy				Lactation		
	Level	LSM	s.e	p-value	Level	LSM	s.e	p-value
Treatment	N	16.252	0.321	0.202	N	16.112	0.345	0.487
	S	16.723	0.293		S	16.240	0.291	
	Level	LSM	s.e	p-value	Level	LSM	s.e	p-value
Lambing status	D	17.751*	0.313	<0.0001	D	17.344*	0.310	<0.0001
	W	15.223*	0.292		W	15.008*	0.316	
	Level	LSM	s.e	p-value	Level	LSM	s.e	p-value
Interaction	N,D	17.334	0.445	0.451	N,D	16.906	0.442	0.154
	N,W	15.170	0.489		N,W	15.318	0.564	
	S,D	18.169	0.442		S,D	17.782	0.438	
	S,W	15.277	0.392		S,W	14.698	0.391	

* = Significant difference (P<0.05)

5.3) Worm egg counts

Worm egg counts over time did not vary between treatments (P=0.645).

Reproductive status however, did significantly affect the WEC. Reproducing ewes consistently produced higher WEC's than non-reproducing ewes (P=0.022) as seen in Table 5.

Table 6: WEC (e.p.g) of non-reproducing (D) and reproducing ewes (W). Time is in weeks after drenching, with week 5 the first week of trial period.

Lambing status	Time (weeks)						
	5	6	7	8	9	10	11
D	9.5	1.7	1.3	4.1	14.9	30.8	36.8
W	43.3	25.9	29.8	83.4	125.4	127.9	157.7

The interaction between supplementation and lambing status was also trending towards significance (P=0.06). Those ewes that did not raise a lamb (D) had lower worm egg counts that those that did (W) while those that received supplement (S) had lower counts compare to those that did not (N). This is seen clearly in Table 6 and Figure 6.

Table 7: Interaction between reproducing (W) and dry ewes (D) with a lysine rumen-bypass supplement either given (S) or withheld (N) of WEC (eggs/gram).

	Time (weeks)						
Interaction	5	6	7	8	9	10	11
N,D	34.54	13.34	10.73	1.92	61.04	90.71	63.95
N,W	16.23	13.65	18.49	133.44	85.40	204.64	221.94
S,D	0.94	0.00	0.00	7.50	0.94	5.62	18.65
S,W	90.53	44.01	44.98	47.69	176.36	73.20	107.21

While not significant over time, during the last few weeks of the trial those ewes fed lysine supplement had lower WEC's than those that were not fed lysine.

Larval differentiation revealed nematode populations to be 100% *Tricostrongylus spp.*

5.4) Bodyweight measurements

Treatment with lysine had no effect on bodyweight at any stage (P>0.05). The interaction between treatment and lambing status also had no effect on bodyweight (P>0.05).

Reproductive status had an effect on bodyweight due obviously to lambing. Dry ewes gained weight during the experimental period and lambing ewes appeared to maintain maternal bodyweight.

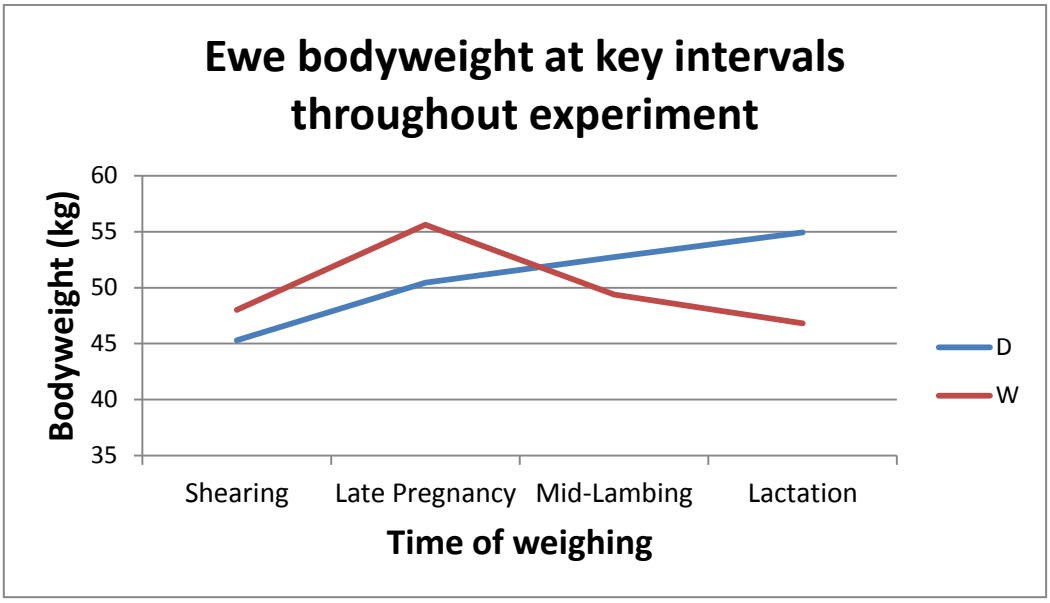

Figure 4: Wet and dry ewe bodyweight at key stages throughout the experiment

6) Discussion

The data from this experiment supports the null hypothesis that there is no difference in reproducing ewes fed rumen-protected lysine in any of the measured categories.

There is an interaction between lambing status and treatment during lactation on the wool growth rate of ewes. The trend (P=0.074) indicates that supplementation with rumen bypass lysine increases wool growth rates in non-reproducing ewes, but not in lactating ewes compared to dry. This outcome was expected as supplementing lysine into diets has increased fibre length in non-reproducing sheep and goats (Reis and Colebrook, 1972; Reis and Tunks 1978; Sahlu and Fernandez 1992) however this has not been observed in reproducing ewes (Stewart *et al.,* 1993; Masters *et al.*, 1993). This supports our results of increased fibre length in dry ewes.

However this same increase was not observed in reproducing ewes. As there have been no studies into lysine and its specific effect on pregnant or lactating ewes' wool growth, solutions to this outcome can be drawn from those studies on Methionine. During reproduction ewes are synthesising many non-wool proteins for use by the foetus, milk production and maternal tissues. In essence, nutrients are partitioned away from wool production. This is supported by Williams *et al.* (1978) and Masters *et al.* (1993) that found no difference in reproducing ewes fed methionine. Instead it is most likely that reproducing ewes are using lysine for milk production as this is where it has been applied in the dairy industry (Misciattelli *et al.*, 2003).

There was no significant interaction during pregnancy. This may be due to an "emergence time" effect in wool production, which describes a delay before change in wool growth can be detected in clipped wool samples (Nagorcka, 1977). The recommended period for wool analysis is 28-day intervals, however due time constraints; this was reduced to 25 and 21 days for pregnancy and lactation respectively. A period of about 7 days may be missed in the data when taking this lag effect into account (Downes and Sharry 1971).

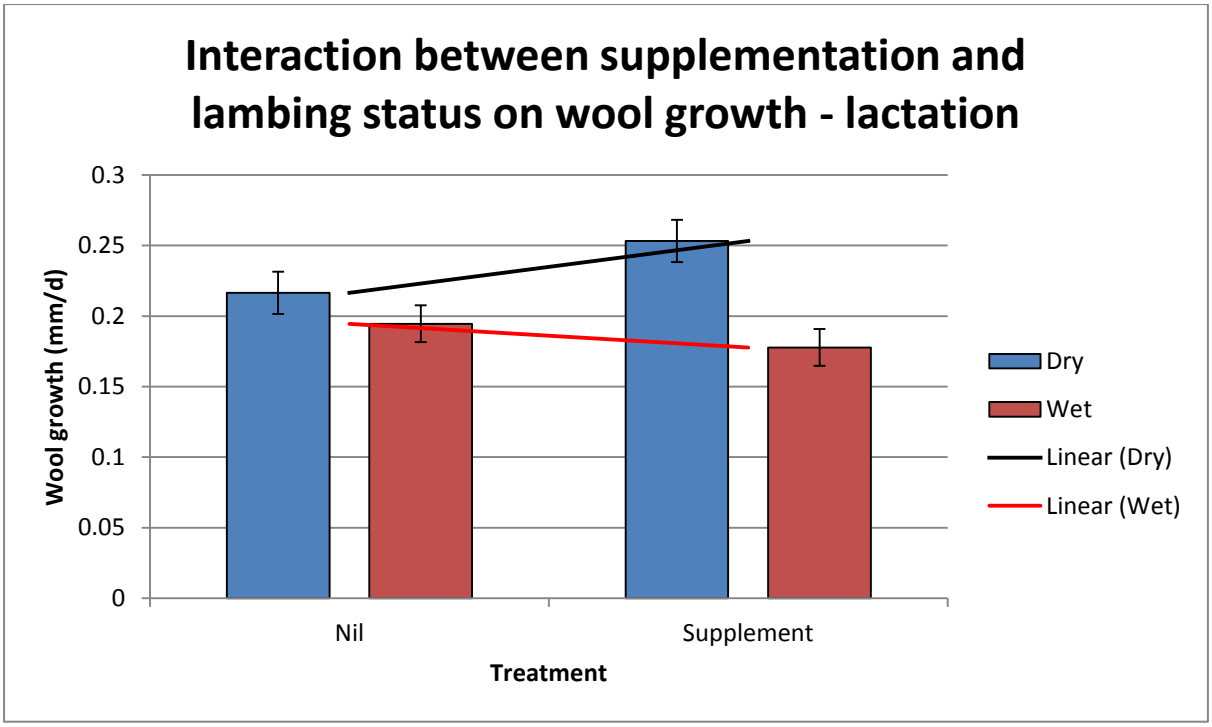

Figure 5: Lactation period supplement interaction

There was a significant difference in the amount of wool produced between reproducing and non-reproducing ewes. Reproducing ewes grew less wool during both pregnancy and lactation (LSM=0.167 mm/day, s.e=0.008 and LSM=0.186 mm/day, s.e= 0.010 respectively) than dry ewes in the corresponding periods (LSM=0.214 mm/d, s.e=0.009 and LSM=0.235 mm/d, s.e=0.010) as expected. This is a result of higher partitioning in reproducing animals (Corbett, 1979). Wool production is not an essential process in terms of animal biology and as a result, is allotted far lower nutrient quantities than other functions (Houdijk *et al.*, 2001). Nutrients are instead being used by foetal maintenance, growth of maternal tissues, milk production and ewe maintenance.

Fibre diameter was also affected by the reproductive status of ewes. Non-reproducing ewes grew thicker diameter wool compared to both pregnant and lactating ewes, as expected. This is also due to partitioning of nutrients away from wool production. Metabolic changes usually produce changes in both growth rate and diameter in about the same proportions, which maintain a fairly constant ratio of length: diameter for each sheep (Downes and Sharry 1971). That is, with decreasing growth rates, micron will also decrease at a rate consistent to growth rates.

There was no significant difference for the interaction between lambing status and treatment during pregnancy or lactation for fibre diameter. However there was a slight indication that lactation interaction may have had a slight trend, with a relatively low p-value (P=0.154) during the lactation period. It was expected that fibre diameter would increase in lysine supplemented ewes as this feature has been observed in other studies (Reis and Colebrook, 1972; Reis and Tunks 1978; Sahlu and Fernandez 1992). In this case it was perhaps not observed due to high rates of partitioning in reproducing ewes and being non-limiting in the dry ewes. While lysine is not used directly in wool fibres, it was hoped that an increased concentration would mean that methionine as well as other amino acids would be freed from protein synthesis requirements elsewhere and be used for wool growth. Reis and Tunks, (1978) tell us that lysine is important for the production of other non-wool proteins. While this may have been the case in our experiment, levels were obviously not high enough to make a difference in wool. According to Nagorcka, (1977) "emergence time" is even more sensitive for measuring fibre diameter compared to growth rates. However this would have only been a factor during pregnancy as trial length ensured that by lactation wool fibre differences would have been detectable.

There was a significant difference in WEC in terms of reproductive status with dry ewes having lower counts than reproducing ewes (P=0.022). This was to be expected as it is typical for a post-parturient rise in parasite egg output to occur in lactating ewes which does not occur in non-lactating ewes (O'Sullivan and Donald, 1970).

Worm egg counts also had a trend (P=0.06) for reproducing ewes fed lysine supplementation to have a lower worm burden than non-supplemented ewes. While not statistically significant, counts for non-supplemented ewes looked much higher, particularly in weeks 10 and 11 as seen in Figure 6. This is indication that perhaps a significant interaction would have been observed if WEC's were higher.

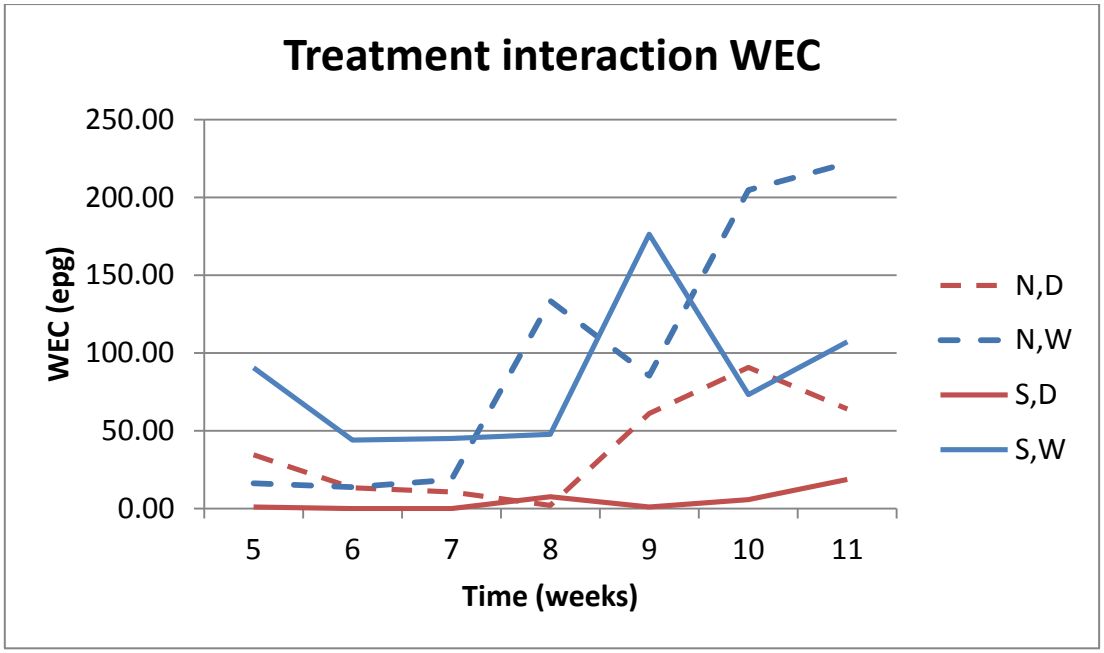

Figure 6: WEC treatment effect of lysine supplementation given (S) or withheld (N) and whether ewe reared a lamb (W) or was dry (D). Time is in weeks after drenching with week 5, the first week of treatment

The reproduction/ treatment trend that developed over the course of the trial is believed to be a result of lysine being used to suppress gastrointestinal worm populations through the immune system. Protein has been shown to lower worm populations in reproducing ewes (Kahn *et al.,* 2003) while relatively little is known about the effect on immune suppression by specific amino acids.

Comments on experimental design

Previous research has shown 3 week prior to parturition to be ideal measurement period. However due to lag effect in wool measurement as described by Nagorcka, (1977) perhaps 6 weeks prior to lactation (Kahn et al. 2003) may have been more effective at showing an interaction during the pregnancy period.

Due to low ration weight (300g) of supplementation ewes would often leave a small amount of lysine after each feeding. A different administration technique of supplement would be advised such as inclusion of supplement in a molasses lick. Stephenson *et al.* (1991) had successful results using this method. A lick technique would also be less labour intensive. Including the supplement in the pellet as opposed to manual mixing and administration

would also be less labour intensive. However consideration of the effects the pelleting process may have on bypass characteristics and viability would need to occur. The pressures and heat created by the pelleting process may affect the copolymer coating of LysiPEARL.

When looking at WEC's, artificial infection or higher natural infection may have shown greater effects of the supplement. Low worm egg numbers were observed and perhaps significance would have been obtained with higher counts.

Higher trial numbers (n>60) would have been beneficial as numbers did not allow single and twin bearing ewes to be analysed within the model.

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8) Appendix

MATRIX VALUES: LysiPEARL™ BRAND

NUTRIENT	QUANTITY
Dry Matter %	99
PROTEIN	
Crude Protein % DM	40
RDP % DM	18.4
RUP % DM	21.6
RUP (%CP)	54
Soluble Protein (%CP)	5
ADIP %	0
NDIP %	0
AMINO ACIDS	
Lysine (%RUP)	100
Methionine (%RUP)	0
Arginine (%RUP)	0
Histidine (%RUP)	0
CARBOHYDRATES	
ADF %	0
NDF %	0
peNDF (%NDF)	0
NFC%	0
FAT	
Ether Extract % DM	50.2
C 14:0, % of TFA	1.99
C 16:0, % of TFA	63.15
C 18:0, % of TFA	34.86
ENERGY VALUES	
Metabolizable Energy - 3X	0.416
Net Energy Lacation - 3X	0.332
Net Energy Gain - 3X	0.229
Net Energy Maintenance	0.332
MINERAL	
Ash % DM	9.8
Chloride %	9.8

FEED FRACTIONS	PARAMETERS
NRC Model	
Category	Plant Protein Concentrate
Energy Equation Class	
PAF	1
TDN % DM	27.88
DE Mcal/kg	0.99
Protein A % CP	0
Protein B % CP	46
Protein C % CP	54
Protein Digestion Rate %/h	4.5
RUP Digestion %	95
CP Digestibility	1
NDFP Digestibility	0
Fat Digestibility	0.2
Lysine % CP	100
Methionine % CP	0
Arginine % CP	0
Histidine % CP	0
CPM and CNCPS 5.0/6.1 Models	
Protein A (%CP)	0
Protein B1 (%CP)	5
Protein B2 (%CP)	95
Protein B3 (%CP)	0
Protein A Rate (%/h)	0
Protein B1 Rate (%/h)	40
Protein B2 Rate (%/h)	5.55
Protein B3 Rate	0
Protein A Intestinal Digestibility	0
Protein B1 Intestinal Digestibility	100
Protein B2 Intestinal Digestibility	90
Protein B3 Intestinal Digestibility	0

* Internal data on file

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