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# **NUTRIENT UTILISATION, GROWTH AND CHEMICAL BODY COMPOSITION OF PRE-WEANED LAMBS REARED ARTIFICIALLY**

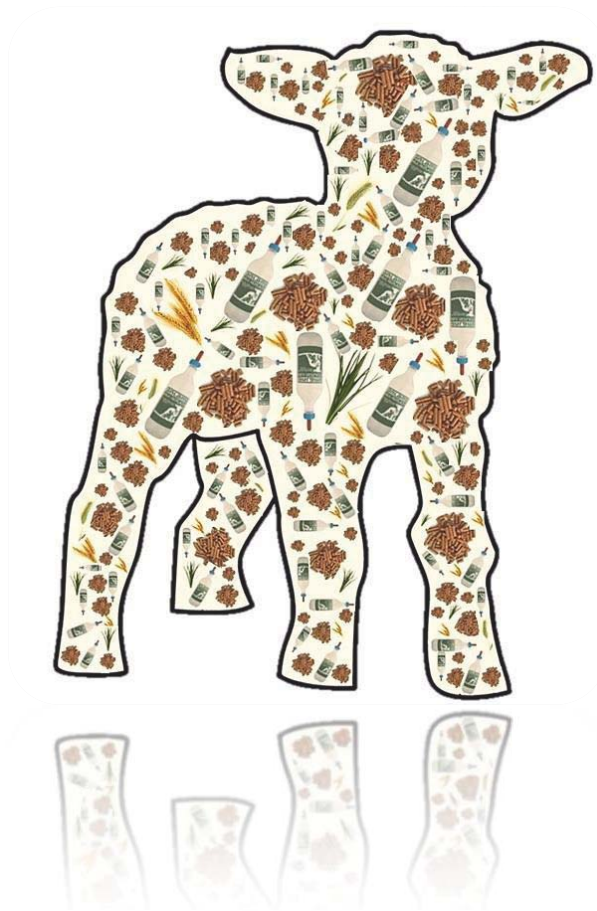
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## **Effects of feeding milk replacer and pellets**

A thesis presented in partial fulfilment of the requirements for the degree of

Doctor of Philosophy in Animal Science

at Massey University, Palmerston North, Manawatū, New Zealand



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*“Dietary inputs are of value only as long as they increase kilograms of lamb weaned,  
improve fibre quality or quantity, or positively impact lifetime production”*

(Hatfield et al, 1995)

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## ABSTRACT

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Understanding how nutrient supply controls lamb growth is important in improving the efficiency of nutrient utilisation. Estimation of metabolisable energy (ME) requirements for lamb maintenance and growth pre-weaning has been limited to milk-only fed lambs. This is due, at least in part, to the difficulty of measuring pasture intake in pre-weaned lambs, which restricts the determination of nutrient balances and nutrient use efficiencies. The aims of this thesis were to: 1) evaluate the effect of various milk and pellets combinations on lamb growth, organ development, body composition and utilisation of energy for maintenance and growth, 2) derive equations for predicting feed intake, and 3) develop a growth simulation model for use as a tool to develop feeding strategies for lambs. Lambs were offered various diet combinations from age one day until slaughter at 18 kg live weight (LW). Addition of solid feed to the milk diet of pre-weaned lambs improved their growth rates, efficiency of gain and enhanced rumen development. Increasing daily ME intake from 1.5 times maintenance to *ad libitum* at a constant protein to energy ratio did not alter the total chemical body composition of the lambs fed to a fixed LW. Increasing the crude protein content of milk replacer, and therefore the corresponding protein to energy ratio, increased average daily gain and efficiency of gain in lambs. Further, the protein content in the empty bodies of lambs increased whilst fat content decreased. Growth and body composition of lambs were unaffected by altered pellet protein content. The study also showed that lambs fed in excess of their protein and energy requirements reached maximum potential protein deposition rates. Based on a model developed, overestimating the maintenance energy requirements of milk-only fed lambs underestimated their daily fat deposition rates and underestimating the maintenance requirements of lamb offered milk and *ad libitum*

access to pellets over estimated their daily fat deposition. A greater percentage increase in fat deposited in gain increased the energy requirements for gain in the lambs. This study has contributed to the knowledge on rearing lambs artificially with various combinations of milk and pellets. The findings will provide a useful platform for future studies aiming to develop feeding strategies to improve pre-weaning lamb growth.

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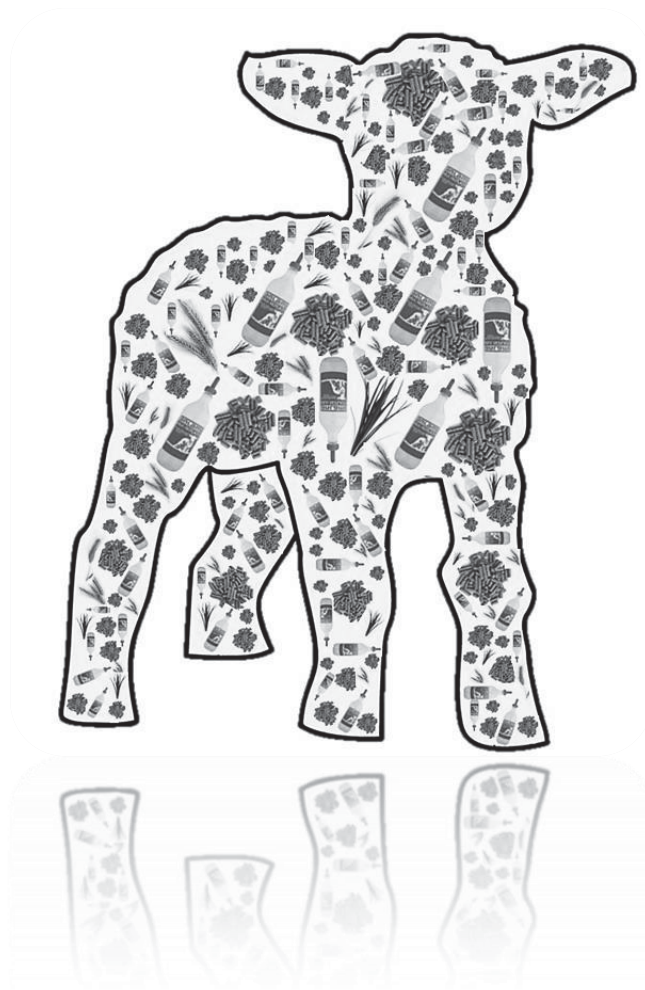




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## Chapter 1    General Introduction

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## 1.1 Overview

The increasing consumer awareness of healthy food choices has resulted in meat producers shifting to the production of leaner meat animals. Moreover, if the aim is to achieve a target live weight for the least feed consumed, a faster-growing animal is more efficient from a nutritional perspective than a slower growing animal. Accordingly, most animal nutritionists have focused on improving growth efficiency through the development of methods which allow animals to convert nutrients into leaner meat products (Gerrits *et al.*, 1996). However, in order to manipulate body composition, adequate knowledge of animal nutrient requirements and the influence of these nutrients on body composition are essential. An understanding of the growth response of an animal to variations in nutrient supply is a prerequisite in determining its nutrient requirement, predicting their performance and ultimately improving efficiency of nutrient utilisation (Schroeder and Titgemeyer, 2008).

The main source of nutrients for the neonate lamb is milk (Treacher and Caja, 2002), accounting for approximately 70% of the lamb's live weight gain in the first 3 to 12 weeks of life (Doney *et al.*, 1984). However, when solid feed consumption begins, normally around 3 – 4 weeks of age, the degree to which milk yield affects lamb growth is poorly understood (van der Linden *et al.*, 2010). Under grazing conditions, lambs obtain their energy intake from both milk and pasture. Therefore, an accurate estimate of the energy intake of individual lambs is an important requirement for an effective nutritional management.

Estimation of milk and pasture intake of naturally suckling lambs at pasture is inherently difficult to quantify as neither of these dietary components can be measured

accurately. Despite this, several techniques have been tried to accurately measure lamb milk intake and pasture intake (Doney *et al.*, 1979; Moore, 1996). Most of these techniques have their distinctive advantages and disadvantages as well as some degree of errors associated with them, which also make it difficult to accurately measure lamb feed intake under grazing conditions and accordingly relate it to their growth.

The majority of lambs in New Zealand are raised for their meat and, therefore, increasing meat production efficiency is one way of minimising the cost of lamb production (Ramsey *et al.*, 1998). However, due to the difficulties associated with measuring feed intake in young lambs under grazing conditions, there is sparse information on the efficiency of lamb growth in New Zealand, with little work undertaken in the last 30 years. Morris and Kenyon (2014) reported that lamb growth rates have improved by 50 g/day since the 1980s, which may imply that the efficiency with which they utilise the energy in their diet for growth and maintenance as well as the partitioning of the energy intake into protein and fat in body tissues has altered. However, this is not known. Additionally, estimations of maintenance and growth energy requirements of lambs have typically been limited to young lambs subsisting on either milk only or solid feed only.

Growth in itself is complex in nature and the growth process is driven mainly by genetics, the environment, and by nutrition (Bastianelli and Sauvant, 1997; Halas *et al.*, 2004). The complexity of the growth process is further highlighted when growth rates of lambs tend to vary among individual animals, even under controlled conditions (Owens *et al.*, 1993). This makes accurate growth predictions under specified or changing environmental conditions quite challenging (Purchas, 1986). Thus, in order to better understand and predict animal growth performance, most animal nutritionists

have resorted to the use of mathematical models. By incorporating existing knowledge into these models, an increased understanding of the response of animals to feed (nutrient) intake can be acquired.

Feed, and particularly energy intake, is the single most important input and is a major component of production costs (Ramsey *et al.*, 1998). By knowing and anticipating the changing nutritional needs of lambs during the growth period, combined with the dynamic interaction between feed intake and growth, a useful basis for farmers to plan their feeding programs and lower feed costs when managing pre-weaned lambs can be provided. Therefore, the aims of this research were to evaluate the effects of a variety of milk and pellet combinations on growth and organ development, body composition and utilisation of energy for maintenance and growth. In addition, aims were to derive equations for predicting feed intake, and to develop a growth simulation model which can be used as a tool for developing feeding strategies for lambs.

Specific aims of this thesis were to:

- evaluate the effects of several dam and offspring traits on milk production and lamb growth from birth to weaning and to determine how much of the variation in twin lamb growth was explained by those traits (Chapter three),
- determine how much of the variation in lamb growth could be explained under controlled feed intake conditions and examine the effects of varying energy intake on growth, organ developments, body composition and metabolisable energy utilisation (Chapter four),
- determine energy and nitrogen metabolisability of milk and pellets when fed together, to compare the growth and chemical body composition of lambs fed varying levels of pellets in addition to milk, and to estimate the maintenance and

growth energy requirements, and the CP:ME requirements for growth of lambs fed milk replacer and pellets to a fixed live weight (Chapter five),

- determine the effects of different protein to energy ratios on lamb growth, body composition and nutrient utilisation (Chapter six),
- develop a methodology for predicting milk intake and solid feed intake of lambs using faecal chemical components (Chapter seven) and
- to develop a model to simulate lamb growth and body composition based on the data collected in the experimental part of the thesis (Chapter eight).

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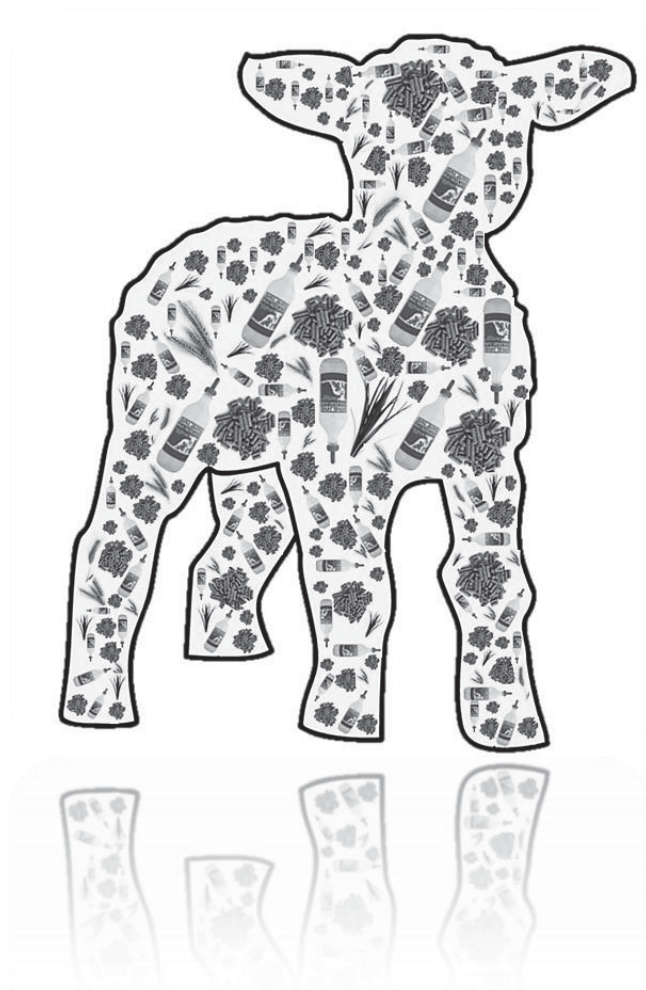




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## Chapter 2      Literature review

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## **2.1 Preamble**

Fast growth of the neonate lamb in the early post-partum period is primarily dependent on the efficient utilisation of the ewe's milk (Gibb and Treacher, 1978; Snowder and Glimp, 1991). Milk intake has its greatest effect on the live weight gain (LWG) of lambs up to about 12 to 16 weeks of age (Gibb and Treacher, 1980; Doney *et al.*, 1984). For this reason, a significant amount of research has been undertaken to investigate the relationship between ewe milk yield and lamb growth. Accordingly, this literature review focuses on the factors affecting lamb growth with specific reference to the nutrition of the pre-weaned lamb. Further, factors affecting ewe milk production and composition will be reviewed. However, for the purpose of this thesis, attention is focused on dam nutrition, ewe live weight and body condition. Furthermore, protein and energy requirements and utilisation of the pre-weaned lambs will be reviewed. Lastly, the various methods of measuring lamb milk and pasture intake; their general assumptions, advantages as well as the experimental errors associated with them will also be discussed. This review concentrates specifically on ovine species. However, where appropriate, references have been made to other species.

## **2.2 Factors Affecting Lamb Growth**

The growth of an animal is dependent on two main factors: its genetic potential and the external environment within which it grows and expresses its gene combination (Parratt and Young, 1983). Because the weaning weight of lambs is of prime economic importance to the sheep industry, it is vital to understand the factors that affect growth pre-weaning. The weaning weight of lambs is dependent upon lamb parameters

including birth weight, age, sex, litter size and dam parameters such as milk yield, age, size, and nutrition with many of these factors being interrelated.

### **2.2.1 Lamb's birth weight**

Birth weight reflects fetal development during pregnancy (Sava *et al.*, 2011) and is also a predictor of postnatal growth (Holland and Odde, 1992). Birth weight is altered by various prenatal environmental factors (Owens *et al.*, 1993), being greatly influenced by litter size (Gardner *et al.*, 2007). A study by Greenwood *et al.* (1998) showed that Suffolk  $\times$  (Finn  $\times$  Dorset) male lambs with low birth weight ( $< 2.9$  kg) took a longer time to reach positive weight balance or to achieve a net gain in weight 24 h postpartum, compared to their larger counterparts ( $> 4.3$  kg). Faster growth rates were observed in high-birth-weight lambs than in low-birth-weight lambs during the early postnatal period (first 2 weeks of birth) even when both were allowed *ad libitum* access to feed. However, after the period of adaptation, lambs given *ad libitum* access to feed grew faster to a 20 kg live weight than the restricted lambs regardless of birth weight (Fig. 2.1). Combined, this suggests that when nutritional and environmental deficiencies are minimal, the influence of birth weight on lamb growth is predominant only during the early postnatal stages of life (deBaca *et al.*, 1956; Greenwood *et al.*, 1998; Greenwood and Bell, 2002). This concurs with Kenyon *et al.* (2011a) who reported that the relationship between birth weight and LWG diminishes as lambs grow. However, early studies by Schinckel and Short (1961) and Taplin and Everitt (1964) reported significant effects of birth weight on postnatal growth of lambs reared artificially up to 16 and 20 weeks of age.

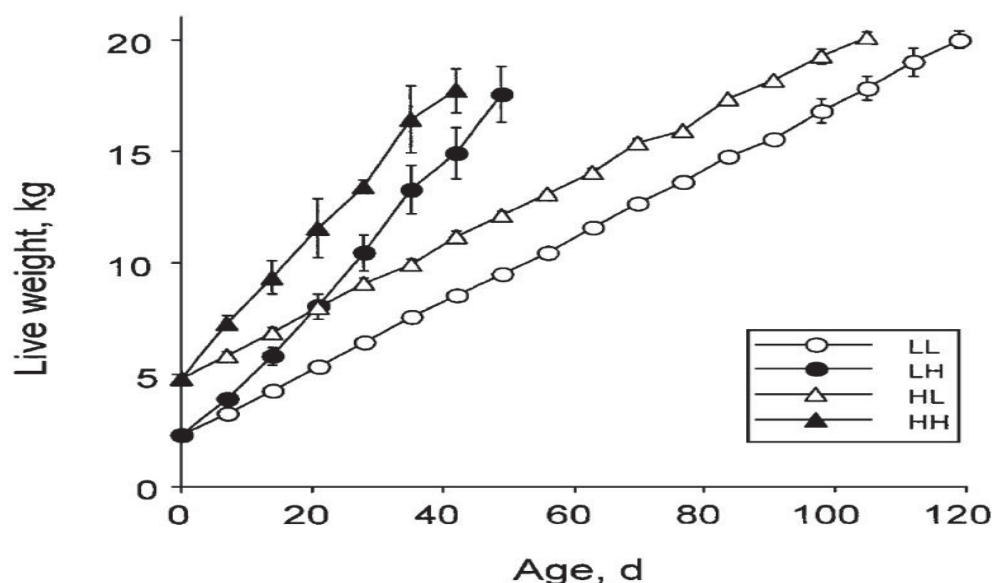


Figure 2.1 Growth of slowly reared lambs of low (LL,  $n = 16$ ) and high (HL,  $n = 12$ ) birth weight and of rapidly reared lambs of low (LH,  $n = 16$ ) and high (HH,  $n = 12$ ) birth weight, individually reared from birth to approximately 20 kg live weight (LW). Source: Greenwood *et al.* (1998)

### 2.2.2 Sex of lamb

Ram lambs tend to be heavier at birth than ewe lambs (Sava *et al.*, 2011). The birth weights of twins of different sex combinations follows the same trend with the male-male pairs being the heaviest, followed by male-female pairs and female-female pairs been the lightest (Gardner *et al.*, 2007). The physiological basis for weight differences between sexes at birth is related to the activation of Mullerian inhibiting substance which is triggered by the sex-determining region Y gene on the Y-chromosome (SRY) (Haqq *et al.*, 1994) and androgens (Kim *et al.*, 1972; de Zegher *et al.*, 1999), which have sex-specific effects on the fetal growth (Loos *et al.*, 2001).

Weight gain differences in favour of ram lambs have also been observed by numerous workers during the post-natal growth periods (Peters and Heaney, 1974; Penning *et al.*,

1980; Notter *et al.*, 1991; Gardner *et al.*, 2007). The higher growth rate in males than in females is at least partially explained by the higher concentrations of androgen. This difference tends to increase with age, thus causing marked differences in the growth rates of the sexes (Fisher *et al.*, 2010).

### **2.2.3 Birth and rearing rank**

Birth and rearing rank affects ewe milk production and consequently lamb growth rates. During lactation, ewes suckling multiple lambs produce more milk than those suckling single lambs (Lloyd, 1963; Peart *et al.*, 1972; Joyce *et al.*, 1976; Muir *et al.*, 2000a; Dillon *et al.*, 2003) (Fig 2.2). However, the amount of milk available to each multiple-suckled lamb is less than that available to a single-suckled lamb (Moffat *et al.*, 2002a; Morgan *et al.*, 2007). This is because the extra milk produced by multiple-bearing ewes (15% to 50% for twins; 60 % to 100% for triplets and 155% for quadruplets) is not proportional to the number of extra lambs (twins - (Peart *et al.*, 1972; Peart *et al.*, 1975a; Joyce *et al.*, 1976; Geenty and Sykes, 1983; Snowden and Glimp, 1991; Hatfield *et al.*, 1995) or triplets and quadruplets - (Peart *et al.*, 1972; Joyce *et al.*, 1976; Gallo and Davies, 1988). Furthermore, in some studies, the increase in milk yield available to multiples in comparison to singletons has been non-significant (Peart, 1967; Moffat *et al.*, 2002b). Consequently, single-reared lambs tend to have higher growth rates than multiple born and reared lambs during the first 4 weeks of life, even under conditions of high ewe milk production (Peart *et al.*, 1975a; Moffat *et al.*, 2002b; Morgan *et al.*, 2007; Kenyon *et al.*, 2011b). Similarly, multiple-born lambs that are reared singly grow faster than their counterparts born and reared as multiples (Morgan *et al.*, 2007; Kenyon *et al.*, 2011a). As lactation progresses, however, growth rates of twin lambs begin to equalise

to that of single lambs (Peart, 1967) due to earlier foraging behaviour, which consequently compensates somewhat (Geenty, 1979; Dimsoski, 1999).

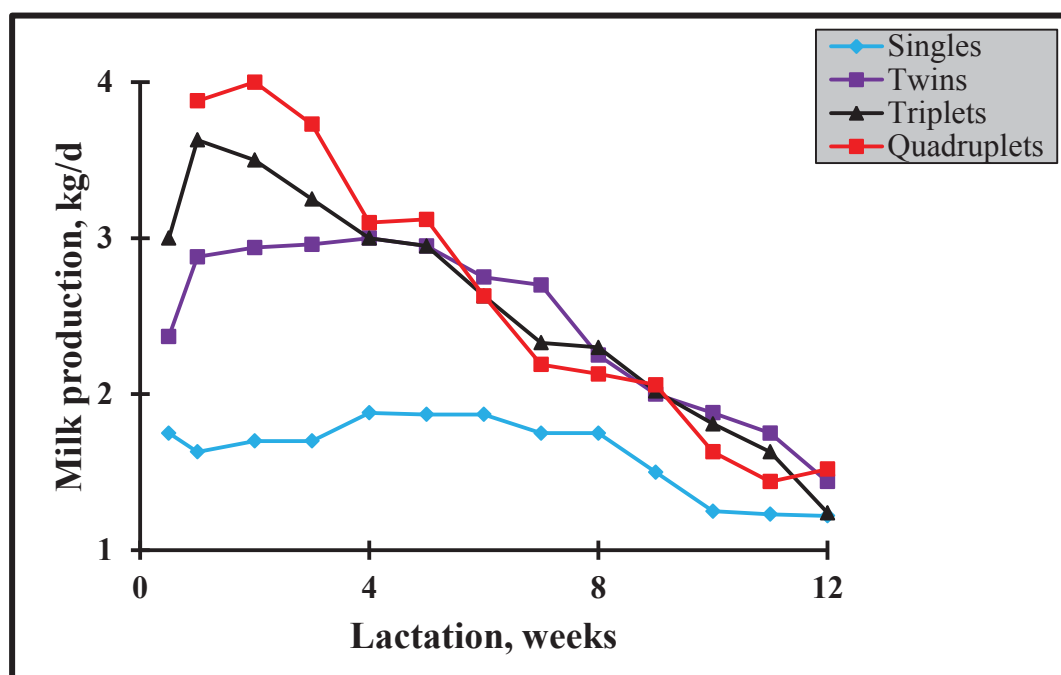


Figure 2.2 Milk yield pattern for Finnish Landrace x Blackface cross ewes suckling singles, twins, triplets and quadruplets. During the first 12 weeks of lactation. Source: Adapted from Peart *et al.* (1972).

#### 2.2.4 Genetic factors

Although lamb growth rate is affected by ewe milk production, it is also affected by lamb genotype and hybrid vigour (Peeters *et al.*, 1992; Muir *et al.*, 2000b). Approximately 50% of the difference in lamb weight at weaning is due to the lamb's genotype for growth and the remaining 50% is attributed to ewe milk production (Yates and Pattie, 1970). A considerable amount of variation in ewe milk production also exists within (Burris and Baugus, 1955) and between ewe breeds (Muir *et al.*, 2000b; Morgan *et al.*, 2006; Morgan *et al.*, 2007). Although lamb genotype is a major contributing

factor to weaning weight differences, optimum ewe lactational performance is required for lambs to completely express their superior genotype (Yates and Pattie, 1970).

### **2.3 Lamb nutrition**

Pre-ruminant lambs are unable to digest solid food until about 3 – 4 weeks of age (Treacher and Caja, 2002) and hence their dam's milk is key to their survival and growth. A typical lactation curve increases up to a peak and then decreases as lactation progresses (in keeping with the nutrient requirements of animals) (Fraga, 2013), with milk production being highest for most ewe breeds in the first 2 – 4 weeks of lactation (Peart *et al.*, 1972; Geenty and Jagusch, 1974; Peart *et al.*, 1975b; Geenty, 1979; Rhind *et al.*, 1992; Sakul and Boylan, 1992). After peak production, lactation begins to decline at a steady rate as lambs progressively consume increasing amounts of solid feed and milk intake per kg live weight (LW) declines, particularly if solid feed (usually pasture) is available (Corbett, 1968; Peart *et al.*, 1975b; Peart *et al.*, 1979).

The pattern of lactation reflects the ability of lambs to consume milk secreted rather than their dams' ability to secrete milk, especially in early lactation for ewes suckling singletons (Moore, 1966; Peart, 1967; Rattray, 1986). Therefore, lambs that require more milk stimulate greater production by the lactating dam, with multiple-born lambs stimulating greater yields as earlier stated. However, if pasture is limiting in late lactation, lambs will suckle more at the expense of ewe body reserves which can result in an accelerated decline in milk production (Rattray, 1986).

### **2.3.1 Ewe Milk Production**

Knowledge of the factors that influence milk production could lead to the development of strategies to enhance lamb growth.

#### **2.3.1.1 Nutrition in Pregnancy and Lactation**

The ewe's plane of nutrition is an important factor influencing milk yield (Barnicoat *et al.*, 1956) with energy intake being the main nutritional determining factor (Pulina *et al.*, 2005; Kenyon *et al.*, 2011a; Galvani *et al.*, 2014), particularly during pregnancy and lactation. Dams fed at maintenance during pregnancy produced lower milk yields than their counterparts fed *ad libitum* (Wallace, 1948; van der Linden *et al.*, 2007). Additionally, severe feed restriction during late pregnancy can result in delayed onset of lactation and consequently, a substantial reduction in the rate of milk production and lamb growth (McCance and Alexander, 1959). In the study by Banchero *et al.* (2006) it was demonstrated that a 30% reduction in metabolisable energy (ME) intake of pregnant Merino ewes reduced colostrum production significantly, while in the studies of Barnicoat *et al.* (1956) and Peart (1967) *ad libitum* feeding during pregnancy helped sustain milk production until the later stages of lactation.

Ewe nutrition during early lactation is crucial for both initial and total milk yield (Peart, 1970). Poor persistency of lactation occurring in ewes may reflect an inability to obtain adequate nutrition and / or an insufficient body reserve to support the demands of lactation. Restricted feeding during the first 3 to 4 weeks of lactation reduced milk yield during the period of restriction. However, the effect was more visible in lean compared with fat ewes suckling twins (Peart, 1970; Coop *et al.*, 1972; Jagusch *et al.*, 1972). Removal of nutrient restriction before ewes reach peak milk production enables ewes to

utilise their body reserves to restore milk yields to normal levels and this can sometimes override the effects of restricted nutrition in late gestation (Peart, 1967; 1968b; Maxwell *et al.*, 1979; Gibb and Treacher, 1982; Rattray *et al.*, 1982).

In summary, nutrient restriction prior to parturition or during lactation will lead to lowered milk yields in ewes rearing either single or twin lambs, particularly the latter. Optimum feeding during lactation is crucial to high milk production of ewes, although maximum milk yield is dependent on adequate nutrition in both late pregnancy and lactation (Barnicoat *et al.*, 1956; Peart, 1967; 1968a).

#### ***2.3.1.2 Effect of Ewe Live weight on Milk Yield***

Studies examining ewe LW at mating or during pregnancy have tended to concentrate on lambing performance, lamb birth weights and / or growth rates (Geisler and Fenlon, 1979; Russel *et al.*, 1981; Kenyon *et al.*, 2004; Kenyon *et al.*, 2012a; Kenyon *et al.*, 2012b) while little is known about the effects of ewe LW on milk yield. Studies examining LW variations between breeds (Gardner and Hogue, 1966; Geenty, 1979) or within breeds (Peart, 1970) during lactation have shown that larger or heavier ewes tend to produce more milk than their smaller or lighter counterparts. Additionally, ewes born to heavy Romney dams (average 60.8 kg) produce more milk than those born to light Romney dams (average 42.5 kg) (van der Linden *et al.*, 2009). Contrary to these findings, no effects of ewe LW on milk yield have been reported for ewes under optimal grazing conditions (Barnicoat *et al.*, 1949; Barnicoat *et al.*, 1956; Bencini and Purvis, 1990). Furthermore, when there is sufficient body reserve to support lactation, milk yield of heavy and light ewes does not differ (Peart, 1970) suggesting that the ability of ewes to convert their body reserves to milk is critical during the lactation period.

Therefore, a positive correlation between ewe LW and milk yield may indicate either better feeding levels or greater maternal energy reserves available for milk production (Barnicoat *et al.*, 1956; Peart, 1970; Peart *et al.*, 1975b). Nonetheless, if feed intake is limiting during lactation, heavy ewes will maintain substantially greater milk production than light ewes (Peart, 1968b).

Combined, these few reports relating ewe LW to milk production indicate the benefits of having heavier ewes on milk production especially when grazing conditions are not optimal. However, these reports have predominantly focused on ewe LW during lactation with a paucity of data on the effects of ewe LW pre-breeding on milk production. Therefore, more research is needed in this area to determine the effects of ewe LW, particularly at mating, on milk production.

#### **2.3.1.3 Effect of Body Condition Score on Milk Yield**

Body condition score (BCS) typically has a positive relationship with fetal growth, lamb birth weight and growth to weaning (see review by Kenyon *et al.* (2014)). However, its relationship with milk production varies. There have been reports of a positive effect of BCS on milk production when BCS was measured in late pregnancy and in lactation (Peart, 1970; Gibb and Treacher, 1980; Hossamo *et al.*, 1986) whereas no effect of BCS on milk production has been reported when BCS was measured pre-breeding, mid and late gestation (Gibb and Treacher, 1982; Hossamo *et al.*, 1986; Oregui *et al.*, 2004). Furthermore, a negative effect of BCS on milk production has been reported in ewes under maintenance feeding during lactation (Pulina *et al.*, 2012).

Under restricted nutrition, high BCS ewes (BCS of 3.0) were able to mobilise body reserves, which compensated in part for the lower feeding levels ultimately resulting in

them producing more milk than their low BCS counterparts (BCS 1.5 to 2) when BCS was measured during lactation (Peart, 1970; Gibb and Treacher, 1980). However, when feeding levels were high in lactation, the effect of late pregnancy BCS in the range of 2 to 3 had no impact on milk production (Gibb and Treacher, 1982). This is because unrestricted feeding conditions may not require ewes to utilise their body reserves to support lactation (Kenyon *et al.*, 2011b) and hence the potential benefits of high BCS will not be observed. When feeding levels are adequate to sustain lactation, low body fat ewes will eat more than their high body fat counterparts and hence the differences in their milk yield will be small (Cowan *et al.*, 1980).

Although high BCS ewes will sustain better production than low BCS ewes when feeding conditions are limiting, optimum feeding levels of ewes during lactation is important for high milk production irrespective of the BCS of ewes. Nevertheless, further work is still required to quantify the impacts of BCS on milk yield, especially at mating.

### **2.3.2 *Effects of ewe nutrition on growth of lambs***

It has been established that alterations to maternal nutrition during pregnancy can affect fetal growth (Barker, 2003) and subsequently the postnatal growth of the resultant lambs (Ford *et al.*, 2007; Gardner *et al.*, 2007; Tygesen *et al.*, 2008; Kenyon *et al.*, 2009). Maternal under-nutrition in early to mid-gestation is less likely to have negative consequences on the birth weights and subsequent growth rates of lambs than under-nutrition during late gestation (Hawkins *et al.*, 2000; Rae *et al.*, 2002; Gardner *et al.*, 2005; Gardner *et al.*, 2007). Maternal nutrition at the latter part of gestation is particularly crucial to lamb birth weight (Robinson *et al.*, 1977; Gardner *et al.*, 2007) as fetal growth is greatest during this period (Gardner *et al.*, 2007) and severe nutrient

deprivation at this time alters both lamb birth weight and subsequent growth rates of lambs (Fig. 2.3) (Taplin and Everitt, 1964; Robinson *et al.*, 1977; Borwick *et al.*, 2003; Tygesen *et al.*, 2007; Husted *et al.*, 2008). However, as seen with ewe milk yield, *ad libitum* feeding following nutrient restriction in early gestation can nullify the effect of the earlier undernourishment and lead to optimal postnatal lamb performance (Fig 2.3; (Taplin and Everitt, 1964).

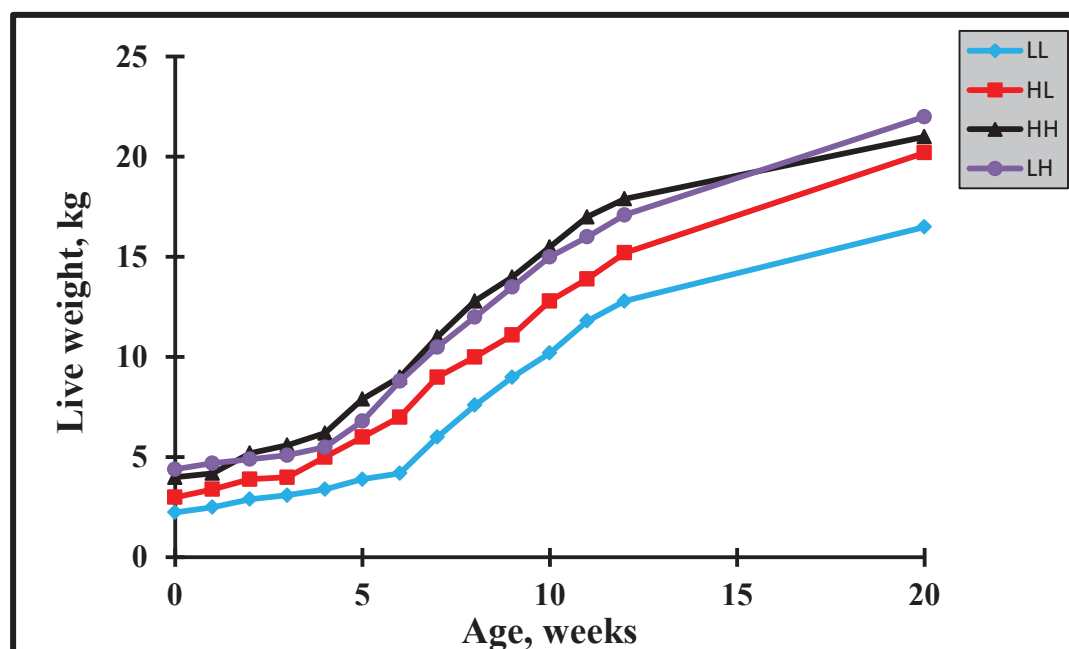


Figure 2.3 Live weights of lambs whose dams were subjected to four nutritional regimes during pregnancy (HH, a high plane throughout pregnancy; HL, a high plane for the first 90 days followed by a low plane to lambing; LH, a low plane for the first 90 days followed by a high plane until lambing; LL, a low plane throughout pregnancy). Source: Adapted from Taplin and Everitt (1964).

### 2.3.3 Ewe milk Composition

Ewe's milk is richer in protein, fat and lactose than cow's or goat's milk (Bencini and Pulina, 1997). Nevertheless, the composition of ewe's milk varies among breeds with

the stage of lactation and the level of nutrition. The percentage composition of the major milk components (protein, fat and lactose) of various ewe breeds are listed in Table 2.1. Ewe milk composition can also vary with environmental factors such as management decisions. These factors can be manipulated by the farmer to produce high-quality milk (Bencini and Pulina, 1997) (Fig. 2.4). Additionally, changes in the quantity of one component can sometimes affect the quantities of the other components (Cant *et al.*, 1993; Pulina *et al.*, 1995).

Table 2.1 Percentage composition of ewes' milk (protein, fat and lactose) obtained from various studies

| Breed                                       | Protein (%) | Fat (%)      | Lactose (%) | Reference                           |
|---------------------------------------------|-------------|--------------|-------------|-------------------------------------|
| Border Leicester × Merino                   | 3.76 – 5.35 | 9.34 – 10.40 | 5.25 – 5.94 | Morgan <i>et al.</i> (2006)         |
| Cheviot                                     | 5.71        | 6.64         | 4.48        | Barnicoat <i>et al.</i> (1956)      |
| Coopworth × Merino                          | 3.70 – 5.42 | 9.85 – 10.97 | 5.17 – 5.87 | Morgan <i>et al.</i> (2006)         |
| East Friesian × Merino                      | 3.90 – 4.98 | 8.38 – 9.34  | 5.23 – 5.85 | Morgan <i>et al.</i> (2006)         |
| Finnsheep × Merino                          | 3.67 – 5.68 | 9.36 – 10.42 | 5.02 – 6.05 | Morgan <i>et al.</i> (2006)         |
| Rambouillet, Columbia, Polypay, and Suffolk | 5.0 – 7.2   | 4.1 – 10.2   | 3.1 – 5.3   | Snowder and Glimp (1991)***         |
| Romney                                      | 5.31        | 5.76         | 4.44        | Barnicoat <i>et al.</i> (1956)      |
| Romney                                      | 4.9         | 7.3          | 5.2         | Blair <i>et al.</i> (2010)          |
| Romney                                      | 4.7–4.9     | 6.3 – 6.8    | 5.1 – 5.2   | Paten <i>et al.</i> (2013)          |
| Romney                                      | 4.7         | 7.8 – 8.1    | 5.2 – 5.3   | van der Linden <i>et al.</i> (2009) |
| Romney, Corriedale and Dorest               | 4.7 – 4.9   | 8.7 – 9.1    | 3.7 – 3.8   | Geenty (1979)***                    |

\*\*\* indicates the percentage pooled means for all the different breeds used in the study.

### *Milk fat*

The content of fat in ewes' milk varies in response to environmental stimulus (Barnicoat *et al.*, 1956; Snowden and Glimp, 1991). It is easily altered by factors such as the number of lambs suckling, stage of lactation and the dietary intake of the ewe (Gardner and Hogue, 1964; Snowden and Glimp, 1991; Horton *et al.*, 1992; Sklan, 1992; Paten *et al.*, 2013). While several authors (Peart *et al.*, 1979; Torres-Hernandez and Hohenboken, 1979; Morgan *et al.*, 2006) have reported an increase in the concentration of fat as lactation advances, others (Peart *et al.*, 1972; 1975b; Snowden and Glimp, 1991) have reported a progressive decline in fat concentration from early to mid-lactation, followed by an increase in late lactation. In dairy cows, increased fat content is normally associated with decreased protein content as a result of the decreased ability of the mammary gland to use amino acids (Cant *et al.*, 1993).

Feed restriction during pregnancy coupled with low milk yield increased fat concentration in milk due to increased utilisation of body fat reserves (Geenty and Sykes, 1986). However, increased feed intake in late gestation increased the level of body fatness during lambing which in turn increased the fat content in milk (Cowan *et al.*, 1980).

### *Milk protein*

The protein content in the diet of ewes affects the quantity and the partition of nitrogenous substances in the milk (Cowan *et al.*, 1981; Bencini and Pulina, 1997). Milk protein, as with milk fat, tends to increase as lactation progresses (Morgan *et al.*, 2006). However, while protein is less affected by ewe breed and the number of suckling lambs (Peart *et al.*, 1972; 1975b), nutrition restriction of ewes during lactation decreases milk

proteins substantially indicating a reduction in nutrient supply during the periods of restriction (McDowell, 1991). Milk protein concentration is positively affected by ewe LW gain from mid-pregnancy to lamb weaning (Bencini and Purvis, 1990; Morgan *et al.*, 2006) although increased body fatness at lambing has been shown to decrease the protein content in milk (Cowan *et al.*, 1980).

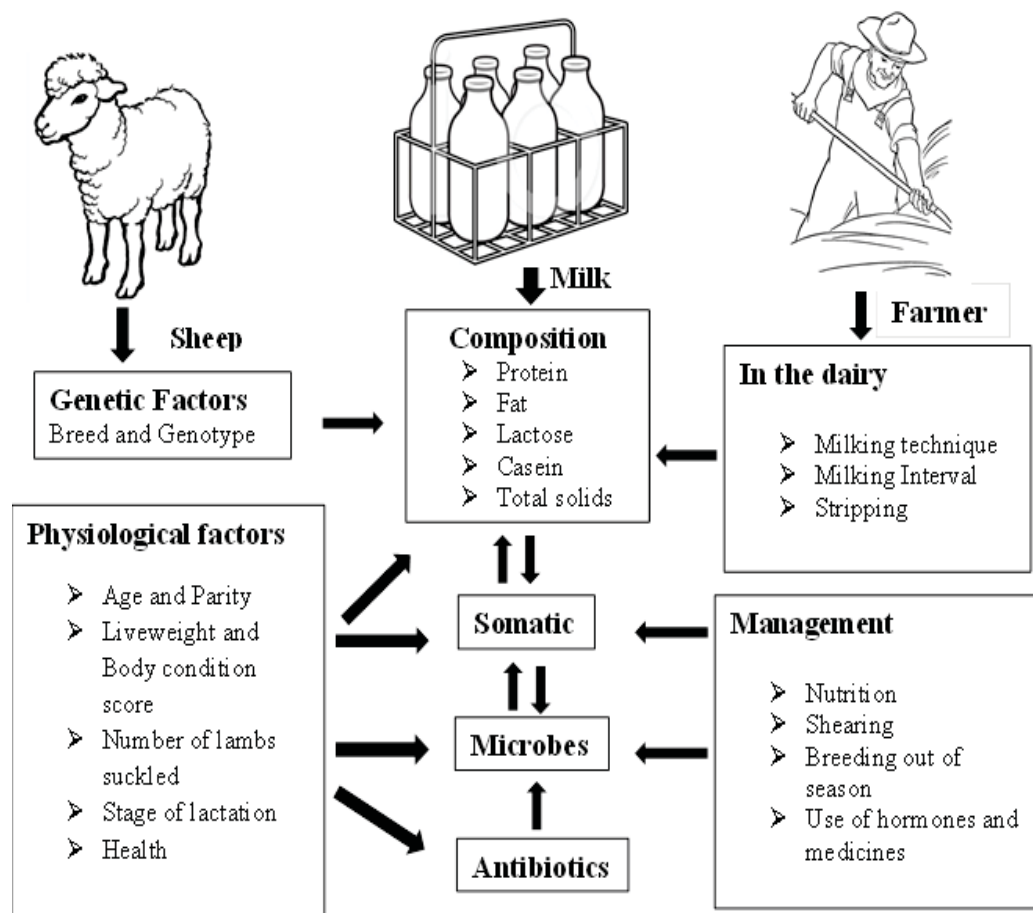


Figure 2.4 Factors affecting sheep milk composition. Farmer inputs include those controlled by the farmer to a certain extent (genetic factors) and those totally controlled by the farmer (management); others depend solely on the sheep's physiological factors. Adapted from (Peart *et al.*, 1972; Bencini and Pulina, 1997; Paten *et al.*, 2013).

### *Milk lactose*

The lactose concentration in ewes' milk shows a gradual decline from early to late lactation (Morgan *et al.*, 2006; Hunter *et al.*, 2015). In goats, a high concentration of lactose in milk is indicative of high mammary gland metabolic activity (Nielsen *et al.*, 2001) and ultimately high milk yield. This is because lactose synthesis and secretion are responsible for the majority of water in milk (Shennan and Peaker, 2000). It is, therefore, unsurprising that lactose content decreases with progressing lactation as mammary gland metabolic activity decreases. Subjecting ewes to different energy intake during pregnancy can increase the milk lactose concentration of their offspring (van der Linden *et al.*, 2009; Blair *et al.*, 2010).

In summary, several factors can affect the various components of ewes' milk (Fig 2.2). However, adoption of the appropriate management procedures in combination with healthy ewes is essential in obtaining good quality milk (Bencini and Pulina, 1997). There is general consistency in the levels of the major milk components between various breeds (Table 2.1) and the number of suckling lambs (Peart *et al.*, 1972; 1975b; Snowden and Glimp, 1991) and this has led to the conclusion that milk composition and the ability to alter composition may be of little concern in commercial lamb production systems (Morgan *et al.*, 2006). However, before such a conclusion can be made, knowledge of how each affects lamb growth is needed.

#### **2.3.4 Ewe milk yield and lamb growth**

Low and sometimes unclear relationships between milk intake and growth have been reported during early and late lactation (Geenty and Jagusch, 1974; Geenty, 1979; Geenty and Dyson, 1986; Muir *et al.*, 1998; Muir *et al.*, 2000b; van der Linden *et al.*,

2010c). For example, Geenty and Dyson (1986) reported coefficient of determination ( $r^2$ ) values of 0.25 and 0.20 between lamb LWG and ewe milk yield during the first six and twelve weeks of lactation respectively in ewes suckling twins. Similarly, ewe milk yield at best explained only 26% of the variation in singleton lamb growth rate on a weekly basis during the first seven weeks of lactation (van der Linden *et al.*, 2010c).

These findings suggest that either the singleton lambs are unable to consume all the milk produced by their dams (Robinson *et al.*, 1968; Snowden and Glimp, 1991; Muir *et al.*, 1998) or the oxytocin technique used for measuring milk yield in the studies of Muir *et al.* (1998) and Hunter *et al.* (2015) measured potential ewe milk volume but not actual lamb milk intake and, therefore, overestimated lamb milk intake (which will be discussed in a later section) (Doney *et al.*, 1979; Geenty and Sykes, 1986; Muir *et al.*, 2000b). Based on these findings, it is not too surprising that lamb growth rates, particularly in non-diary breeds, were poorly predicted by milk yield during the first six to seven weeks of lactation (Geenty and Jagusch, 1974; Geenty, 1979; Geenty and Dyson, 1986; van der Linden *et al.*, 2010c). Additionally, the poor relationship between milk yield and lamb growth rate indicates that solid feed intake by lambs may be an important contributing factor affecting pre-weaning lamb weight gain (Geenty and Dyson, 1986; Hatfield *et al.*, 1995).

### **2.3.5 Ewe milk composition and lamb growth**

Geenty (1979) found that milk fat yield had no noticeable effect on lamb growth rate whereas lactose, protein, and solid-non-fat percentages positively affected lamb growth. The correlation between lamb live weight and the respective protein and lactose yields were  $r = 0.57$  and  $r = 0.52$  over 12 weeks of lactation (Geenty, 1979). Additionally, although ewe milk yield explained about 73% and 71% of the variation in the growth of

single and twin-reared lambs respectively, there was no relationship between lamb growth and either milk protein or fat percentage for singles or twins lambs (Torres-Hernandez and Hohenboken, 1980). However, the growth of single-reared lambs was best predicted by the model containing both milk yield and milk protein percentage ( $R^2 = 0.86$ ) with only a slightly improvement in a model containing both milk yield and fat percentage ( $R^2 = 0.74$ ). While, inclusion of any of protein or fat percentage did not improve the accuracy in predicting growth in twins ( $R^2 = 0.71$ ) (Torres-Hernandez and Hohenboken, 1980).

The lack of difference in the protein and fat yields of five different ewe breeds suckling single or twin lambs (Slen *et al.*, 1963) and a negative correlation between lamb weight and milk fat and protein percentages (Walkom *et al.*, 2016), have led to the conclusion that neither of the two components had a major influence on lamb weight gain and thus it may be more advantageous to increase ewe milk yield rather than milk quality when aiming to improve lamb growth during lactation.

### **2.3.6 Solid feed intake and lamb growth**

Twin lambs consume more pasture at an early age than singles due to lower milk availability (Geenty and Dyson, 1986). Therefore, at 6 weeks of age about 50% of twin lambs' energy intake come from pasture in contrast to approximately 22% for singles (Fig. 2.5) (Geenty *et al.*, 1985; Geenty, 2010). Further, lambs weaned at 3 weeks of age consume at least twice as much pasture as their suckling counterparts (Langlands and Donald, 1975; Williams *et al.*, 1976). However, this greater pasture intake did not compensate for their lack of milk intake (Williams *et al.*, 1976; Gibb *et al.*, 1981). Typically, about 4.7 kg of pasture is required to obtain the same amount of metabolisable energy that will be provided by consuming a litre of milk in order to

maintain the same total net energy gain (Penning and Gibb, 1979). Nevertheless, the quantity of pasture required will depend on the digestibility of the pasture, the age of the lamb and the stage of rumen development. Although pasture is not as nutrient-dense as ewe's milk when it is excessively substituted for milk intake later in lactation, lamb growth rate can be adversely affected due to the reductions in digestible energy and nitrogen (N) intakes (Joyce and Rattray, 1970). Conversely, lambs that receive less milk can have improved growth rates from 4 weeks to weaning, providing they have access to high-quality solid feed (Geenty *et al.*, 1985; Morgan *et al.*, 2007).

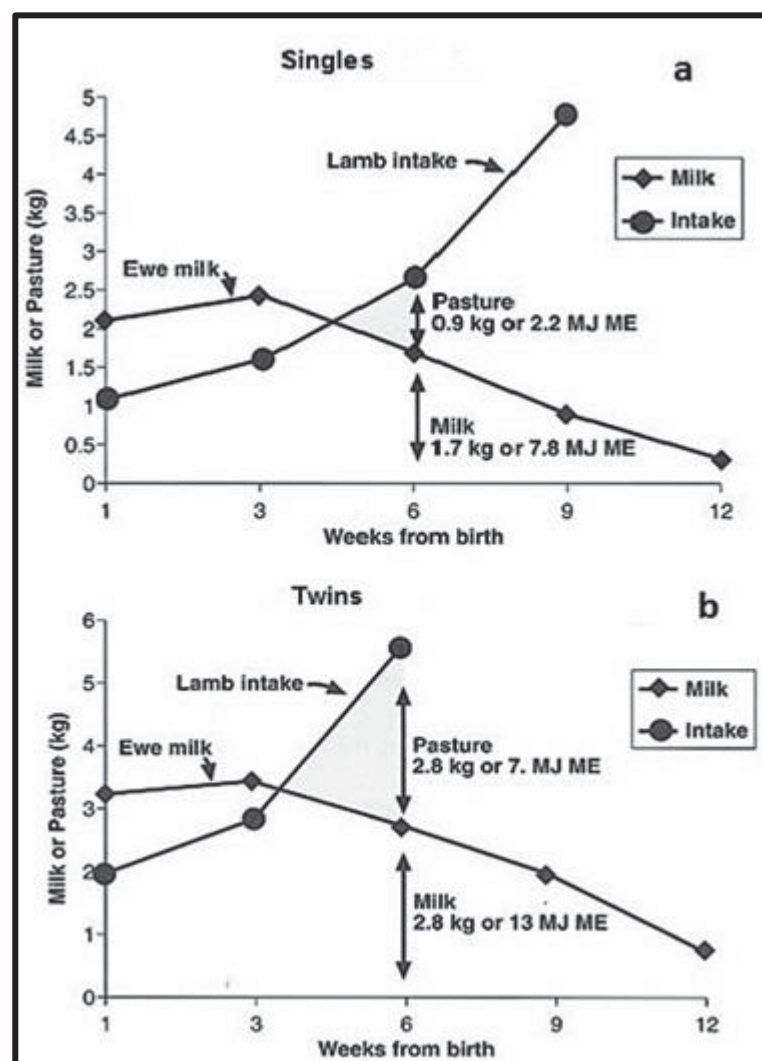


Figure. 2.5 Transition showing decreasing ewe milk and increasing pasture intake by (a) single and (b) twin lambs during the first 12 weeks of lactation. Source: (Geenty *et al.*, 1985; Geenty, 2010).

In summary, lamb growth is dependent on both ewe milk yield and composition in the first few weeks of life. However, under the circumstances in which it is difficult to predict lamb growth especially when ewe milk yield or composition does not reflect lamb LWG, a limitation in knowledge of lamb milk intake and how it contributes to growth is highlighted. Solid feed also plays an important role in the growth of lambs in early life. Therefore, ensuring a smooth transition from liquid to solid feeding may allow lambs to consume and digest sufficient solid feed in later lactation to support growth.

## **2.4 Energy requirements and utilisation in lambs**

### **2.4.1 Energy balance**

Not all the energy consumed from the diet of lambs is available for use. During digestion, energy is lost by means of excretion in various forms (solid, liquid and gaseous) (Fig. 2.6). The digestible energy (DE) is the gross energy (GE) intake less faecal energy excreted. Faecal energy loss is the most important and variable loss of energy, thus, DE is a better measure of energy available to support production than GE (McDonald *et al.*, 2011). Urinary and gaseous (methane) losses are taken into account when the metabolisable energy (ME) is being calculated. Therefore, ME represents the energy available for use by lambs.

Urinary energy excreted contains both nitrogenous and non-nitrogenous compounds whilst methane (CH<sub>4</sub>) results from microbial fermentation of feeds in the rumen and large intestines (Johnson and Johnson, 1995; Lassey *et al.*, 1997; Haque *et al.*, 2014).

Methane emissions are closely related to the level of feed intake and diet type (Johnson and Johnson, 1995). At maintenance level of feeding, about 5% to 10% of GE or 11 to 13% of DE is lost as methane in the ruminant lamb (Jentsch *et al.*, 1969; Kempton and Leng, 1979; Lassey *et al.*, 1997; Pelchen and Peters, 1998). Thus, in digestibility studies where CH<sub>4</sub> cannot be measured directly, it can be estimated as 8% of GE intake (McDonald *et al.*, 2011). Alternatively, ME can be estimated as  $0.81 \times \text{DE}$  based on the assumption that energy losses from urine and methane make up 19% of DE (McDonald *et al.*, 2011).

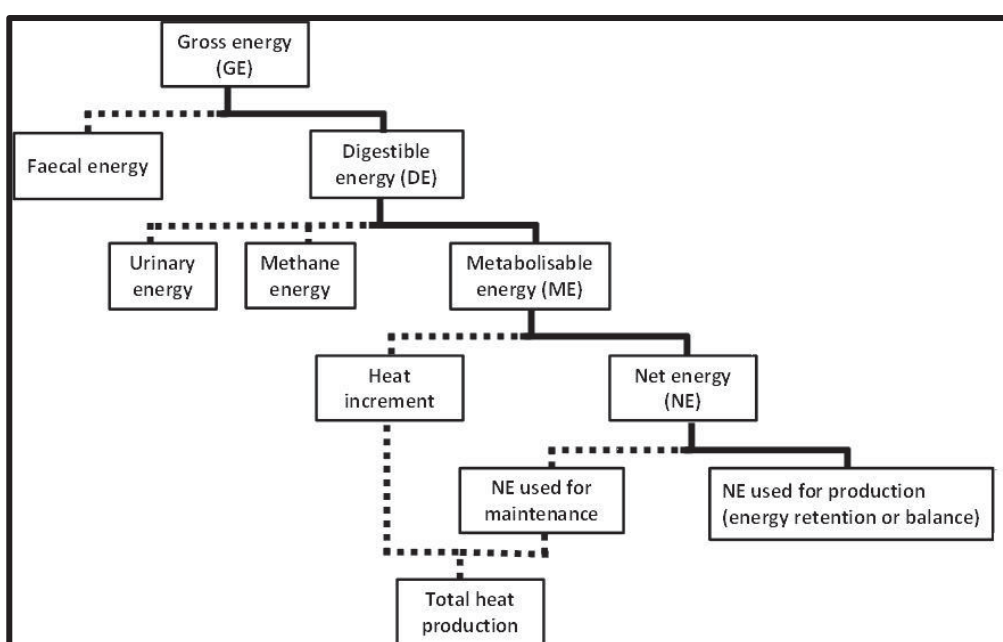


Figure 2.6 The partition of gross energy in lambs. Digestible energy (DE) is gross energy intake (GE) less faecal losses; metabolisable energy (ME) is DE less urinary and methane losses; net energy (NE) is ME less heat increment of feeding. NE can be further subdivided into net energy for maintenance and production. Adapted from (Birkett and de Lange, 2001; McDonald *et al.*, 2011).

#### **2.4.2 ME requirement for maintenance**

Maintenance energy is defined as the ME intake per day when the animal is in zero energy balance (zero energy retention and zero energy loss) (Dawson and Steen, 1998). The energy required for maintenance generally depends on the size, age and sex of the animal, the physical work it has to do and the quality of feed given to the animal (Sykes and Nicol, 1983; Nicol and Brookes, 2007). Metabolisable energy for maintenance ( $ME_m$ ) is calculated as a function of metabolic live weight ( $LW^{0.75}$ ) since the increase in  $ME_m$  requirement is not directly proportional to LW (Walker and Faichney, 1964a; Sykes and Nicol, 1983).

Maintenance energy requirements of lambs vary with the age of lambs and the type of diet (CSIRO, 2007; NRC, 2007). Estimation of  $ME_m$  requirements in lambs have either been from very young lambs (up to 3 weeks of age) fed milk diets only (Jagusich and Mitchell, 1971; Walker and Norton, 1971b; Chiou and Jordan, 1973) or early weaned lambs consuming solid feed only (Mitchell and Jagusch, 1972; Thomson *et al.*, 1979; Alam *et al.*, 1991). ARC (1980) recommends a value of 0.40 MJ ME/kg  $LW^{0.75}$  per day for maintenance for lambs subsisting on liquid diets. Similarly, Sanz Sampelayo *et al.* (1995) reported a  $ME_m$  value of 0.39 MJ ME/kg  $LW^{0.75}$  per day for 2-month old lambs also fed a milk-only diet. While higher  $ME_m$  values have been reported in housed lambs consuming milk only (0.50 to 0.61 MJ / kg  $LW^{0.75}$ ) (Jagusich and Mitchell, 1971; Chiou and Jordan, 1973; Degen and Young, 1982) and for early weaned lambs (4 to 7 weeks of age) consuming pasture (0.70 and 0.80 MJ / kg  $LW^{0.75}$ ) (Fennessy *et al.*, 1972; Mitchell and Jagusch, 1972). Generally, it is expected that lambs reared indoors have a lower maintenance requirement than grazing lambs (Sykes and Nicol, 1983) due to minimal use of energy for activity (Luo *et al.*, 2004). Thus, allowances must be made

for movement and feed prehension under field conditions although they are increased by only 10% (Sykes and Nicol, 1983).

While differences in maintenance requirement have been attributed to differences in body composition or proportions of water, protein and fat in LWG (Gareett, 1971; Webster, 1979; Webster, 1993), there has been substantial evidence which suggests that energy expenditures of visceral organs constitute a major proportion of total animal energy expenditures (Smith, 1970; Smith and Baldwin, 1974; Ferrell *et al.*, 1976; Canas *et al.*, 1982). While, the liver and GIT constitute approximately only 10% of the total body weight, these tissues combined account for approximately 40 to 50% of the total energy expenditure (Ferrell, 1988). It can be, therefore, hypothesised that diets which increase the weights of visceral organs in lambs may increase their maintenance energy requirements. However, more research is needed to elucidate this.

In summary, the pre-mentioned studies indicate the significant variability in maintenance energy requirements in young lambs. Nevertheless, these estimations have typically been limited to young lambs subsisting exclusively on milk or solid feed with little work undertaken in New Zealand in the last 30 years. Therefore, more research is needed to evaluate the maintenance energy requirements of lambs consuming both liquid and solid diets concurrently.

### **2.4.3 ME requirement for growth**

Metabolisable energy requirement for growth (ME<sub>g</sub>) is primarily determined by the proportion of fat and protein contributing to weight gain (SCA, 1990; NRC, 2007; Deng *et al.*, 2012). ME<sub>g</sub> is higher in growing animals that gain more fat than those that gain more protein. The efficiency of *in vivo* net synthesis of fat is higher than that of protein

(Luo *et al.*, 2004; Nicol and Brookes, 2007; NRC, 2007). Protein deposition has a significant influence on LWG due to the associated water deposited with protein as lean tissue (Roy, 1980). Lean tissue consist primarily of protein (20%) and water (75%) (Babiker *et al.*, 1990). The relationship between water and protein results in less feed energy being required to deposit 1 g of lean tissue than 1 g of adipose tissue (van Milgen and Noblet, 2003). Thus, ME<sub>g</sub> per kg LWG is much smaller in animals growing more lean than fat tissues.

Pre-weaned lambs generally have a greater proportion of protein than fat in their weight gain (Walker and Norton, 1971b; Searle *et al.*, 1972) and therefore, their ME<sub>g</sub> tends to be lower than in growing or mature sheep. For example, for pre-weaned milk-fed male lambs of 15 – 20 kg LW growing at 200 g/day, ARC (1980) recommends an ME intake of 6.4 – 7.8 MJ / kg LWG whereas recommendations of 25 MJ ME per kg LWG have been reported for 25 kg weaned rams (Geenty and Rattray, 1987; Nicol and Brookes, 2007).

In summary, there is little data on the growth energy requirements of pre-weaned lambs, especially in lambs below 20 kg fed either milk only or milk and solid feed concurrently. Furthermore, as LWG is related to protein or fat deposition, it would be worthwhile and more accurate to estimate ME<sub>g</sub> of pre-weaned lambs using their protein and fat depositions in gain. Therefore, more research to determine ME<sub>g</sub> is warranted.

#### **2.4.4 Efficiency of ME utilisation (k)**

The most important measurements when evaluating a diet for energetic use are the ME value and the efficiency of ME utilisation (k) (McDonald *et al.*, 2011). The k value is calculated as the net energy (NE) output divided by ME intake. According to the ARC

(1980) ME system, ME intake above maintenance is used with constant efficiency in promoting energy deposition. Therefore, the efficiency of utilisation of ME for maintenance ( $k_m$ ) is measured between fasting and the point at which there is zero energy retention (ARC, 1980). Graphically, it is the slope of the regression line relating energy retention to ME intake (Jagusch and Mitchell, 1971; Fennessy *et al.*, 1972; Mitchell and Jagusch, 1972; Dawson and Steen, 1998). The point of interception of the equation on the x axis gives an estimate value of the  $ME_m$  (Fig. 2.7) (Fennessy *et al.*, 1972; Dawson and Steen, 1998).

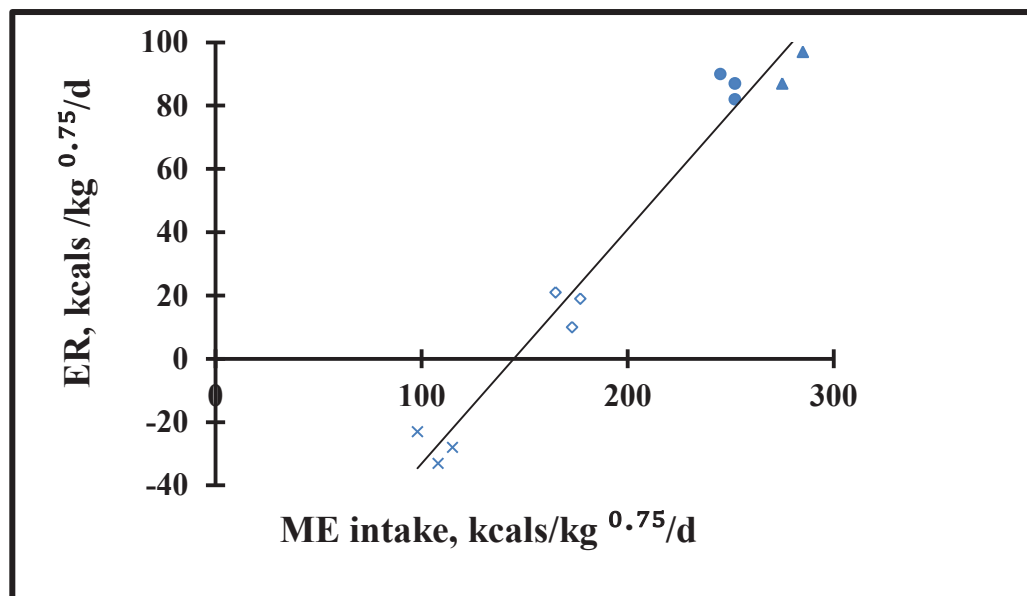


Figure 2.7 The efficiency of metabolisable energy (ME) utilisation for maintenance in milk-fed lambs calculated as the slope of the regression line relating energy retention (ER) to ME intake. Adapted from Jagusch and Mitchell (1971).  $ER = 0.77 ME - 111.75$

#### 2.4.5 Efficiency of milk ME utilisation

Young lambs subsisting on a milk only diet generally have higher efficiencies than those ruminating (ARC, 1980). The efficiency with which ME from milk is utilised for

either maintenance or gain varies with experimental conditions. Studies with milk-fed lambs suggest that ME utilisation for maintenance and growth usually depends on the type of milk fed to lambs; typically a range of values from 68.6 to 77% have been obtained (Table 2.2). The higher fat content in ewe's than cow's milk may account for the higher efficiency of ewe milk utilisation by lambs (Jagusich and Mitchell, 1971).

Table 2.2 Efficiency of utilisation of metabolisable energy for growth in milk-fed lambs

| Type of milk | Efficiency ( $k_g$ ), % | Reference                    |
|--------------|-------------------------|------------------------------|
| Cow's milk   | 71.0                    | Walker and Jagusch (1969)    |
| Ewe's milk   | 76.9                    | Jagusich and Mitchell (1971) |
| Cow's milk   | 68.6                    | Walker and Norton (1971b)    |
| Cow's milk   | 71.0                    | Penning <i>et al.</i> (1977) |
| Ewe's milk   | 70.7                    | Degen and Young (1982)       |

#### **2.4.6 Efficiency of solid food ME utilisation**

Solid feed intake of suckling lambs in the first 21 days is considered to be negligible (less than 30 g/day) (Owen *et al.*, 1969; Walker and Hunt, 1981; Lane *et al.*, 1986). Therefore, few studies (Gardner *et al.*, 1964; Fennessy *et al.*, 1972) have investigated the efficiency of converting ME from non-milk sources to gain in young suckling lambs. The efficiency of utilisation of ME for growth ( $k_g$ ) by older lambs (5 to 14 months of age) is usually above 40% (Ratnayake and Joyce, 1974; Degen and Young, 1982), while, values above 50% have been reported in lambs given high-quality diets (Graham and Searle, 1972; Joyce *et al.*, 1972; Ratnayake and Joyce, 1974). For young lambs (3 to 8 weeks of age) fed different types of solid feed, values of 13.2% to 29% (Fennessy *et al.*, 1972; Mitchell and Jagusch, 1972; Thomson and Cammell, 1979) have

been obtained. Processes that change the physical form of pasture (such as grinding, cubing and pelleting) greatly improve the efficiency of ME utilisation in young lambs (Thomson and Cammell, 1979). Young lambs (about 14 weeks old) fed ground and pelleted lucerne metabolised about 53% of their energy intake as compared to 28% in non-processed lucerne (Thomson and Cammell, 1979)

The lower  $k_g$  value obtained for young lambs in comparison to older lambs could indicate that either a greater proportion of the total energy from solid feed is used in maintenance at the expense of gain (Penning *et al.*, 1977), or more protein is being deposited at the expense of fat (Ratnayake and Joyce, 1974). The latter was indicated by Ratnayake *et al.* (1974) as a process that is less efficient in ruminants. Additionally, the greater efficiency of solid food utilisation in older lambs in comparison to young lambs is partly due to the digestibility of the feed used (Thomson, 1972; Ratnayake and Joyce, 1974), and partly due to the production of volatile fatty acid (VFA) in the rumen which stimulates rumen papillae development from where the end products of digestion are absorbed (Tamate *et al.*, 1962; van Houtert, 1993).

Solid feed intake is the basis for rumen development in milk-fed ruminants (Wardrop and Coombe, 1961; Tamate *et al.*, 1962). Most early weaned lambs are abruptly weaned onto solid feed. However, this process does not offer these lambs adequate time to adapt their metabolism to nutrients produced by digestion in the rumen (Fennessy *et al.*, 1972). By administering intraruminal VFA, Tamate *et al.* (1962) observed extensive papillary development in the rumen of 3-day old calves. This suggests that if lambs were introduced to solid feed diets very early in life, their rumen development could be advanced and their capability to utilise solid feed more efficiently later in life may be improved. However, this is not known.

#### **2.4.7 Estimating ME requirements and efficiency of ME utilisation**

The ME intake is generally partitioned into energy retained (ER) and energy associated with maintenance ( $ME_m$ ):

$$ME \text{ intake} = ME_m + \left( \frac{1}{k_g} \right) ER$$

Where  $k_g$  = efficiency of utilisation of ME for growth of body energy

A simple model partitions energy into production, e.g. growth ( $k_g$ ), and maintenance purposes (Birkett and de Lange, 2001). However, in order to model the use of energy intake more accurately, ER is further partitioned into fat (Fd) and protein (Pd) deposition (Kielanowski, 1966; 1976). Multiple linear regression can be used to derive the different efficiencies that relate ER as fat ( $k_f$ ) and protein ( $k_p$ ) (Kielanowski, 1966). Based on the assumption that the only determining factors of energy balance are the energy cost in maintenance, fat and protein deposition (Kielanowski, 1966; Ørskov and McDonald, 1970), ME intake can be estimated as:

$$ME \text{ intake} = ME_m + \left( \frac{1}{k_f} \right) Fd + \left( \frac{1}{k_p} \right) Pd$$

or

$$ME \text{ intake} = a + \beta_1 LW^{0.75} + \beta_2 Pd + \beta_3 Fd$$

Where  $LW^{0.75}$  is metabolic live weight and the regression coefficients  $\beta_1$ ,  $\beta_2$  and  $\beta_3$  represent the ME required for maintenance, and ME required to deposit 1 g of protein and 1g of fat respectively (Ørskov and McDonald, 1970).

## **2.5 Protein requirements and utilisation in lambs**

Protein is usually the most expensive component of the lamb's diet. Therefore, when feeding pre-weaned lambs, accurate estimations of their protein requirements is necessary to ensure the right balance between growth and prevention of excess N excretion. For the remainder of this review, protein and nitrogen (N) will be used interchangeably.

### **2.5.1 Nitrogen balance**

Nitrogen balance is the difference between N intake less faecal and urinary N excretions (Owens and Zinn, 1988). Endogenous faecal N losses are often related to dry matter intake. The loss of faecal nitrogen of milk-fed lambs has been shown to be between 0.1 to 0.14 g N/100 g dry matter ingested or 2.61 to 4.46 g N/100 g dry matter excreted (Walker and Faichney, 1964a; b) while for lambs fed pellets in addition to milk, it was 0.22 g/100 g of dry matter ingested (Hodge, 1965). The higher figure for the lambs fed milk and pellets may reflect an increase in metabolic faecal nitrogen that usually comes about as a result of the increase in live weight rather than a reduction in digestibility of protein (Hodge, 1965). The majority of N intake is excreted in the urine. While only approximately 10% of the ingested N is lost as faecal N, approximately 40% to 50% of the digested N is lost through urine N excretions in young lambs consuming milk only diets (Walker and Faichney, 1964a; b). Older lambs of about 30 kg to 35 kg LW consuming solid feed lose 30% of their total N intake in faecal excretion and up to 70% of the digested N is excreted as urine (Galvani *et al.*, 2009; Nie *et al.*, 2015).

Generally, increased N intake is associated with increased losses in faecal and urinary N, although relatively higher losses in the urine with lower N intake have also been

observed in lambs fed at sub-maintenance levels (Walker and Faichney, 1964a; Jagusch and Mitchell, 1971). However, urinary N losses are greater for lambs consuming solid feed than those consuming liquid diets. Consequently, the inclusions of solid feed to pre-ruminant diets either when reared by the dam or artificially have been associated with reduced efficiency of N retention due to higher faecal and urinary N losses (Santra and Karim, 1999; Labussiere *et al.*, 2009). Nevertheless, studies in veal calves have shown that the provision of low-protein (88g /kg DM) solid feed results in a high marginal efficiency of N utilization, which was explained by an increased supply of digestible N, urea recycling and increased post-absorptive N utilization for growth (Berends *et al.*, 2012; Berends *et al.*, 2014). Therefore, the low N efficiency obtained by Santra and Karim (1999) and Labussiere *et al.* (2009) could be related to the CP content of the solid feed offered (16% to 27%). The milk replacer offered to veal calves contained 21.2% CP (Berends *et al.*, 2012; Berends *et al.*, 2014). However, satisfactory growth rates have been achieved with milk replacers containing 23% to 26 % CP content in young lambs and calves (Chiou and Jordan, 1973; Blome *et al.*, 2003). It is uncertain if low-protein solid feed would stimulate N recycling if high-protein milk replacers were fed; this warrants further studies.

### **2.5.2 Protein requirement of the young lamb**

In older lambs, approximately 60% to 80% of the protein ingested in a feed as N is utilised by the microorganisms in the rumen during fermentation resulting in the production of microbial crude protein (MCP) (Nolan and Dobos, 2005). The remaining 20% - 40% escapes the fermentation process and is absorbed as amino acids (AA) through the small intestines and is referred to as undegradable protein (UDP) (Nolan and Dobos, 2005). Both MCP and UDP contribute to the lamb's metabolisable protein

(MP) supply. In the young lamb, protein requirements are usually estimated in terms of digestible protein intake and the efficiency of use of the apparent digestible protein leaving the stomach (ADPLS) due to the absence of significant microbial activity in the rumen (ARC, 1980; SCA, 1990; CSIRO, 2007).

The ADPLS requirements of lambs according to the ARC (1980) is calculated as the sum of the protein retention, protein retained in wool and the endogenous urinary N as protein divided by 0.80 whereas crude protein (CP) requirements are the ADP requirements divided by 0.92 (Eq. 2.1).

$$\text{CP requirement (g/day)} = \frac{6.25 \times (R_N + \text{Wool}_N + U_{N(E)})}{d_N \times k_{N(U)}} \quad (2.1)$$

Where:  $R_N$  = N retention in the fleece-free LW gain (g /day);

$U_{N(E)}$  = endogenous urinary N (g /day);

$d_N$  = apparent digestibility of N (0.92 for milk protein) and

$k_{N(U)}$  = efficiency of utilisation of absorbed N (0.80 for milk protein).

Protein requirements are generally estimated by measuring the response in N retention to changes in protein intake (Black *et al.*, 1973; Black and Griffiths, 1975) . Protein requirements vary with LW, breed, stage of maturity, diet type, environmental conditions etc and therefore, the protein requirements of lambs of similar age or live weight may not be a constant, but will be represented by a range of values (Walker and Jagusch, 1969). Protein requirements for growing lambs vary from 100 g/ kg DM intake to 285 g/ kg DM intake for milk-fed lambs and from 100 to approximately 170g/ kg DM intake for lambs greater than 15 kg LW (Table 2.3).

Table 2.3 Estimates of protein requirements obtained from feeding trials of growing lambs

| Live weight at start of study (kg) | Study period (weeks) | Growth phase | Diet type                     | Estimated Crude Protein requirement (g/ kg DM) | Reference                   |
|------------------------------------|----------------------|--------------|-------------------------------|------------------------------------------------|-----------------------------|
| 3 – 8                              | 3                    | Suckling     | Spray-dried cow's milk        | 285                                            | Walker and Norton (1971a)   |
| 4                                  | 8.6                  | Suckling     | Ewe's milk and Concentrate    | 180 <sup>†</sup>                               | Santra and Karim (1999)     |
| 4 – 5                              | 4.3                  | Suckling     | Dried skim milk               | 240 – 260                                      | Chiou and Jordan (1973)     |
| 5                                  | 2 - 13               | Suckling     | Ewe's milk and Concentrate    | 143 <sup>†</sup>                               | Karim <i>et al.</i> (2001b) |
| 5                                  | -                    | Suckling     | Milk protein only             | 200 <sup>‡</sup>                               | ARC (1980)                  |
| 8                                  | 8                    | Suckling     | Spray-dried cow's milk        | 285                                            | Walker and Faichney (1964a) |
| 10                                 | -                    | Suckling     | Milk protein only             | 124 <sup>‡</sup>                               | ARC (1980)                  |
| 12                                 | 8                    | Weaned       | Pelleted concentrate ration   | 135 – 140                                      | Jordan and Hanke (1970)     |
| 15                                 | 5*                   | Weaned       | Barley and white fish meal    | 110 – 190                                      | Ørskov <i>et al.</i> (1971) |
| 16                                 | 8                    | Weaned       | Pelleted concentrate ration   | 135 – 140                                      | Ranhotra and Jordan (1966)  |
| 18 – 21                            | 5 – 9                | Weaned       | Lucerne                       | 265                                            | Mitchell and Jagusch (1972) |
| 20                                 | 4 – 5*               | Weaned       | Barley, soya bean and cassava | 175                                            | Andrews and Ørskov (1970a)  |
| 20                                 | -                    | Suckling     | Milk protein only             | 72 <sup>‡</sup>                                | ARC (1980)                  |
| 25                                 | 4 – 5*               | Weaned       | Barley, soya bean and cassava | 150                                            | Andrews and Ørskov (1970a)  |
| 30 – 35                            | 4 – 5*               | Weaned       | Barley, soya bean and cassava | 125                                            | Andrews and Ørskov (1970a)  |

<sup>†</sup>Crude protein requirement from concentrate diet only

\*Age of lamb at the start of the experiment

<sup>‡</sup>Requirements based on lambs growing at 200g /day

Table 2.3 indicates that the protein requirements of lambs are usually estimated based on either milk or solid feed intake, with few studies (Santra and Karim, 1999; Karim *et al.*, 2001a) examining lambs fed both milk and solid feed and, therefore, further work may be required. Young lambs usually begin to consume solid feed at 2 to 3 weeks after birth at which time they begin to acquire the typical rumen microbes (Fonty *et al.*, 1987). However, relatively little is known about dietary protein utilisation during the transitional period of lamb growth. Protein obtained from milk and solid feed may affect N utilisation, maintenance and growth requirements differently, as well as chemical body composition of body weight gain.

### **2.5.3 Protein and energy interrelationship**

The protein and energy dependent phases of growth describe the relationship between energy and protein supply on protein deposition (Chowdhury and Ørskov, 1997; Titgemeyer, 2003; Schroeder and Titgemeyer, 2008). For monogastric animals in the protein-dependent phase, protein supply is most limiting to growth (Campbell and Dunkin, 1983; Chiba *et al.*, 1991). As shown in Figure 2.8, increasing protein intake from level A to B, when energy is not limiting (high-energy intake), increases protein deposition linearly (D to E) until energy limits the response (Fig. 2.8). Similarly, when protein intake is adequate or in excess of the animal's requirement (A), any further increase in protein intake (B) no longer affects deposition (C). However, an increase in energy intake will linearly increase protein deposition (energy-dependent phase) to the point where the protein supply again becomes limiting (D) (Fig.2.8).

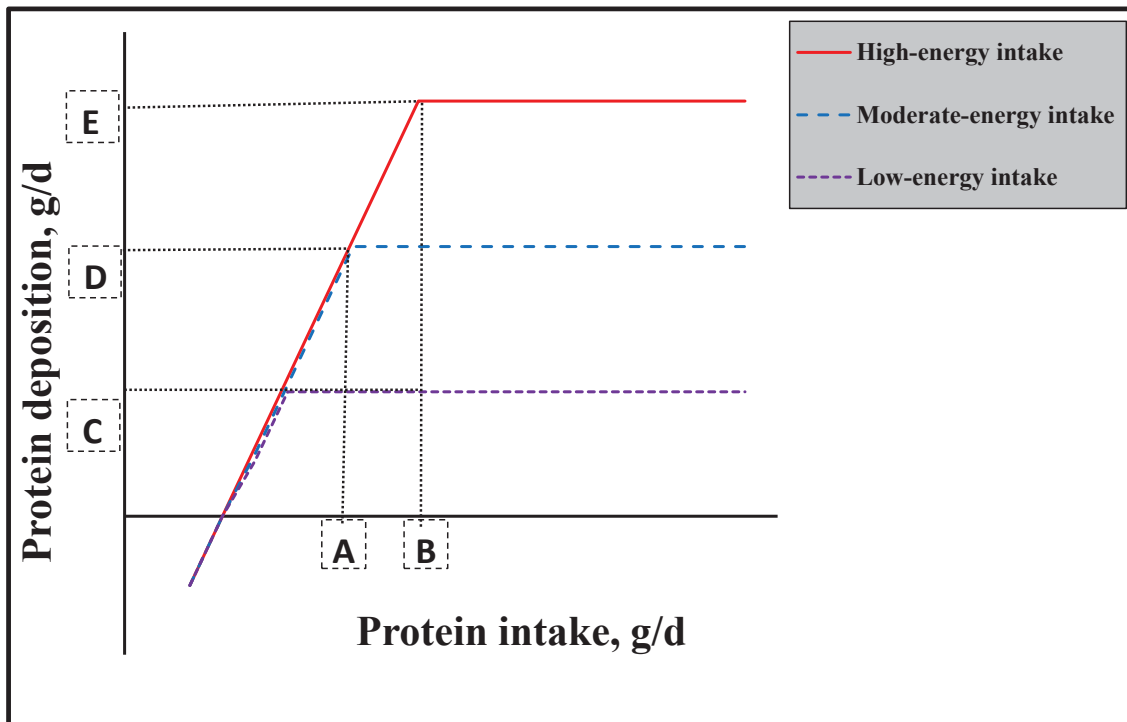


Figure 2.8 Protein- and energy-dependent phases of growth. Protein deposition of animals on the inclined lines would be limited by protein intake whereas that of animals on the plateau surfaces would be limited by energy intake. (Campbell *et al.*, 1985; Titgemeyer, 2003; Schroeder and Titgemeyer, 2008) (Adapted).

Two assumptions are implied based on the relationship between energy and protein supply (Schroeder and Titgemeyer, 2008):

- 1) Specific AAs are required at certain energy levels. Therefore, protein requirements are related to energy supply. The linear relationship between protein deposition rate and energy intake may enable protein requirements to be expressed relative to energy intake.
- 2) When protein is limiting, increases in energy intake will not affect protein deposition. Therefore, energy intake does not affect the efficiency of protein or AA utilisation.

In ruminants, however, the study of protein and energy interrelationship is quite complex due to ruminal metabolism. An increase in dietary energy typically increases microbial protein synthesis in the rumen, which in turn, increases the protein supply to

the animal (Titgemeyer, 2003) provided there is enough dietary N. Some studies have indicated the existence of the protein- and energy-dependent phases of growth in growing or of intragastrically maintained ruminants (Andrews and Ørskov, 1970b; Black and Griffiths, 1975; Lindberg and Jacobsson, 1990). However, other studies were unable either to demonstrate the two separate phases of growth or observe a response to one of the phases. For example, Ørskov *et al.* (1999) observed that when protein intake did not exceed an animal's requirement, the energy-dependent phase was not demonstrated. Similarly, studies with pre-ruminant calves and growing steers showed that protein deposition increased linearly with energy intake although protein was limiting (Donnelly and Hutton, 1976; Hovell *et al.*, 1983; Gerrits *et al.*, 1996). The protein-and energy interdependence does not always apply to ruminant animals. Therefore, the assumption that the efficiency of protein utilisation is unaffected by energy intake may not be entirely true for lambs and likely warrants further investigation.

## **2.6 Estimation of lamb milk and solid feed intake**

### **2.6.1 *Measuring lamb milk intake***

Several techniques, namely lamb weighing, hand or machine milking following oxytocin injection, suckling behaviour, and isotope dilution, have been utilised to measure or estimate milk intake (Robinson *et al.*, 1968; Macfarlane *et al.*, 1969; Doney *et al.*, 1979; Cameron, 1998; van der Linden *et al.*, 2010b). Each will be briefly outlined below with their known limitations.

The lamb weighing method is based on weighing the lamb before and after a suckling event. The weight differential between suckling is recorded as lamb milk consumption

(Robinson *et al.*, 1968; Doney *et al.*, 1979). This method is labour intensive and may lead to underestimation of milk intake if the handling of animals impairs milk ejection or suckling (Dove and Freer, 1979).

The isotope dilution technique is based on the relationship between water turnover and milk intake. Milk is approximately 85% water and the oxidation of hydrogen in milk solids produces additional 10% metabolic water. Therefore, milk produces approximately 95% water after metabolism of milk by the lamb (Yates *et al.*, 1971). Using this knowledge, measurement of water turnover can be indicative of milk intake (Macfarlane *et al.*, 1969). Measuring water turnover, involves the separation of the lamb from its dam after a period of suckling to allow the suckled milk equilibrate in the lamb's stomach. A blood sample is taken from the lamb to determine the baseline body water, after which a known concentration of a tracer is injected intramuscularly. The lamb remains separated from its dam without water (for about 2 hours), to allow equilibration of the tracer. A second blood sample is taken. The animals are weighed and returned to pasture and recaptured after 7–10 day interval for a repetition of the procedure. Water turnover is estimated by the decrease in the tracer concentration in body fluids. The tracer used is generally tritiated water (TOH) (Macfarlane *et al.*, 1969; Dove and Freer, 1979) or deuterium oxide (D<sub>2</sub>O) (Pettigrew *et al.*, 1987; Auchtung *et al.*, 2002; van der Linden *et al.*, 2010b). In some instances however, the combination of TOH and D<sub>2</sub>O (double-isotope technique) have been used to measure milk intake in ruminants (Holleman *et al.*, 1975; Dove, 1988).

This technique of measuring milk intake is convenient to use, avoids excessive disturbance or handling of animals, and it eliminates errors due to variation in the degree of udder emptying (Doney *et al.*, 1979; Dove and Freer, 1979). Nevertheless, it fails to take into account non-milk sources of water when milk ceases to be the only source of water to animals (i.e. drinking or solid feed intake). It can, therefore, overestimate actual milk intake (Yates *et al.*, 1971; Dove and Freer, 1979).

The suckling behaviour method relies on the assumption that the rate of milk transfer is positively correlated with the time spent suckling (Cameron, 1998); thus the longer and more often the offspring suckled, the more milk it consumed (Fletcher, 1971). Although this method eliminates the interference of humans with the natural behaviour patterns of the dam and her offspring, its accuracy and reliability are questionable. Variations in the suckling ability of offspring; motivation for suckling including hunger and learning; the existence of non-nutritive suckling; dam's experience, physiology and ability to release milk; variation in milk composition and the methods for measuring suckling behaviour, are all confounding factors that have to be overcome in order to accurately measure milk consumption using this method (Cameron, 1998).

Several studies measuring milk production and lamb growth have used ewe's milk yield as a tool to measure lamb milk intake on the assumption that lambs consume all of the milk produced by their dam (Gibb and Treacher, 1980; Van der Linden *et al.*, 2010a; Paten *et al.*, 2013); however, this might not always be the case. Ewe milking techniques, including, hand (Coombe *et al.*, 1960; Moore, 1962; Gardner and Hogue, 1966) or machine milking (Corbett, 1968; Gibb and Treacher, 1980) following stimulated let-down by intravenous injection of oxytocin, tend to empty the udder to a greater degree than the lamb can actually achieve (Robinson *et al.*, 1968). More often than not, these

methods of measurement will, therefore, tend to overestimate lamb milk intake (Robinson *et al.*, 1968; Muir *et al.*, 2000b). Use of ewe udder dimensions to measure ewe milk production has also proven to be an inaccurate predictor of milk yield due to inconsistencies in results obtained (van der Linden *et al.*, 2010b; van der Linden *et al.*, 2011).

Comparisons of the various methods of estimating lamb milk intake have been undertaken (Coombe *et al.*, 1960; Robinson *et al.*, 1968; Doney *et al.*, 1979; van der Linden *et al.*, 2010b). It is generally accepted that these methods tend to either underestimate or overestimate lamb milk intake and thus do not give a true reflection of intake. Therefore, it would be very useful to identify alternative means to accurately measure lamb milk intake.

### **2.6.2 *Measuring lamb solid feed intake***

Solid feed intake is an important component of nutrient intake affecting both pre- and post-weaning growth performance of grazing ruminants. However, it is inherently difficult to estimate solid feed intake, under grazing conditions (Moore, 1996; Peripolli *et al.*, 2011) as there are no easy and accurate methodologies (Boval *et al.*, 2003; Penning, 2004). Attempts to measure pasture intake by ruminants have led to a variety of techniques. These include the use of external (chromium oxide, rare earths) and/or internal (lignin, indigestible fibres, chromogen, acid insoluble ash, faecal protein) markers, near infrared reflectance spectroscopy (NIRS) to estimates of faecal output, ingestive behaviour, disappearance of herbage mass, prediction from forage characteristics and animal performance (Cordova *et al.*, 1978; Dove and Mayes, 1991; Moore, 1996; Poppi, 1996; Azevedo *et al.*, 2014; David *et al.*, 2014).

The problem of incomplete recoveries, as well as variable results obtained from studies using external markers, makes it difficult to validate the external marker technique of estimating intake (Van Keulen and Young, 1977; Thonney *et al.*, 1979; Penning and Johnson, 1983). The n-alkanes technique of measuring intake was proposed to eliminate the errors associated with incomplete recoveries (Mayes *et al.*, 1986b). The n-alkanes technique involves the use of an internal and external marker concurrently; naturally-occurring alkanes, together with orally-administered synthetic alkanes allowing for the estimation of total intake and diet composition simultaneously (Dove and Mayes, 1996). Nonetheless, the method overestimates intake in comparison to other methods (Ferri *et al.*, 2008) and pasture intake estimates vary (Smit *et al.*, 2005). The n-alkane technique is by far the only method that has been used to estimate feed intake in suckling lambs (Mayes *et al.*, 1986a). However, the technique can only be used to estimate pasture intake as it is based on the use of saturated aliphatic hydrocarbons (n-alkanes) present in plant cuticular wax.

Prediction of intake from faecal composition has been used to estimate pasture intake in growing and adult sheep. This is known as the faecal index technique. The faecal crude protein (fCP) method is the most popular index technique used. This method is based on direct relationships between the amount of faecal crude protein produced and the amount of organic matter intake (Azevedo *et al.*, 2014). It relies on the assumption that the amount of crude protein in faecal excreta per unit of organic matter (OM) ingested is constant, or that faecal N excretion is directly related to the intake of a determined feed (Lancaster, 1949; Strozinski and Chandler, 1972). Pasture intake is then estimated using linear regression equations. The inclusion of additional faecal components such as faecal neutral detergent fibre (fNDF) and faecal acid detergent fibre (fADF) in the

regression models have been shown to improve prediction accuracy of intake using the fCP method (Holloway *et al.*, 1981; Peripolli *et al.*, 2011; Azevedo *et al.*, 2014; David *et al.*, 2014).

The combination of milk and pasture in the diet of pre-weaned lambs makes it difficult to estimate feed intake under grazing conditions. Nevertheless, studies using the fCP fNDF or fADF methods to estimate pasture intake have been promising in comparison with others (Smit *et al.*, 2005; Ferri *et al.*, 2008; Schneider *et al.*, 2011). Both milk and pasture contain crude protein and OM thus, it is proposed that the use of faecal index technique may be a favourable option in estimating feed intake in pre-weaned lambs and warrants investigation

## **2.7 Perspective and proposal**

Studies of ewe milk energy intake and lamb growth rate have mostly reported a poor relationship between measured milk energy intake and lamb growth. It is, therefore, of interest to gain a greater understanding of lamb milk and solid feed intake and their influence on lamb growth pre-weaning. It is also of interest to better understand the maintenance and growth energy requirements of lambs consuming both milk and solid diets concurrently. Improved accuracy of measuring milk and solid feed intake would be an advantage. Furthermore, growth in animals is a complex process and a greater understanding of the growth process will be obtained when all available information and concepts are synthesised, transformed into algorithms, and integrated into a growth simulation model.

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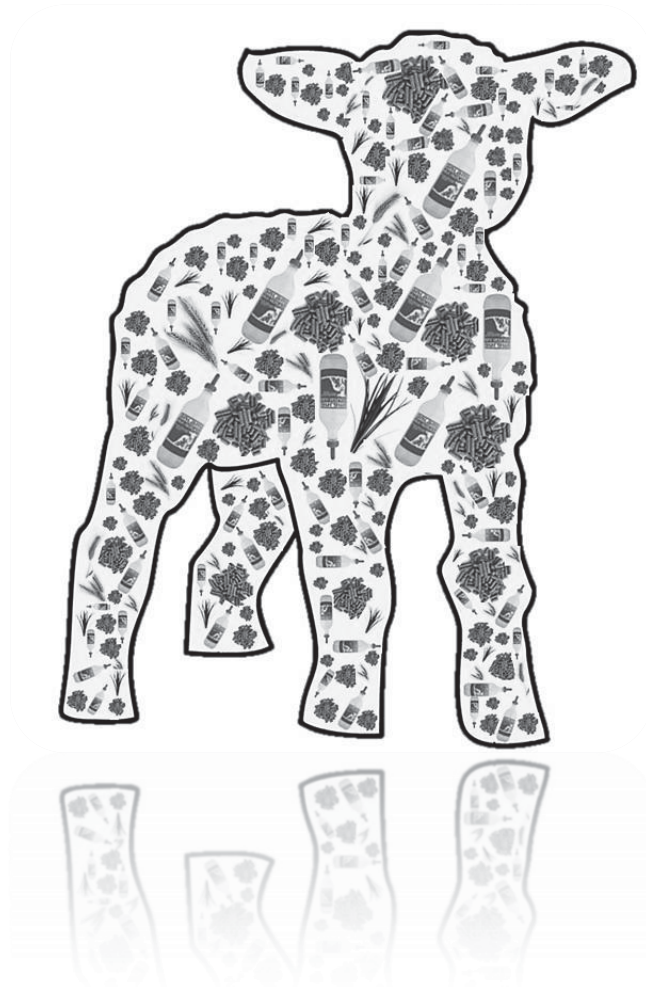
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## Chapter 3 Relationships between Prenatal Ewe Traits, Milk Production and Pre-weaning Performance of Twin Lambs

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Chapter based on the following publication:

**DANSO, A. S., MOREL, P. C. H., KENYON, P. R., and BLAIR, H. T.** 2016. Relationships between prenatal ewe traits, milk production, and preweaning performance of twin lambs. *Journal of Animal Science*, 94 (8): 3527 – 3539.

## ABSTRACT

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There is limited information on factors affecting twin lamb growth pre-weaning which limits the options available to farmers to actively manage lamb growth. Data from two multi-year experiments involving 402 twin-bearing Romney ewes were used to evaluate the effects of prenatal ewe traits (live weight at mating and set stocking, and body condition score at mating and set stocking) and combined twin lamb birth weight on ewe milk production and lamb growth from birth to weaning as well as the proportion of variation in twin lamb growth that could be explained by these variables. Additionally, the effect of accumulated ewe milk yield (MY, days 0 to 42) and accumulated milk components (protein, fat, lactose) on twin lamb growth were investigated. The effects of prenatal variables on MY, birth weight and combined twin lamb live weight gain from day 0 to 42 ( $LWG_{0-42}$ ) were inconsistent across the two experiments. In addition, prenatal ewe traits ( $P < 0.05$ ) explained less than 30% of the variation in MY and lamb growth from birth to weaning in both experiments. Combined twin lamb birth weight was positively ( $P < 0.001$ ) correlated with MY,  $r = 0.34$  and  $0.43$ , in experiments one and two, respectively. Combined twin lamb  $LWG_{0-42}$  was dependent on ewe MY ( $r^2 = 0.43$ , for experiment one;  $r^2 = 0.30$ , for experiment two). Lactose, fat and milk CP yields explained 47% and 42% of the variation in lamb  $LWG_{0-42}$  in experiment one and two, respectively. Lactose and milk CP yield positively affected ( $P < 0.05$ )  $LWG_{0-42}$  in experiment one and two, respectively. Fat yield had a positive relationship with  $LWG_{0-42}$  in experiment one and a negative relationship with  $LWG_{0-42}$  in experiment two. In conclusion, the measured prenatal ewe traits had a minimal effect on milk yield and twin lamb growth to weaning. Milk yield and composition explained the greatest proportion of variation in  $LWG_{0-42}$ . This suggests

that farmers should select ewes with higher milk yields to maximize twin lamb growth to weaning. However, less than 50% of the variation in  $LWG_{0-42}$  and weaning LW was explained by the measured ewe and lamb parameters. Therefore, further studies are required to determine additional ewe or lamb variables that control variation in twin lamb growth.

### 3.1 Introduction

A faster growing lamb will achieve a target live weight for less feed consumed than a slower growing lamb, making it more efficient (Peeters *et al.*, 1992). Therefore, an understanding of the factors that affect lamb growth will help develop strategies to increase production efficiency. Known factors include: ewe live weight and body condition at mating and during pregnancy (Geisler and Fenlon, 1979; Kenyon *et al.*, 2012); nutrition of the dam during pregnancy and lactation (Snowder and Glimp, 1991; Kenyon *et al.*, 2009); lamb birth weight (Greenwood *et al.*, 1998; Kenyon *et al.*, 2011a); birth rank (Joyce *et al.*, 1976; Muir *et al.*, 2000) and level of nutrient intake (Degen and Benjamin, 2005; Morgan *et al.*, 2007). In addition, studies have examined the impact of ewe milk production on lamb growth and have reported positive effects, especially in early lactation as expected (Snowder and Glimp, 1991; Mekoya *et al.*, 2009).

However, information on the proportion of variation in lamb growth (especially twin born and reared lambs) explained by ewe milk production, live weight and body condition, and lamb birth weight is lacking. Kenyon *et al.* (2011a) examined the effects of some dam and lamb traits on lamb growth rate but they explained only 25% of the variation in lamb live weight gain from birth to weaning. Their study however did not include ewe milk production. Furthermore, little is known about the proportion of variation in milk yield explained by pre-natal ewe traits.

The objectives of the present study were firstly to evaluate the effects of several dam and offspring traits on milk production and twin lamb growth from birth to weaning using data from two multiple-year studies and secondly to determine how much of the variation in twin lamb growth was explained by these same traits.

## 3.2 Materials and methods

The studies were conducted at the Massey University Keeble Sheep and Beef farm, 5 km south of Palmerston North, New Zealand and were approved by the Massey University Animal Ethics Committee.

### 3.2.1 *Experiment one*

#### *Background*

The design of the experiment has been previously reported by Kenyon *et al.* (2009). Briefly, two groups of 450 Romney ewes (group 0 [G0]; heavy; live weight [LW] =  $60.8 \pm 0.18$  kg; body condition score [BCS]  $3.02 \pm 0.03$  and light [L]; LW =  $42.5 \pm 0.17$  kg; BCS =  $1.97 \pm 0.03$ ) were selected at mating. The G0 dams were artificially inseminated and randomly allocated to one of two pastoral feeding regimens (pregnancy maintenance or *ad libitum*) between days 21 and 140 of pregnancy (P21 – 140). From day 140 of pregnancy until weaning, all G0 dams and their offspring (group 1[G1]) were allowed *ad libitum* access to pasture (Kenyon *et al.*, 2009). The average pre- and post-grazing herbage masses between P21 and P140 were  $1330 \pm 140.0$  and  $804 \pm 133.4$  kg DM/ha for the maintenance regimen, and  $2304 \pm 156.8$  and  $1723 \pm 149.7$  kg DM/ha for the *ad libitum* regimen (Kenyon *et al.*, 2009). This resulted in four G0 dam groups (Fig. 3.1).

#### *Present study*

After weaning, all G1 female progeny were managed as one group under commercial conditions for the remainder of their lifetime (van Der Linden, 2007; Asmad *et al.*, 2014). The G1 ewes were bred yearly (approximately 19 months of age in 2007; 32 months in 2008; 43 months in 2009; 55 months in 2010 and 68 months in 2011) after

synchronisation with a progesterone controlled internal drug released device (CIDR, 0.3 g progesterone, Pharmacia & UpJohn, Auckland, New Zealand), to Suffolk rams for five days. The present study utilised 259 sets of twin-bearing G1 ewe milk production records and their lamb (group 2 [G2]) growth data from 2007 to 2011 (71 ewes in 2007, 51 ewes in 2008, 54 in 2009, 45 in 2010 and 38 in 2011). The age of the G1 ewes at the time of weaning of the fifth set of lambs was approximately 77 months. The ewes were milked once a week, for six consecutive weeks in spring (September to November) each year. The first milking commenced at day 7 (range of 5–9 days) after parturition. The LW and BCS (scale 0 to 5, including half units; Jefferies (1961)) of the G1 ewes were recorded at mating (m; day 0 of pregnancy) and set stocking (ss; day 140 of pregnancy with mating as day 0) for each year. During the lactation period pasture covers were maintained above 1200 kg DM /ha (van der Linden *et al.*, 2009; Asmad *et al.*, 2014) indicating feeding levels were not restricted (Morris and Kenyon, 2004).

Milk yield was measured using the oxytocin method followed by machine and hand stripping (van der Linden *et al.*, 2009; Paten *et al.*, 2013). On the day of milking, ewes were milked in the morning and again approximately five hours later. The time of each milking and the quantity of milk obtained were recorded. The G2 lambs were separated from their dams between the first and second milking to prevent suckling. Daily milk yield was calculated by multiplying the yield at 2nd milking by the proportion of 24 hours from 1st to 2nd milking (van der Linden *et al.*, 2010b; Paten *et al.*, 2013).

Ewe milk was analysed for fat, lactose and crude protein (CP) percentages by infrared spectroscopy using Milkoscan FT120 (Foss, Hillerod, Denmark) calibrated for sheep milk (DairyNZ, Hamilton, New Zealand) (DairyNZ, Hamilton, New Zealand) (Paten *et al.*, 2013).

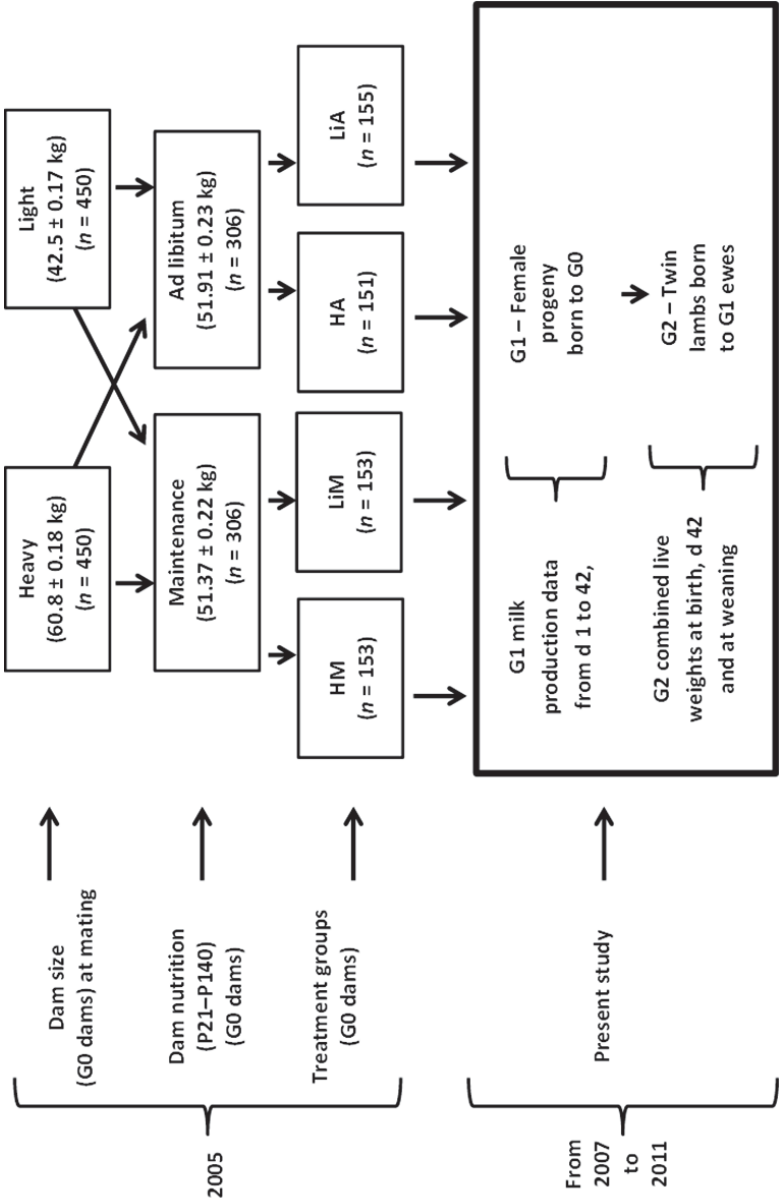


Figure 3.1. Experimental design and overview of dam size and nutrition studies of group 0 (G0) dams whose female offspring (group 1 [G1]) and grand offspring (group 2 [G2]) are used in Exp. 1. Group 0: dams were either heavy or light at mating and were randomly allocated to receive pregnancy maintenance or *ad libitum* feeding treatment from Day 21 to 140 of pregnancy (P21–P140), resulting in 4 treatment groups (heavy-*ad libitum* [HA], heavy-maintenance [HM], light-*ad libitum* [LiA] and light-maintenance [LiM]).

The energy content in milk (MJ kg milk yield<sup>-1</sup>) was calculated based on CP and fat percentages in the milk using the equation (Holmes *et al.*, 2002):

$$(0.376 \times \text{Fat}\%) + (0.209 \times \text{CP}\%) + 0.976$$

Weekly ewe milk and composition (fat, lactose, CP and energy) yields between two milking periods were calculated as the average daily milking multiplied by seven days. Accumulated milk and composition yields over the 42-day period was calculated as the sum of the weekly yields.

The effects of the size and nutrition treatments of G0 dams on the milk production and growth performance of G1 ewes have been published (van der Linden *et al.*, 2009; Van der Linden *et al.*, 2010a; Kenyon *et al.*, 2011a). However, the relationships between individual G1 ewe live weight and body condition score at breeding and set stocking, milk production and G2 twin lamb growth performance from birth to weaning have not yet been investigated. The present study examined the relationships between individual G1 ewe LW and BCS at mating and set stocking, G1 ewe milk production and G2 combined twin lamb growth performance from birth to weaning.

### **3.2.2 Experiment two**

#### *Background*

The design of the trial has been previously described by Kenyon *et al.* (2011b). Briefly, Romney dams (G0; LW of  $66.3 \pm 0.18$  kg, BCS of  $2.96 \pm 0.02$ ) were subjected to different feeding regimens during mid-to-late pregnancy. From Day 21 to 50 of pregnancy (P21-50), the dams were randomly allocated to one of three pastoral feeding regimens (submaintenance, maintenance or *ad libitum*). The average pre- and post-grazing forage mass between P21 and Day 50 of pregnancy (P50) were  $996 \pm 89.3$  and

814  $\pm$  54.2 kg DM/ha for the submaintenance regimen, 1,479  $\pm$  107.7 and 1,112  $\pm$  59.4 kg DM/ha for the maintenance regimen and 2,331  $\pm$  82.0 and 1,649  $\pm$  54.2 kg DM/ha for the *ad libitum* regimen. Dams were then reallocated to either pregnancy maintenance (MP50-140) or *ad libitum* (AdP50-140) feeding during days 50 to 140 of pregnancy (P50–140). The average pre- and post-grazing pasture covers during the period between P50 and P140 were 1,450  $\pm$  83.9 to 1,011  $\pm$  32.8 kg DM/ha for the M regimen and 1,828  $\pm$  76.0 to 1,301  $\pm$  37.8 kg DM/ha for the A regimen. From P140 through to weaning, all G0 dams and their lambs (G1) were provided with *ad libitum* pasture. The design resulted in six treatment groups) (Fig. 3.2).

#### *Present study*

The present study utilized 143 twin-bearing G1 ewe milk production and their lambs' (G2) growth data from 2011 to 2013 (52 ewes in 2011, 43 ewes in 2012 and 48 ewes in 2013). The G1 ewes were synchronized (at approximately 18 months of age) with a progesterone controlled internal drug released device (CIDR, 0.3 g progesterone, Pharmacia & UpJohn, Auckland, New Zealand) for six days each year and bred using semen from Romney rams over a period of 17 days. The ewes were milked once a week, starting from day 7  $\pm$  1 postpartum, for six consecutive weeks in spring (September to November) each year. Milk production and composition were recorded weekly and their total yields were estimated as described in experiment one. Pasture covers during lactation were maintained above 1200 kg DM /ha (Paten *et al.*, 2013).

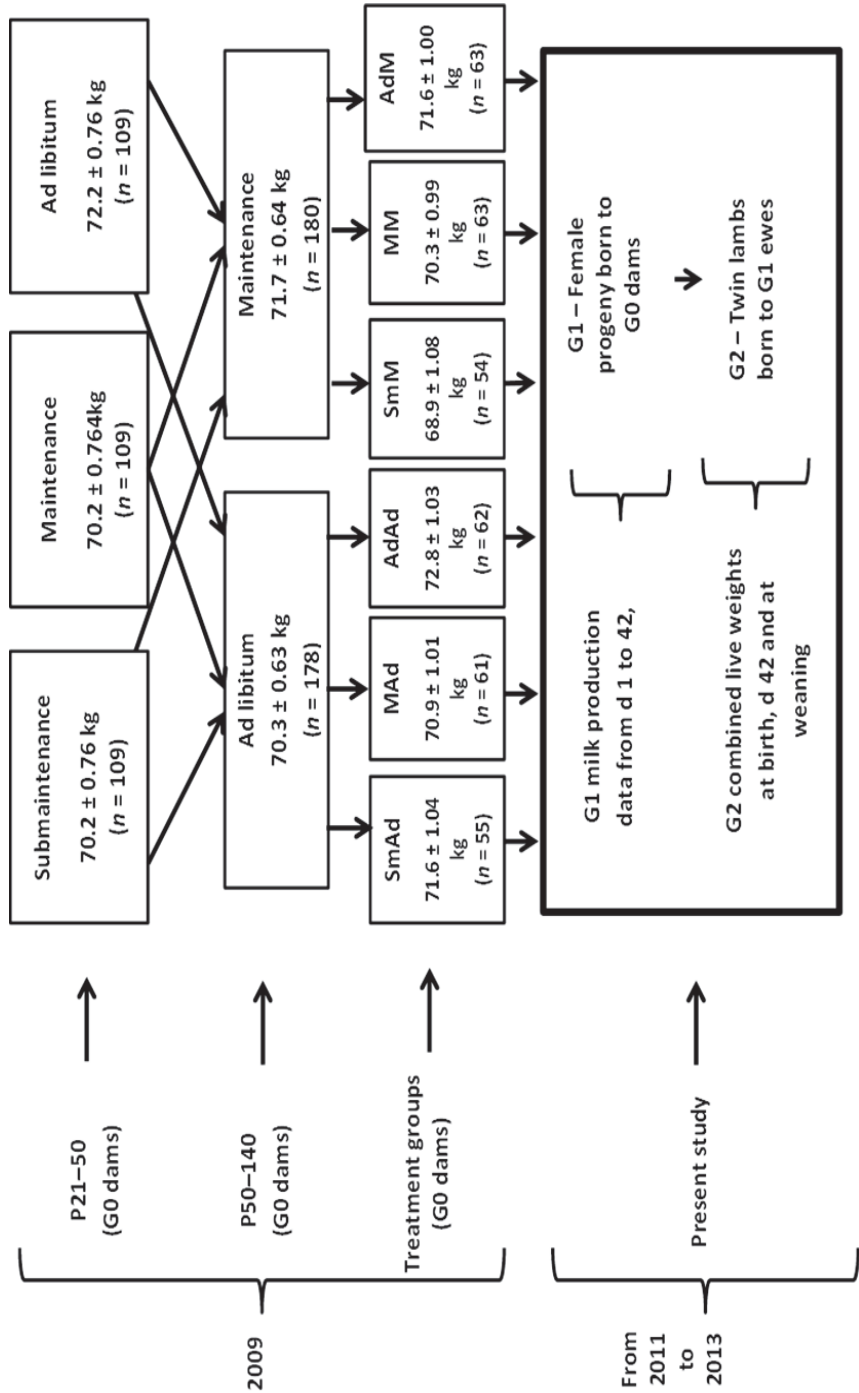


Figure 3.2 Experimental design and overview nutrition during early and mid-to-late pregnancy of group 0 (G0) dams whose female offspring (group 1 [G1]) and grand offspring (group 2 [G2]) are used in Exp. 2. SmM = submaintenance fed from d 21 to 50 of pregnancy (P21-50) and then maintenance fed from d 50 to 140 of pregnancy (P50-140); MM = maintenance fed throughout pregnancy, from d 21 to 140; AdM = *ad libitum* fed from d 21 to 50 of pregnancy and then maintenance fed from d 50 to 140 of pregnancy; SmAd = submaintenance fed from d 21 to 50 of pregnancy; MAd = maintenance fed from d 21 to 50 of pregnancy and then *ad libitum* fed from d 50 to 140 of pregnancy; AdAd = *ad libitum* fed throughout pregnancy, from d 21 to 140. Adapted from Paten *et al.* (2013)

The effects of G0 dam nutrition treatments during early and mid-to-late pregnancy on the milk production of G1 ewes and growth performance of G2 lambs have been published (Paten *et al.*, 2013). Again, the relationships between individual G1 ewe live weight and body condition score at breeding and set stocking, milk production and G2 twin lamb growth performance from birth to weaning have not been published. Therefore, the present study examined the relationships between individual G1 ewe LW and BCS at mating and set stocking, G1 ewe milk production and G2 combined twin lamb growth from birth to weaning.

### ***3.2.3 Offspring Experiment one and two***

The G2 lambs born to ewes in the two experiments were ear tagged and identified to their dams. They were weighed individually within 24 hours of birth, weekly for the 6-week milking period and at weaning. The ranges of weaning ages ( $Age_w$ ) in experiment one were: 54 days to 93 days for 2007; 95 days to 106 days for 2008; 80 days to 101 days for 2009; 102 days to 110 days for 2010 and 77days to 83 days for 2011. The  $Age_w$  ranges in experiment two were: 75 days to 80 days in 2011; 89 days to 94 days in 2010 and 104 days to 112 days in 2013.

Only complete twin sets to weaning were included in the study. For the purpose of this study, lambs' birth weight (Bwt) and live weight at day 42 ( $LW_{42}$ ) and at weaning ( $LW_w$ ) refer to the combined weight of the pair. Birth weight was subtracted from  $LW_{42}$  and  $LW_w$  to obtain the combined twin lamb weight gains from birth to day 42 ( $LWG_{0-42}$ ) and combined twin lamb live weight gains at weaning ( $LWG_{0-w}$ ) for each year.

### 3.2.4 *Statistical analysis*

The accumulated ewe milk yield (MY) and composition (protein, fat, lactose), lamb Bwt,  $LW_{42}$ ,  $LW_w$ ,  $LWG_{0-42}$  and  $LWG_{0-w}$  data for each experiment was first analysed with a general linear model with fixed effects of treatment groups, year of lambing the sex combinations (MM, MF and FF) of lambs in each set (Proc GLM; (SAS, 2013)). Ewe LW at mating ( $LW_m$ ), ewe BCS at mating ( $BCS_m$ ), ewe LW at set stocking ( $LW_{ss}$ ) and ewe BCS at set stocking ( $BCS_{ss}$ ) were included in the model as covariates. The cross products between the fixed effects and the covariates were also included in the models to test for the homogeneity of the slopes. As there were no significant ( $P > 0.05$ ) interactions between the fixed effects and the covariates (data not shown), it was concluded that the relationships between accumulated MY and composition, lamb Bwt,  $LW_{42}$ ,  $LW_w$ ,  $LWG_{0-42}$  and  $LWG_{0-w}$  data, and ewe  $LW_m$  and  $BCS_m$  or ewe  $LW_{ss}$  and  $BCS_{ss}$  were similar across years and treatment groups, thus, the treatment effects of each experiment were not presented. A model was fitted to  $LW_{42}$  and  $LWG_{0-42}$  with the sex combinations of lambs in each set as a fixed effect and MY as covariate and cross products between the fixed effects and the covariates were also included in the models to test for the homogeneity of the slopes in both experiments. There were no significant ( $P > 0.05$ ) effects of sex and the cross products between sex and MY on the dependent variables in both experiments (data not shown), only the covariate was significant ( $P < 0.001$ ). Therefore, only the overall relationships between  $LW_{42}$ ,  $LWG_{0-42}$  and MY are presented. The relationships between the different independent variables in each experiment were investigated by fitting simple linear regressions or multiple linear regressions (stepwise selection method) across all years and treatment groups. Estimates of Pearson's correlation coefficients between ewe variables ( $LW_m$ ,  $BCS_m$ ,  $LW_{ss}$ ,  $BCS_{ss}$  and accumulated MY) and lamb growth measurements (Bwt,  $LW_{42}$ ,  $LWG_{0-42}$ ,  $LW_w$ ,

LWG<sub>0-w</sub> and Age<sub>w</sub>) were calculated to determine the effect of each measured ewe and lamb trait on the dependent variables independent of other measured traits.

Simple linear regression analyses of accumulated MY and milk energy content on LWG<sub>0-42</sub> for each experiment were calculated.

Stepwise regression was used to choose important ewe (LW<sub>m</sub>, BCS<sub>m</sub>, LW<sub>ss</sub>, BCS<sub>ss</sub>) and lamb (Bwt) effects on accumulated MY over the 42-day period for each experiment. Combined lamb Bwt was included in the model as a covariate to correct for the residual effect from LW<sub>ss</sub> (proxy correction for conceptus weight). Variables that contributed the greatest to the variation explained were fitted first, followed by other variables that improved the model (forward selection). Only variables with significant effects ( $P < 0.05$ ) remained in the regression models. Regression analyses of ewe parameters (LW<sub>m</sub>, BCS<sub>m</sub>, LW<sub>ss</sub>, BCS<sub>ss</sub>, MY) and lamb Bwt on LWG<sub>0-42</sub> and milk components (fat yield, lactose yield and CP yield) on LWG<sub>0-42</sub> were carried out using the stepwise method to select the variables that best explained the variation in LWG<sub>0-42</sub> (PROC REG; SAS, 2013). As milk energy content was a linear component of fat yield and crude protein yield, it was excluded from the multiple regression analysis. Forward regression was used to select the ewe (LW<sub>m</sub>, BCS<sub>m</sub>, LW<sub>ss</sub> and BCS<sub>ss</sub>) and lamb (Bwt, LW<sub>42</sub> and Age<sub>w</sub>) traits that best explained the variation in LW<sub>w</sub> and LWG<sub>0-w</sub>.

### 3.3 Results

The means and range of values of the data sets used in experiments one and two are presented in Table 3.1. The values were not adjusted for year or ewe age. A considerable difference between years was apparent with respect to Age<sub>w</sub> of the lambs resulting from the use of second and third cycle ewes for milking.

Pathway models were constructed using estimates of unadjusted Pearson correlations for each experiment to show the relationships between ewe and lamb variables (Fig. 3 and 4 for experiments one and two, respectively). Prenatal predictions were variable, with only ewe  $LW_{ss}$  being positively correlated with Bwt and MY in both experiments. Ewe  $LW_m$ ,  $BCS_m$  and  $BCS_{ss}$  showed either negative or no relationship with Bwt and MY across experiments.

Lamb Bwt was positively correlated with accumulated MY,  $LW_{42}$  and  $LW_w$  in both experiments. Birth weight was only correlated with  $LWG_{0-42}$  and  $LWG_{0-w}$  in experiment two. Accumulated MY showed a consistent positive relationship with  $LW_{42}$  and  $LW_w$  in both experiments

Table 3.1 Descriptive statistics of the ewe and lamb data sets from the two experiments

| Variable                               | Experiment one ( <i>n</i> = 259) |       |       | Experiment two ( <i>n</i> = 143) |       |       |       |       |
|----------------------------------------|----------------------------------|-------|-------|----------------------------------|-------|-------|-------|-------|
|                                        | Mean                             | SD    | Min   | Max                              | Mean  | SD    | Min   | Max   |
| Ewe parameters <sup>1</sup>            |                                  |       |       |                                  |       |       |       |       |
| LW <sub>m</sub> , kg                   | 68.19                            | 9.25  | 50.50 | 100.5                            | 71.66 | 10.33 | 50.50 | 93.0  |
| BCS <sub>m</sub>                       | 2.95                             | 0.69  | 1.50  | 5.00                             | 3.14  | 0.7   | 2.00  | 4.50  |
| LW <sub>ss</sub> , kg                  | 76.47                            | 10.34 | 47.50 | 109.5                            | 84.78 | 9.44  | 66.0  | 118.5 |
| BCS <sub>ss</sub>                      | 2.41                             | 0.54  | 1.50  | 4.50                             | 2.76  | 0.52  | 2.00  | 4.50  |
| Accumulated milk production parameters |                                  |       |       |                                  |       |       |       |       |
| Milk yield, kg                         | 114.8                            | 17.41 | 67.42 | 164.1                            | 119.3 | 18.0  | 77.7  | 162.8 |
| Lactose yield, kg                      | 6.06                             | 0.97  | 3.38  | 8.88                             | 6.15  | 0.94  | 4.06  | 8.73  |
| Crude protein yield, kg                | 5.60                             | 0.95  | 3.22  | 8.77                             | 5.84  | 0.85  | 3.73  | 7.97  |
| Fat yield, kg                          | 8.13                             | 1.36  | 4.77  | 11.85                            | 8.05  | 1.65  | 4.64  | 13.21 |
| Energy content yield, MJ               | 534.8                            | 79.07 | 314.3 | 774.7                            | 541.3 | 89.71 | 339.7 | 809.7 |
| Combined lamb parameters <sup>2</sup>  |                                  |       |       |                                  |       |       |       |       |
| Twin lamb Bwt, kg                      | 9.56                             | 1.32  | 5.00  | 12.8                             | 10.38 | 1.40  | 6.70  | 13.90 |
| Twin lamb LW <sub>42</sub> , kg        | 32.47                            | 3.87  | 22.70 | 43.2                             | 31.85 | 4.10  | 19.90 | 45.20 |
| Twin lamb, LWG <sub>0-42</sub> , kg    | 22.91                            | 3.51  | 13.7  | 32.0                             | 21.47 | 3.38  | 13.20 | 34.10 |
| Twin lamb LW <sub>w</sub> , kg         | 54.92                            | 9.03  | 21.90 | 73.00                            | 56.75 | 9.47  | 24.00 | 77.00 |
| Twin lamb LWG <sub>0-w</sub> , kg      | 45.54                            | 8.13  | 11.90 | 62.00                            | 46.37 | 9.01  | 13.90 | 65.40 |
| Twin lambs' Age <sub>w</sub> , days    | 90.20                            | 13.22 | 54.00 | 110.0                            | 92.30 | 13.10 | 75.00 | 112.0 |

<sup>1</sup>Ewe parameters: LW<sub>m</sub> = ewe live weight at mating; BCS<sub>m</sub> = ewe body condition score at mating; LW<sub>ss</sub> = ewe live weight at set stocking; BCS<sub>ss</sub> = ewe body condition score at set stocking

<sup>2</sup>Lamb parameters: Bwt = birth weight; LW<sub>42</sub> = live weight at day 42; LWG<sub>0-42</sub> = weight gain from birth to day 42; LW<sub>w</sub> = live weight at weaning; LWG<sub>0-w</sub> = weight gain from birth to weaning; Age<sub>w</sub> = age at weaning.

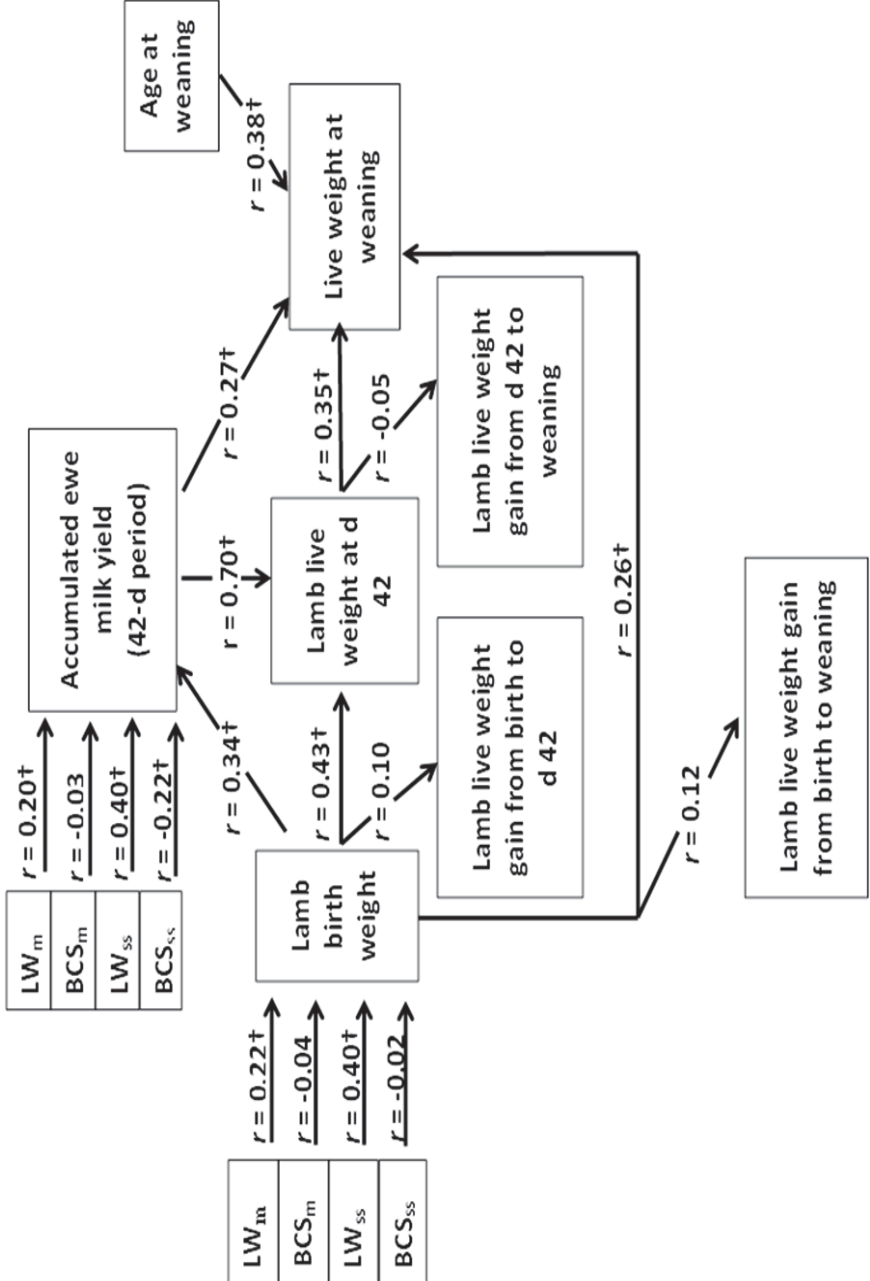


Figure 3.3 Pathway modelling showing the relationships between the measured ewe live weight at mating ( $LW_m$ ), ewe BCS at mating ( $BCS_m$ ), ewe live weight at set stocking ( $LW_{ss}$ ), ewe BCS at set stocking ( $BCS_{ss}$ ), accumulated ewe milk yield over a 42-d period, and combined twin lamb (birth weight [Bwt], live weight at Day 42, live weight gain from Day 0 to 42, live weight gain from Day 42 to weaning, live weight at weaning, live weight gain from birth to weaning, and age at weaning) variables from birth to weaning in Exp. 1 using estimates obtained from Pearson's correlation coefficients.  $^\dagger P < 0.001$

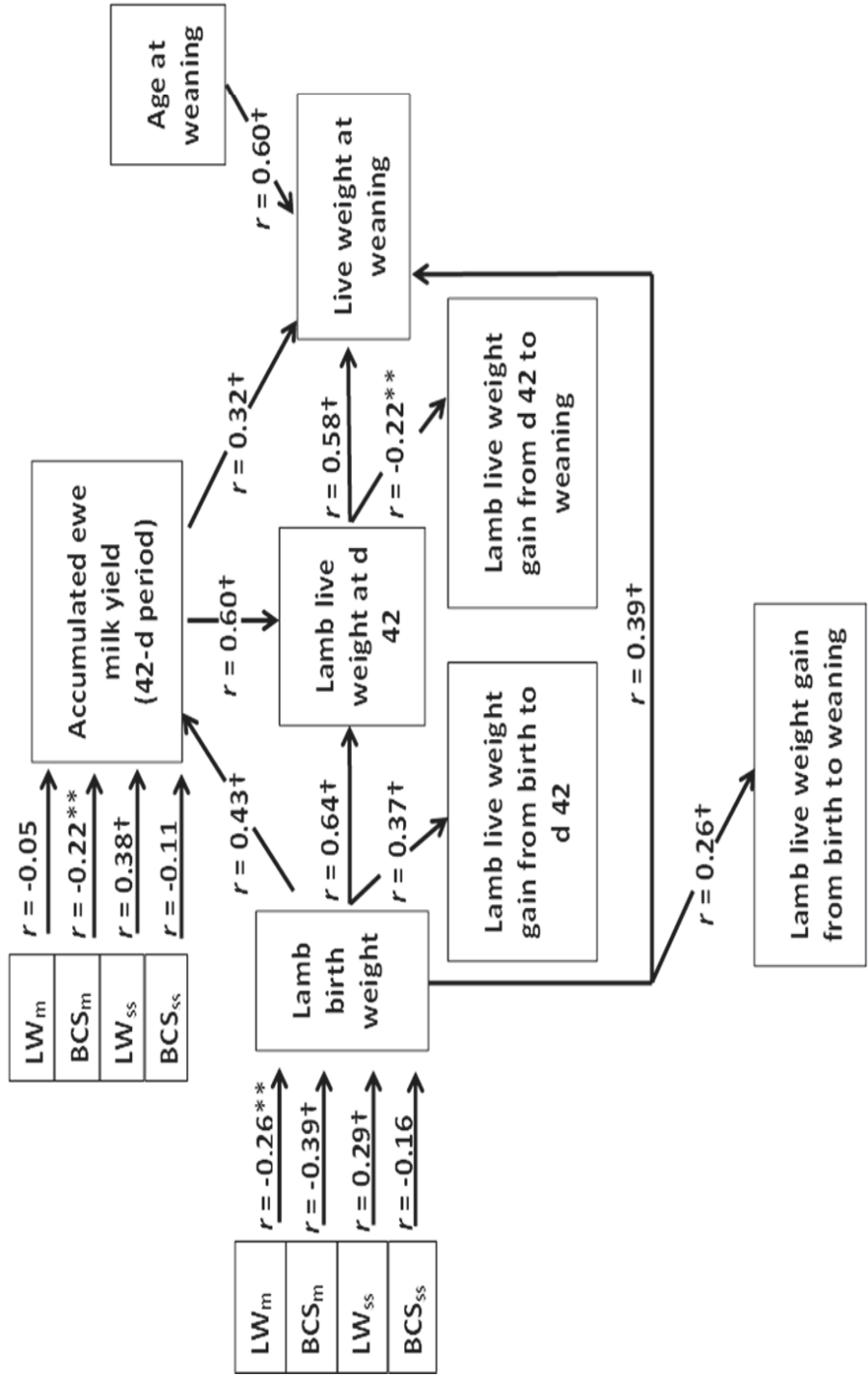


Figure 3.4 Pathway modelling showing the relationships between the measured ewe live weight at mating ( $LW_m$ ), ewe BCS at mating ( $BCS_m$ ), ewe live weight at set stocking ( $LW_{ss}$ ), ewe BCS at set stocking ( $BCS_{ss}$ ), accumulated ewe milk yield over a 42-d period (MY), and combined twin lamb (birth weight [Bwt], live weight at Day 42, live weight gain from Day 0 to 42, live weight gain from Day 42 to weaning, live weight at weaning, live weight gain from birth to weaning) variables from birth to weaning in Exp. 2 using estimates obtained from Pearson's correlation coefficients.  $^{**}P < 0.01$ ;  $^\dagger P < 0.001$

The stepwise method showed that the significant ( $P < 0.05$ ) ewe traits explained less than 30% of the total variation in Bwt in both experiments (Table 3.2). Regression analysis showed a positive ( $P < 0.05$ ) relationship of ewe  $LW_{ss}$  on Bwt in both experiments. Ewe  $BCS_m$  and  $BCS_{ss}$  had negative relationships with Bwt in experiment two. There were consistent positive ( $P < 0.05$ ) relationships of ewe  $LW_{ss}$  and Bwt on accumulated MY in both experiments. Additionally,  $LW_m$  and  $BCS_{ss}$  had a respective positive and negative relationships on accumulated MY in experiment one. Overall, the significant ( $P < 0.05$ ) prenatal ewe parameters and Bwt accounted for only 24% and 28% of the total variation in accumulated MY in experiment one and two respectively.

Total MY produced during the first 42 days accounted for a greater proportion of the variation in total  $LWG_{0-42}$  (43% in experiment one (Fig. 3.5), and 30% in experiment two (Fig. 3.6)). A 1.0 kg increase in accumulated MY was associated with a 130 g and 100 g increase in the twin lambs' total  $LWG_{0-42}$  in experiments one and two, respectively.

Regression analyses showed that ewe milk energy accounted for 43% and 20% of the variation in lamb  $LWG_{0-42}$  in experiments one and two, respectively. The regression equations were:

$LWG_{0-42} = 7.29 (\pm 1.13) + 0.03 (\pm 0.002) \text{ Milk energy } (r^2 = 0.43)$  for experiment one and,

$LWG_{0-42} = 12.31 (\pm 1.56) + 0.02 (\pm 0.003) \text{ Milk energy } (r^2 = 0.20)$  for experiment two.

These results indicate that for every 1 MJ of milk energy intake, the combined LWG of twin lambs in experiment one increased by 30 g whereas a 1-MJ milk energy intake resulted in 20 g increased in LWG for twin lambs in experiment two. Milk lactose, fat

and CP yields explained 47% and 42% of the variation in lamb  $LWG_{0-42}$  in experiment one and two, respectively (Table 3.3). Fat yield had a positive relationship with  $LWG_{0-42}$  in experiment one and a negative relationship with  $LWG_{0-42}$  in experiment two.

The significant ( $P < 0.05$ ) parameters from the stepwise regression explained between 41% to 61% of the variation in  $LW_{42}$  and  $LWG_{0-42}$  in the two experiments (Table 3.4). The single largest variable was accumulated MY, which explained between 43% and 50% of the variation in  $LW_{42}$  and  $LWG_{0-42}$ , respectively, in experiment one, but only 13% to 29% of variation in  $LW_{42}$  and  $LWG_{0-42}$  in experiment two. Combined Bwt explained the greatest proportion (41%) of the variation in  $LW_{42}$  in experiment two. Prenatal variables,  $LW_m$  (in experiment one) and  $BCS_m$  (in experiment two) had negative effects ( $P < 0.05$ ) on  $LW_{42}$  and  $LWG_{0-42}$  explaining 1% and 12% of the variation respectively.

Prenatal variables had no effect ( $P > 0.05$ ) on  $LW_w$  and  $LWG_{0-w}$  in both experiments, with the exception of  $BCS_{ss}$  which had a significant ( $P < 0.001$ ) positive effect on  $LW_w$  and  $LWG_{0-w}$  in experiment one (Table 3.5). Lamb  $LW_{42}$  explained the greatest proportion of the variation in  $LW_w$  and  $LW_{0-w}$  in experiment one (21% and 16%, respectively); while  $Age_w$  explained the greatest proportion of the variation in  $LW_w$  and  $LW_{0-w}$  in experiment two (36% and 32%, respectively). Lamb  $Age_w$  and  $LW_{42}$  were excluded from the stepwise regression model to test if accumulated MY would likely have an effect on  $LW_w$  and  $LWG_{0-w}$  (data not shown). However, the effect of accumulated MY was not significant ( $P > 0.05$ ) in both experiments when lamb  $Age_w$  and  $LW_{42}$  were excluded from the model.

Table 3.2 Multiple linear regression coefficients,  $\pm$ S.E (semi partial  $R^2$ ) of ewe parameters<sup>1</sup> ( $LW_m$ ,  $BCS_m$ ,  $LW_{ss}$  and  $BCS_{ss}$ ) and combined lamb birth weight (Bwt) on accumulated milk yield (MY) and Bwt in experiments one and two using forward stepwise selection

| Variable                      | Intercept         | Input parameters               |                                 |                                |                                 | P-value | $R^2$ |
|-------------------------------|-------------------|--------------------------------|---------------------------------|--------------------------------|---------------------------------|---------|-------|
|                               |                   | $LW_m$                         | $BCS_m$                         | $LW_{ss}$                      | $BCS_{ss}$                      |         |       |
| Experiment one                |                   |                                |                                 |                                |                                 |         |       |
| Order of fitting <sup>2</sup> |                   |                                |                                 |                                |                                 |         |       |
| Bwt, kg                       | 5.70 $\pm$ 0.56   | -                              | -                               | 1<br>0.05 $\pm$ 0.01<br>(0.16) | -                               | <0.001  | 0.16  |
| Order of fitting              |                   |                                |                                 |                                |                                 |         |       |
| MY <sup>4</sup> , kg          | 61.50 $\pm$ 9.76  | 4<br>0.31 $\pm$ 0.15<br>(0.01) | -                               | 1<br>0.33 $\pm$ 0.13<br>(0.16) | 3<br>-8.66 $\pm$ 2.17<br>(0.03) | <0.001  | 0.24  |
| Experiment two                |                   |                                |                                 |                                |                                 |         |       |
| Order of fitting              |                   |                                |                                 |                                |                                 |         |       |
| Bwt, kg                       | 9.41 $\pm$ 1.05   | -                              | 1<br>-0.60 $\pm$ 0.15<br>(0.15) | 2<br>0.05 $\pm$ 0.01<br>(0.06) | 3<br>-0.89 $\pm$ 0.22<br>(0.08) | <0.001  | 0.29  |
| Order of fitting              |                   |                                |                                 |                                |                                 |         |       |
| MY, kg                        | 22.04 $\pm$ 13.35 | -                              | -                               | 2<br>0.62 $\pm$ 0.14<br>(0.10) | 1<br>4.32 $\pm$ 0.6<br>(0.18)   | <0.001  | 0.28  |

<sup>1</sup>Ewe parameters:  $LW_m$  = ewe live weight at mating;  $BCS_m$  = ewe body condition score at mating;  $LW_{ss}$  = ewe live weight at set stocking;  $BCS_{ss}$  = ewe body condition score at set stocking.

<sup>2</sup>Order of fitting: indicates order in which the predictor variable contributed to the equation.

<sup>3</sup>NI = parameter was not included in model.

<sup>4</sup>MY = accumulated ewe milk yield over a 42-day period.

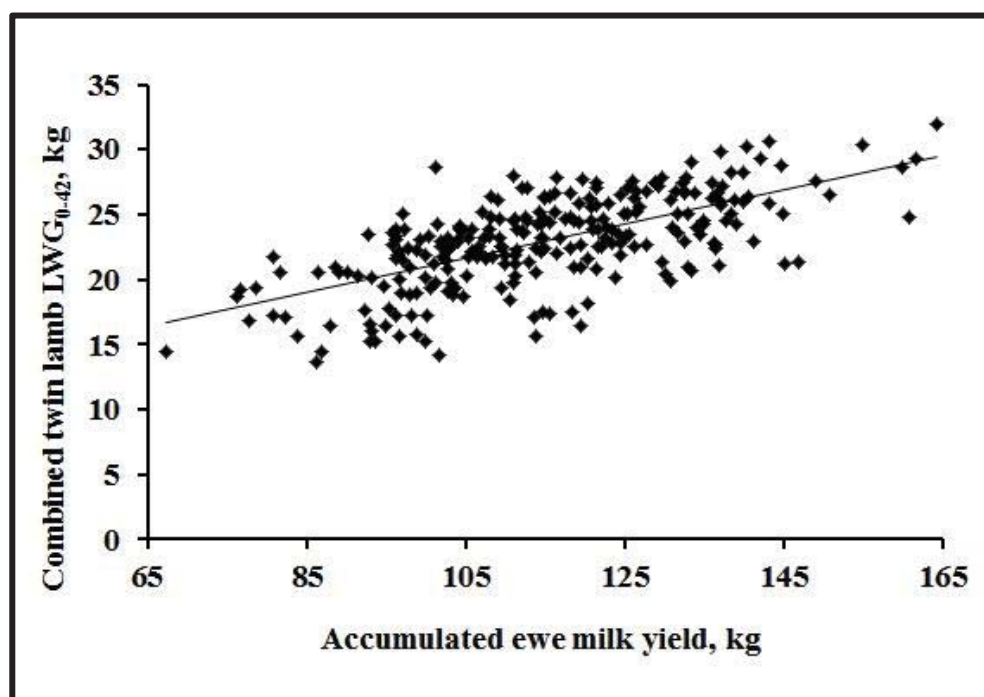


Figure 3.5 Relationship between the accumulated ewe milk yield over a 42-day period and the combined live weight gain (from birth to day 42) (LWG<sub>0-42</sub>; kg) of twin lambs in experiment one.

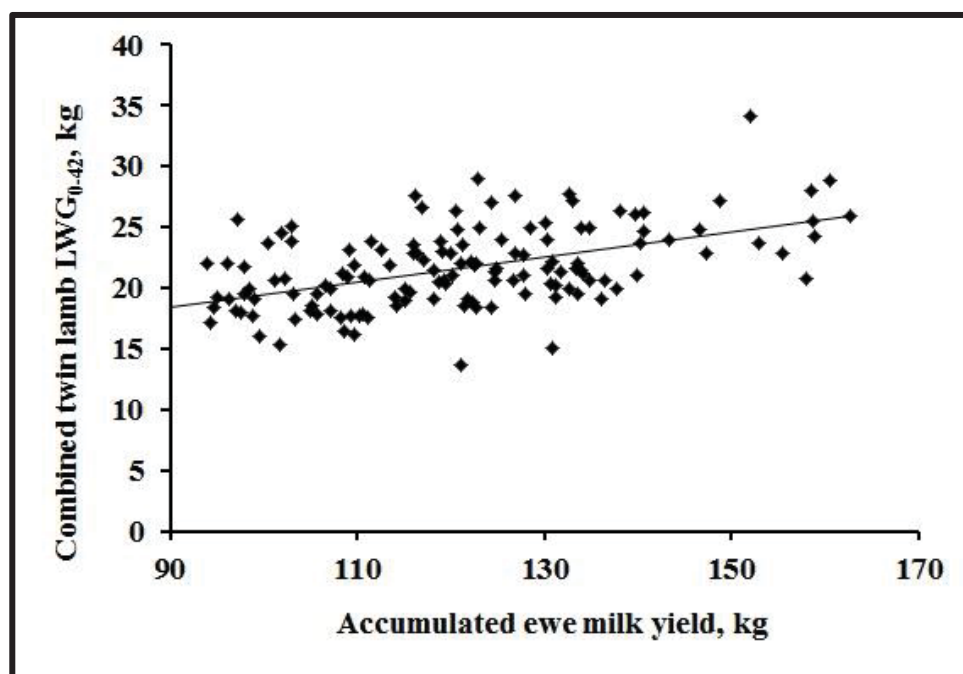


Figure 3.6 Relationship between the accumulated ewe milk yield over a 42-day period and the combined live weight gain (from birth to day 42) (LWG<sub>0-42</sub>; kg) of twin lambs in experiment two.

Table 3.3 Linear regression coefficients,  $\pm$  S.E (partial  $R^2$ ) of milk components on combined twin lamb live weight gain from day 0 to 42 (LWG<sub>0-42</sub>) in experiments one and two using forward stepwise selection

| Parameter                     | Intercept       | Milk components, kg       |                            | Crude protein             | P-value | R <sup>2</sup> |
|-------------------------------|-----------------|---------------------------|----------------------------|---------------------------|---------|----------------|
| Experiment one                |                 | Lactose                   | Fat                        |                           |         |                |
| Order of fitting <sup>1</sup> |                 | 1                         | 2                          |                           |         |                |
| Lamb LWG <sub>0-42</sub> , kg | 6.68 $\pm$ 1.10 | 1.82 $\pm$ 0.21<br>(0.43) | 0.64 $\pm$ 0.15<br>(0.04)  | -                         | <0.001  | 0.47           |
| Experiment two                |                 |                           |                            |                           |         |                |
| Order of fitting              |                 | -                         | 2                          | 1                         |         |                |
| Lamb LWG <sub>0-42</sub> , kg | 6.86 $\pm$ 1.51 | -                         | -0.43 $\pm$ 0.18<br>(0.02) | 3.10 $\pm$ 0.34<br>(0.40) | <0.001  | 0.42           |

<sup>1</sup>Order of fitting: indicates order in which the predictor variable contributed to the equation.

Table 3.4 Linear regression coefficients,  $\pm$ S.E (partial  $R^2$ ) of ewe parameters1 (LW<sub>m</sub>, BCS<sub>m</sub>, LW<sub>ss</sub>, BCS<sub>ss</sub> and MY) and combined twin lamb birth weight (Bwt) on combined twin lamb live weight at day 42 (LW<sub>42</sub>) and live weight gain from day 0 to 42 (LWG<sub>0-42</sub>) in experiments one and two using stepwise regression

| Variable                      | Intercept        | Input parameters           |                            |                  |                   |                           | Bwt                        | P-value | R <sup>2</sup> |
|-------------------------------|------------------|----------------------------|----------------------------|------------------|-------------------|---------------------------|----------------------------|---------|----------------|
| Experiment one                |                  | LW <sub>m</sub>            | BCS <sub>m</sub>           | LW <sub>ss</sub> | BCS <sub>ss</sub> | MY                        |                            |         |                |
| Order of fitting <sup>2</sup> |                  | 3                          |                            |                  |                   | 1                         | 2                          |         |                |
| LW <sub>42</sub> , kg         | 12.25 $\pm$ 1.64 | -0.04 $\pm$ 0.02<br>(0.01) | -                          | -                | -                 | 0.14 $\pm$ 0.01<br>(0.50) | 0.68 $\pm$ 0.13<br>(0.04)  | <0.001  | 0.55           |
| Order of fitting              |                  | 3                          |                            |                  |                   | 1                         | 2                          |         |                |
| LWG <sub>0-42</sub> , kg      | 12.24 $\pm$ 1.64 | -0.04 $\pm$ 0.32<br>(0.01) | -                          | -                | -                 | 0.14 $\pm$ 0.01<br>(0.43) | -0.32 $\pm$ 0.13<br>(0.02) | <0.001  | 0.46           |
| Experiment two                |                  |                            |                            |                  |                   |                           |                            |         |                |
| Order of fitting              |                  |                            | 3                          |                  |                   | 2                         | 1                          |         |                |
| LW <sub>42</sub> , kg         | 15.78 $\pm$ 2.57 | -                          | -1.69 $\pm$ 0.34<br>(0.07) | -                | -                 | 0.09 $\pm$ 0.01<br>(0.13) | 1.08 $\pm$ 0.18<br>(0.41)  | <0.001  | 0.61           |
| Order of fitting              |                  |                            | 2                          |                  |                   | 1                         |                            |         |                |
| LWG <sub>0-42</sub> , kg      | 16.66 $\pm$ 1.99 | -                          | -1.75 $\pm$ 0.32<br>(0.12) | -                | -                 | 0.09 $\pm$ 0.13<br>(0.29) | -                          | <0.001  | 0.41           |

<sup>1</sup>Ewe parameters: LW<sub>m</sub> = live weight at mating; BCS<sub>m</sub> = body condition score at mating; LW<sub>ss</sub> = live weight at set stocking; BCS<sub>ss</sub> = body condition score at set stocking; MY = accumulated milk yield over 42-day period.

<sup>2</sup>Order of fitting: indicates order in which the predictor variable contributed to the equation.

Table 3.5 Multiple linear regression coefficients,  $\pm$ S.E (partial  $R^2$ ) of ewe and lamb parameters on combined twin lamb live weight at weaning ( $LW_w$ ) and live weight gain from birth to weaning ( $LWG_{0-w}$ ) in experiments one and two using forward stepwise selection

| Variable                      | Intercept         | Ewe parameter <sup>1</sup> |         |           | Lamb parameters <sup>2</sup>   |    |     |                                | P-value                        | $R^2$       |
|-------------------------------|-------------------|----------------------------|---------|-----------|--------------------------------|----|-----|--------------------------------|--------------------------------|-------------|
|                               |                   | $LW_m$                     | $BCS_m$ | $LW_{ss}$ | $BCS_{ss}$                     | MY | Bwt | $LW_{42}$                      |                                |             |
| Experiment one                |                   |                            |         |           |                                |    |     |                                |                                |             |
| Order of fitting <sup>3</sup> |                   |                            |         |           |                                |    |     |                                |                                |             |
| $LW_w$ , kg                   | -16.66 $\pm$ 5.94 | -                          | -       | -         | 3<br>2.19 $\pm$ 0.85<br>(0.02) | -  | -   | 1<br>1.14 $\pm$ 0.12<br>(0.21) | 2<br>0.32 $\pm$ 0.04<br>(0.15) | <0.01 0.38  |
| Order of fitting              |                   |                            |         |           |                                |    |     |                                |                                |             |
| $LWG_{0-w}$ , kg              | -20.26 $\pm$ 5.92 | -                          | -       | -         | 3<br>2.07 $\pm$ 0.85<br>(0.02) | -  | -   | 1<br>0.98 $\pm$ 0.12<br>(0.16) | 2<br>0.32 $\pm$ 0.04<br>(0.16) | <0.001 0.34 |
| Experiment two                |                   |                            |         |           |                                |    |     |                                |                                |             |
| Order of fitting              |                   |                            |         |           |                                |    |     |                                |                                |             |
| $LW_w$ , kg                   | 4.58 $\pm$ 5.06   | -                          | -       | -         | -                              | -  | -   | 2<br>0.80 $\pm$ 0.18<br>(0.08) | 1<br>0.29 $\pm$ 0.06<br>(0.36) | <0.001 0.44 |
| Order of fitting              |                   |                            |         |           |                                |    |     |                                |                                |             |
| $LWG_{0-w}$ , kg              | 1.52 $\pm$ 5.12   | -                          | -       | -         | -                              | -  | -   | 2<br>0.60 $\pm$ 0.18<br>(0.05) | 1<br>0.27 $\pm$ 0.06<br>(0.32) | <0.001 0.37 |

<sup>1</sup>Ewe parameters:  $LW_m$  = live weight at mating;  $BCS_m$  = body condition score at mating;  $LW_{ss}$  = live weight at set stocking;  $BCS_{ss}$  = body condition score at set stocking; MY = accumulated milk yield over a 42-day period.

<sup>2</sup>Lamb parameters: Bwt = Combined twin lamb birth weight;  $LW_{42}$  = combined twin lamb live weight at day 42;  $Age_w$  = twin lambs' age at weaning.

<sup>3</sup>Order of fitting: indicates order in which the predictor variable contributed to the equation.

### 3.4 Discussion

The aim of the present study was to evaluate the effects of several dam and offspring traits on milk production and twin lamb growth performance from birth to weaning and to determine the proportion of the variation of lamb growth that could be explained by these parameters. With this knowledge, it may be possible to manipulate pre-natal or post-natal ewe and lamb parameters to increase ewe milk production and/ or increase twin lamb growth.

Ewe live weight at mating had no effect on twin lamb live weight at birth or at weaning or live weight gains between birth and weaning in both experiments and only accounted for 1% of the variation in milk yield, LW<sub>42</sub> and LWG<sub>0-42</sub> in experiment one. This supports previous studies which have reported that ewe live weight pre-breeding or at mating had no effect or accounted for less than 3% of the variation in lamb birth weight or growth to weaning (Oldham *et al.*, 2011; Corner-Thomas *et al.*, 2014). Collectively, the evidence indicates that aiming for heavier ewes at mating is unlikely to increase or alter twin lamb live weight at birth or growth to weaning. However, it is recognised that ewe mating live weight will affect reproductive rate (Steinheim *et al.*, 2002).

Ewe live weight at set stocking had a consistent positive effect on twin lamb birth weight in both experiments although it explained less than 20% of the total variation. This relatively small positive effect on twin lamb birth weight was of similar magnitude to that reported in ewe lambs by Schreurs *et al.* (2010) but in contrast to Corner-Thomas *et al.* (2014) who reported a small negative influence of late pregnancy ewe LW on lamb birth weight when ewe live weight was corrected for predicted conceptus weight. The present study and that of Schreurs *et al.* (2010) did not correct for predicted conceptus weight, thus the analysis potentially was confounded by the weight of the foetuses.

Regardless of this potential relationship, the results indicate that management practices affecting total ewe live weight in late pregnancy will in turn affect twin lamb birth weight. Increasing the birthweight of twin lambs at birth is known to increase lamb survival (Morel *et al.*, 2009).

Ewe live weight at set stocking had a positive effect on milk yield but accounted for less than 20% of the variation in milk yield. Previous studies (Barnicoat *et al.*, 1956; Gardner and Hogue, 1966; Peart, 1970) have shown a positive effect of ewe live weight in late pregnancy on milk yield, while no effect of live weight has been observed under optimal grazing conditions (Barnicoat *et al.*, 1956) and in ewes with adequate body reserves (BCS > 3.0) (Peart, 1970). Combined, these results indicate an advantage of having heavier ewes as they will produce more milk, especially under conditions in which either ewe nutrition during lactation or body condition is not optimal.

The effects of ewe BCS at mating or set stocking on twin lamb birth weight were inconsistent across experiments. Likewise, previous reports relating ewe BCS at breeding or in late pregnancy on birth weight have been inconsistent with positive, negative and no effects being reported (see review by Kenyon *et al.* (2014)). In the present study, twin lamb birth weight was not related to BCS in experiment one whereas the negative effect of BCS at mating and in late pregnancy together accounted for 23% of the variation in birth weight in experiment two. Kenyon *et al.* (2004) observed a negative effect of high (3.5 – 4.0) body condition score on birth weight. This might explain the negative effect observed in experiment two, where a majority of ewes had higher BCS than in experiment one.

Ewe BCS at mating had no impact on milk yield, and in experiment one a small negative effect of ewe set stocking BCS was observed. Likewise, previous reports

relating BCS to milk production have found no effect when BCS was measured pre-breeding (Hossamo *et al.*, 1986) and in late pregnancy (Oregui *et al.*, 2004). Although a positive effect of BCS on milk production has been reported when BCS was measured in late pregnancy under restricted levels of pasture intake (Gibb and Treacher, 1980). Under restricted nutrition, high BCS ewes are able to mobilise body reserves, which compensates in part for the lower feeding levels, ultimately resulting in them producing more milk than their low BCS counterparts (Peart, 1970; Gibb and Treacher, 1980). Unrestricted feeding conditions may not require ewes to utilise their body reserves to support lactation and hence the potential benefits of high BCS will not be observed (Kenyon *et al.*, 2011c). It is possible that the minor or negligible effect of mating BCS on milk production in the current study was due to the *ad libitum* feeding conditions during lactation (van der Linden *et al.*, 2007; Paten *et al.*, 2013).

Considering the negligible effect of BCS on lamb birth weight and ewe milk yield it is unsurprising that BCS at mating had no impact on lamb live weights at day 42 or weaning and live weight gain from birth to weaning in both experiments. Small negative effects on lamb live weight and live weight gains to day 42 in experiment two were observed. Previous studies have reported no effect of mating BCS on twin lamb growth or weaning weight (Kenyon *et al.*, 2011c; Aliyari *et al.*, 2012) whereas Kenyon *et al.* (2004) reported a small positive effect of BCS at mating on lamb growth to weaning ( $r^2=0.22$ ). These few data suggest that BCS at mating may have little influence on lamb postnatal growth, thus not making it a means to substantially alter twin lamb growth to weaning.

Ewe BCS at set stocking had a very small positive effect on lamb live weight and live weight gains to weaning in experiment one but not in experiment two. Kenyon *et al.*

(2012) and Corner-Thomas *et al.* (2015) reported no effect of late pregnancy BCS over the range of 2 to 3 on lamb live weight at weaning under conditions at which ewe nutrition was not restricted in lactation, as in the present experiments. Combined these results indicate that increasing ewe BCS late in pregnancy is unlikely to increase lamb growth, especially under conditions where feed provided during lactation is not limiting.

Ewe live weight is known to be related to ewe BCS, especially in non-pregnant animals (Geisler and Fenlon, 1979; Sanson *et al.*, 1993; Kenyon *et al.*, 2004). The stepwise regression model selected the predictor variable that contributed the most to the prediction equation first in the model. Thus, the inclusion of ewe live weight likely nullified the effect of BCS and vice versa, depending on which predictor variable was first selected. This may explain why in most models either only ewe live weight or only BCS was significant.

The positive effect of twin lamb birth weight on ewe milk yield in both experiments is consistent with previous findings (Burris and Baugus, 1955; Barnicoat *et al.*, 1956). Heavier lambs are likely to consume more milk thereby stimulating greater production by the lactating dam (Moore, 1966b; Peart, 1967). Similarly, twin lamb birth weight was positively correlated with twin lamb live weight gain in the first 42 days of life agreeing with previous findings (Gallo and Davies, 1988). The diminishing correlation between lamb birth weight and later growth rates observed in the pathway model is consistent with the findings of Greenwood *et al.* (1998) who reported that when lamb nutrient intake is not limited during lactation, birth weight influences lamb growth rate only during the early stages of life. O'Connor (1996) showed only a small positive correlation ( $r = 0.19$ ) between birth weight and weaning weight in Scottish Black-face lambs. Therefore, birth weight may have little impact on final saleweight under optimal

feeding conditions. However, the greatest advantage of heavier birth weights in early stage of life is the increase in survival rates of lambs, thus further improving farm profitability (Morel *et al.*, 2008; 2009).

Milk yield explained the greatest proportion of lamb live weight gain at day 42, indicating the importance of milk in the diet of lambs in early life (Coombe *et al.*, 1960; Geenty *et al.*, 1985; Geenty and Dyson, 1986). However, accumulated milk yield to week 6 of lactation did not affect lamb weaning weights in the present study. This was in contrast to Morgan *et al.* (2007) who reported that higher milk yields in early lactation (3 to 4 weeks) gave lambs a growth advantage which lasted until weaning at 12 weeks of age. The latter study however, measured milk yield only during peak production (average yield of week 3 and 4) which may have accounted for the differences in their results and those of the current study. Excluding LW<sub>42</sub> from the stepwise regression model in the current study (data not shown), did not cause milk yield to become a significant causative factor controlling variation in weaning weight. As lambs age, a greater proportion of their nutrition is obtained from pasture to compensate for lower milk intake (Geenty and Dyson, 1986; Degen and Benjamin, 2005). This likely explains the poorer relationships between ewe milk yield and twin lamb growth rates from birth to weaning observed previously (Geenty and Dyson, 1986; Muir *et al.*, 2000) and in the present study, compared to lamb growth in early life. The results from the present study indicate that selection for ewes with higher milk yields is a mechanism to increase lamb growth in early life. Lamb growth differences established in the first 6 to 8 weeks of life affect later growth until about 16 weeks of age (Gibb and Treacher, 1980) as observed in the present study.

Overall, studies to date identify two important points for lamb growth to weaning. During the first few weeks (up to 6 weeks) of life, milk is the most important source of nutrition in early life (Snowder and Glimp, 1991; Morgan *et al.*, 2007). However, after the first few weeks until weaning solid feed intake is more important than milk intake for lamb growth (Peart, 1968; Hatfield *et al.*, 1995). Due to the extensive grazing conditions the ewes and lambs were farmed under in the current studies, milk yield in later lactation (after day 42) and lamb herbage intake were not measured. It is therefore proposed that raising lambs under controlled milk and feed intake conditions may enable the relationship between lamb nutrient intake and growth to be better explained.

The effects of fat yield, crude protein yield and lactose yield on live weight gain from birth to day 42 was inconsistent across the two experiments. The positive effects of fat and lactose yield in experiments one and crude protein yield in experiment two on lamb growth observed in the present study are consistent with those reported by Geenty (1979) and Hatfield *et al.* (1995). The negative effect of fat yield on lamb growth in experiment two was unclear. Contrary to these findings, there have been previous reports of little to no effects of individual milk components on lamb growth, which have led to the conclusion that quantitative milk production is a better indicator of early lamb growth than any of the individual components (Torres-Hernandez and Hohenboken, 1980). Crude protein yield, lactose yield and milk energy yield explained a greater proportion of the variation in twin lamb growth in early lactation compared to that explained by fat yield. The low proportion of variation explained by fat yield observed in both experiments of the current study indicated that growth of twin lambs was more closely related to the solid non-fat concentration than the fat concentration (Moore, 1966a; Scales, 1968). However, the inconsistencies observed in both experiments may

indicate that it is beneficial to increase milk yield rather than milk quality when aiming to improve twin lamb LWG.

### **3.5 Conclusion**

The present study indicated that ewe live weight and body condition score at mating or at set stocking prior to lambing had minimal effects on milk yield and pre-weaning growth of twin lambs. This suggests that under management conditions similar to the present study, altering ewe live weight or body condition at mating and/or in late pregnancy is not a method that farmers could use to substantially improve milk yield and lamb live weights at weaning. These studies also indicate that there is little to no benefit of higher lamb birth weight on lamb growth until weaning under adequate feeding conditions. Ewe milk yield and composition explained the greatest proportion of lamb live weight gain to 42 days of age. However, twin lamb live weight at weaning was not related to milk yield to day 42 of lactation. This is likely due to lambs being less dependent on milk as an energy source after day 42. Overall, less than 50% of the total variation in twin lamb weight gains from birth to weaning and weaning weight was explained by the measured variables. Further research is needed to identify additional factors that affect lamb growth to weaning.

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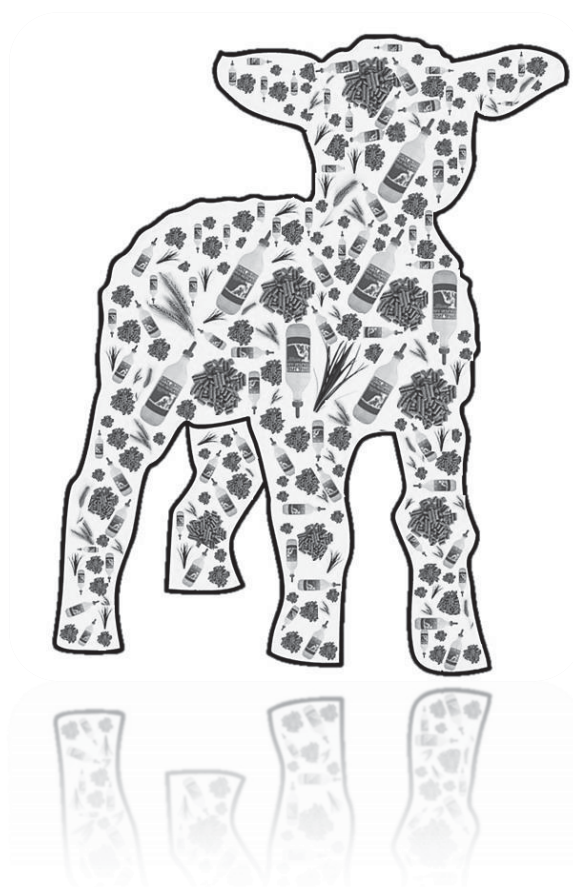
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## Chapter 4      Effects of Pre-Weaning Diet on Growth, Organ Development and Chemical Body Composition of Artificially Reared Lambs

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## FOREWORD

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The previous chapter (Chapter 3) evaluated the effects of several ewe and lamb traits on milk production and twin lamb growth, to determine what proportion of the total variation in lamb growth that could be explained by these measured traits. It was shown that altering ewe LW or BCS prior to either mating or set stocking are not tools farmers could use to increase either ewe milk production and/or twin lamb growth to weaning, whilst selecting for greater milk production could be used as a tool to increase lamb growth. However, milk energy yield explained a maximum of 43% of the total variation in twin lamb growth. Twin lamb weaning weight was not related to 42-day accumulated ewe milk yield, possibly due to lambs not being solely reliant on milk intake as an energy source. It is therefore vital to consider both milk and pasture intake by lambs when evaluating variation in lamb growth. However, estimation of milk and pasture intake under field conditions is difficult as neither of the dietary components can be measured accurately. It was proposed that a more direct control of one or both dietary components will enable the relationship between lamb nutrient intake and growth to be better explained. Chapter 4 therefore examines the proportion of variation in lamb growth that could be explained by milk and solid feed energy intake under controlled feed intake conditions. It also investigates the effect of milk and solid feed on organ development, chemical body composition and the utilisation of metabolisable energy for maintenance and growth. This Chapter is published in part as a Brief Communication in the Proceedings of New Zealand Society of Animal Production. (Danso *et al*, 2014).

## ABSTRACT

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Little is known about the relationship between lamb energy intake and the efficiency of the dietary energy for pre-weaning growth. Rearing lambs under controlled milk and feed intake conditions enables these relationships to be determined. Two feeding regimens were used to create variation in lamb energy intake to investigate what proportion of the variation in lamb growth can be explained. The regimens' effect on organ development, body composition and the utilisation of metabolisable energy for maintenance and growth were also investigated. Sixteen Suffolk twin-born male lambs were removed from their dams at 24 h post-partum and randomly assigned to one of two feeding groups (n=8) under artificial rearing. The first group was bottle-fed entirely on milk replacer (MO) and the second group was bottle fed milk replacer in addition to *ad libitum* access to pellets (MP). Milk and pellet intake were recorded daily and live weight (LW) was recorded at one day of age and every three days thereafter until slaughter at an average of 59 days of age. The data was used to estimate metabolisable energy (ME) requirement for maintenance ( $ME_m$ ) and growth ( $ME_g$ ) and, the efficiency of ME utilisation for growth ( $k_g$ ). Feeding pellets in addition to milk replacer increased ( $P < 0.05$ ) live weight gain (LWG), stomach weights and rumen development in MP lambs but did not change ( $P > 0.05$ ) the gain to feed ratio or the chemical body composition of the lambs. A simple regression model relating ME intake to energy retention resulted in estimates of 0.45 MJ per kg  $LW^{0.75}$  per day for  $ME_m$  and a  $k_g$  value of 0.40. A multiple regression model equating ME intake to metabolic LW ( $LW^{0.75}$ ) and LWG resulted in  $ME_m$  and  $ME_g$  values of 0.45 MJ per kg  $LW^{0.75}$  per day and 12.9 MJ  $kg\ LWG^{-1} \cdot day^{-1}$ , respectively and a  $k_g$  value of 0.36. The  $ME_m$  and  $ME_g$  estimated by the multiple regression model ( $LW^{0.75}$  and LWG) showed a higher  $R^2$  (0.99) and

concordance correlation coefficient (CCC) (0.96) and, a lower relative predictive error (RPE) (5.83%) than the simple linear equation ( $r^2 = 0.83$ ; CCC = 0.91; RPE = 8.15%). The ME requirement for maintenance was similar to previously reported values while, a greater metabolisable energy for growth was shown in the present study than that previously reported for milk-only-fed lambs, which warrants further investigation.

## 4.1 Introduction

Neonate lambs are unable to digest solid food until about three to four weeks of age (Treacher and Caja, 2002), hence ewe's milk is key to their survival and growth. Accordingly, studies investigating lamb growth in early life have typically focused on the relationship between ewe milk production and lamb growth (Snowder and Glimp, 1991; Muir *et al.*, 1998; Morgan *et al.*, 2006; Morgan *et al.*, 2007). However, little is known about the association between ewe milk yield and the efficiency with which lambs use that energy for growth. van der Linden *et al.* (2010) measured ewe milk production and singleton lamb growth during the first two months post-partum and reported a poor relationship between measured milk energy yield, and lamb growth. On a weekly basis, milk yield explained just 26% of the variation in lamb growth (van der Linden *et al.*, 2010). Similarly, an increased milking ability of Poll Dorset ewes showed only a minor effect on the growth rate of lambs (Geenty *et al.*, 1985; Muir *et al.*, 1998). These studies indicate that the relationship between ewe milk production, lamb milk intake and how they contribute to growth is poorly understood.

Under pastoral conditions, lamb growth is driven by nutrient intake from both milk and pasture. Therefore, it is crucial to consider both milk and pasture consumption by lambs when evaluating variation in lamb growth. However, estimation of milk and pasture intake of suckling lambs at pasture can be challenging as neither of the dietary components can be measured directly. Rearing lambs artificially gives an opportunity to better understand the relationship between lamb milk, solid feed intake and lamb growth (Doney *et al.*, 1984) and also to investigate the maintenance and growth energy requirements of lambs. The aims of this study were: 1) to determine how much of the variation in lamb growth that could be explained under controlled feed intake

conditions, 2) to examine the effects of varying energy intake on growth, organ developments and body composition, and 3) to develop regression equations to predict metabolisable energy (ME) for maintenance and growth of pre-weaned lambs fed both milk and solid feed when reared artificially.

## **4.2 Materials and Methods**

The study was conducted at Massey University 5km South of Palmerston North, New Zealand during the months of September to December 2013. The study and animal handling procedures were approved by the Massey University Animal Ethics Committee (MUAEC protocol 13/73).

### **4.2.1 Animals and diet**

Sixteen mixed-sex twin-born Suffolk lamb sets were selected for the study. One male lamb from each set was allowed to suckle its dam for 24 hours post-partum before being separated from the dam and housed in individual indoor pens. The floor space for each pen measured 1.8 m x 1.08 m. Each pen had rubber mats as its base. Lambs were weighed at the start of the study and then every three days thereafter until the end of the study period (58 to 61 days of age. or average 59 days of age).

The lambs were randomly assigned to one of two feeding groups (n=8 per group). One group received milk replacer only by bottle feeding (MO) while the second group received the same amount of milk replacer in addition to *ad libitum* access to pellets (MP). Liquid milk replacer (Milligan's Feed Ltd, Oamaru, New Zealand) was prepared daily with water at 37°C in a ratio of 1 part milk replacer to 5 parts water (as per manufacture's recommendation based on age). The quantity of milk replacer fed and the

frequency of feeding were adjusted as lambs grew and aged. The initial amount of milk powder was 120 g/day for 6 kg lambs which was gradually increased to 210 g/day over the average 59-day study period however feeding frequency decreased from 4 feeds at day at age one day to 2 feeds per day at age 58 to 61 days. Lamb Start Mix (Reliance Feeds, Canterbury, New Zealand) was offered to MP lambs from 1 to 18 days of age to familiarise the lambs with the pellets. Performance Pellets (Reliance Feeds, Canterbury, New Zealand) were then offered from 19 days of age until the end of the study. Random samples of 50 g taken from each bag of milk replacer and pellets were pooled and kept at -20°C pending analysis.

#### ***4.2.2 Slaughter***

All lambs were euthanized at the end of the study (58 to 61 days of age. or average 59 days of age). Lambs were weighed after being fasted overnight prior to slaughter by captive bolt and exsanguination. They were then skinned, eviscerated and various carcass parameters were measured. Weights of the head, skin and feet (HSF), hot carcass and organs were recorded. The stomach and intestines were weighed before and after removal of contents to determine gut fill. Weights of the liver and kidney were also recorded. The carcass and organs were stored at -20°C until further analysis.

#### ***4.2.3 Body tissues sampling***

Body components were separated into three fractions: 1) carcass; 2) organs (comprising of all internal organs) and HSF. The fractions were each first cut into small blocks before being minced separately in a commercial butcher's mincer, once through a 10 mm grinding plate and twice through a 3 mm grinding plate. Prior to mincing, the frozen body components were re-weighed to account for moisture losses. Subsamples of

the minced tissues from the carcass and organs were collected into plastic containers, weighed, and freeze-dried.

#### **4.2.4 Proximate Analysis of diet and body tissues**

All subsamples of the body tissues (carcass, organs, and HSF) per lamb and diet samples were analysed at the Massey University Nutrition laboratory (IANZ accredited to ISO 17025) for:

- 1) nitrogen (N) by the Leco total combustion method (AOAC method 968.06);
- 2) dry matter (DM) by using a convection oven at 105°C (AOAC methods 930.15 and 925.10);
- 3) ash was determined in a furnace at 550°C (AOAC method 942.05) and
- 4) gross energy (GE) (for all samples except HSF) by bomb calorimetry.

The concentration of fat in both pellets and body tissues were determined by the Soxtec extraction method (AOAC method 991.36) whilst milk fat was determined using the Mojonnier extraction method (AOAC 954.02). The concentration of starch in both pellets was determined by the  $\alpha$ -amylase method (AOAC 996.11). Neutral detergent fibre (NDF), acid detergent fibre (ADF) and lignin concentrations in both pellets were determined using the Tecator Fibretec System following the method described by Robertson and Van Soest (1981) (AOAC method 2002.04). Fats, ash, starch, N and DM were all determined following the AOAC (1990) method. The composition of the milk and the two pellets are presented in Table 4.1.

Table 4.1 Chemical analysis of milk replacer, start mix and performance pellets as fed to lambs.

| Chemical composition     | Diet          |                        |                                  |
|--------------------------|---------------|------------------------|----------------------------------|
|                          | Milk replacer | Start mix <sup>1</sup> | Performance pellets <sup>1</sup> |
| Dry matter, %            | 96.3          | 88.7                   | 88.8                             |
| Ash, %                   | 5.8           | 7.9                    | 9.4                              |
| Protein, %               | 24.4          | 16.9                   | 17.5                             |
| Fat, %                   | 26.6          | 1.4                    | 4.4                              |
| Lactose, % <sup>2</sup>  | 38            | -                      | -                                |
| Minerals, % <sup>2</sup> | 6             | -                      | -                                |
| GE <sup>3</sup> , kJ/g   | 22.5          | 15.3                   | 15.4                             |
| Starch, %                | -             | 36.4                   | 34.7                             |
| NDF <sup>4</sup> , %     | -             | 13.8                   | 14.3                             |
| ADF <sup>5</sup> , %     | -             | 4.8                    | 5.1                              |
| Lignin, %                | -             | 0.8                    | 0.9                              |

<sup>1</sup>Composed of barley, soya bean meal, canola, peas, wheat, maize, oats, molasses, vegetable oil, grass seed meal, minerals, vitamins, prebiotics and essential oils

<sup>2</sup>Values presented as stated by the manufacturer

<sup>3</sup>GE = Gross energy

<sup>4</sup>NDF = Neutral detergent fibre

<sup>5</sup>ADF = Acid detergent fibre

#### 4.2.5 Histological studies

Square tissue samples measuring 1 cm x 1 cm were taken from the right side of the cranial ventral sac of the rumen of each animal within 30 minutes of slaughter. The tissues were washed to remove any digesta under cold tap water, placed in Tissues-Loc cassettes, and fixed immediately in a 10% formalin (buffered) solution for 48 hours. The samples were cut into two fingers measuring 1cm x 0.5 cm each. The tissues were immersed into 10% NB formalin and stored overnight. Each tissue was embedded in paraffin wax and then cut into two sections, each measuring 3µm thick. The sections were placed on slides and stained with haematoxylin and eosin and kept for histological examination. The slides were observed under a light microscope. Rumen papillae (n = 10 papillae per lamb) length and width were measured at 2.5x magnification. Papillae length was defined as the distance from the tip to the base of the papillae. The width of

the papillae was measured and recorded. The papillae circumference was determined by measuring the contour line of each papilla using ImageJ software (Rasband, 1997).

#### **4.2.6 Calculations**

Milk and pellet intake were determined as daily amount given less refusal. Total ME intake was calculated as the sum of ME in milk and pellets (for MP lambs). ME intake was calculated as  $0.91 \times \text{gross energy (GE) intake}$  (Roy, 1980) for milk and 13.5 and 13.9 MJ/kg dry matter intake (DMI) for Start Mix and Performance pellets respectively as stated by the manufacturer.

Average daily gain (ADG) was calculated as live weight gain (LWG) over the study period divided by the number of experimental days (average 59 days) and the gain to feed ratio was calculated as the kilogram LWG divided by the kilogram dry matter intake (DMI). Empty body weight (EBW) was calculated as the sum of the weight of the whole body components: HSF, carcass, and organs. EBW did not include blood weight. The organs include all internal organs (e.g., heart, lungs, kidneys, and the empty gastrointestinal system).

Crude protein was calculated as  $N \times 6.25$  for all samples. Total energy retained (TER) was calculated from the body composition of the whole empty body (all fractions put together) with the assumption that EBW equalled live weight (LW) at the start of the study and, all lambs at the start of the study contained 1.95% fat and 16.6% protein (Greenwood *et al.*, 1998) with respective energy values of 39.3 MJ/kg and 23.6 MJ/kg (SCA, 1990).

Rumen papilla was considered to be cylindrical in shape with one closed end, thus the lateral papillae surface area was calculated as:

Surface area of papillae (cm<sup>2</sup>) =  $2\pi rL + \pi r^2$  (Hill *et al.*, 2005)

Where  $r$  is the radius in cm and  $L$  is the length in cm

Maintenance energy requirement ( $ME_m$ ) was estimated for the pooled data set using two regression equations. Firstly, ME intake was regressed on TER with the assumption that the intercept determined the amount ME intake used for maintenance:

$$\text{ME intake} = ME_m + \left(1/k_g\right) \text{TER} \text{ (Birkett and de Lange, 2001)}$$

Where  $k_g$  = efficiency of utilisation of ME for growth

Secondly, ME intake from the two feeding groups as well as the initial (i) and final (f) LW data were used to derive parameters for the maintenance (m) and growth (g) energy requirements.

$$\text{ME intake} = ME_m + ME_g$$

It was assumed that the  $ME_m$  requirement was proportional to the metabolic live weight ( $LW^{0.75}$ ) of the lambs. The total metabolic live weight ( $TLW^{0.75}$ ) over the study period for each lamb was calculated using an integral equation:

$$TLW^{0.75} = \frac{\int_{LW_i}^{LW_f} LW^{0.75}}{ADG}$$

$$TLW^{0.75} = \frac{\left(\frac{LW_f^{1.75} - LW_i^{1.75}}{1.75}\right)}{ADG}$$

$$ME_m = a * TLW^{0.75}$$

ME<sub>g</sub> was proportional to the LWG over the experimental period

$$LWG = LW_f - LW_i$$

$$ME_g = b * LWG$$

To derive the factors *a* and *b*, a multiple regression was fitted to the entire data set with the intercept set to zero.

$$ME \text{ intake} = a * TLW^{0.75} + b * LWG$$

#### **4.2.7 Statistical analysis**

One lamb from the MP group died on day 42 of the experiments as a result of a rupture in the abomasum and therefore, all its intake and growth data up to day 42 was excluded from the statistical analysis. Growth performance was analysed using the generalised linear model procedure (Proc GLM) in SAS (2013) with the fixed effect of the feeding group. The carcass and organ weights were analysed using Proc GLM with the fixed effect of the feeding group and with EBW as a covariate to determine if any treatment effects on slaughter and body compositional parameters were due to differences in the EBW.

Repeat measure analysis was used to analyse the chemical composition of the tissues (carcass and organs) using the Proc MIXED procedure of (SAS, 2013) with the fixed effect of the feeding group and tissue (carcass and organs), EBW as a covariate and lamb within the feeding groups as a random effect. The interactions between fixed effects and covariate were examined to test the homogeneity of the slopes. Repeat measure analysis was performed on the histological dimensions using Proc MIXED in

(SAS, 2013) with the fixed effect of the feeding group and lamb within the feeding groups as a random effect.

The maintenance and growth energy requirements of the pooled data set were estimated using the Proc REG procedure of SAS. Relative prediction error (RPE) (Fuentes-Pila *et al.*, 1996; Fuentes-Pila *et al.*, 2003), the concordance correlation coefficient (CCC) (Lin, 1989; Nickerson, 1997) and the coefficient of determination ( $R^2$ ) were used to evaluate the goodness of fit of the equation developed. The RPE and CCC were calculated using the following equations:

$$RPE = \frac{MPE}{\hat{A}}$$

$$CCC = \frac{2SAP}{S_A^2 + S_P^2 + (\hat{A} - \hat{P})^2}$$

Where  $P_i$  is the predicted value as calculated by the multiple regression equations and  $A_i$  is the observed value of lamb<sub>*i*</sub>. Means, standard deviations, and covariance of  $A_i$  and  $P_i$  were calculated using the following equations:

$$\text{Mean prediction error (MPE)} = \sqrt{MSPE}$$

Mean square prediction error

$$(MSPE) = \frac{1}{n} \sum_i^n (P_i - A_i)^2$$

$$\hat{A} = \frac{1}{n} \sum_i^n A_i; \hat{P} = \frac{1}{n} \sum_i^n P_i$$

$$S_A^2 = \frac{1}{n} \sum_i^n (A_i - \hat{A})^2 ; S_P^2 = \frac{1}{n} \sum_i^n (P_i - \hat{P})^2$$

$$S_{AP} = \frac{1}{n} \sum_i^n (A_i - \hat{A}) (P_i - \hat{P})$$

## 4.3 Results

### 4.3.1 *Intakes and gains of lambs*

The average initial LW was not different ( $P > 0.05$ ) among feeding groups (Table 4.2), however, the final LW of MP was 5 kg greater ( $P < 0.05$ ) than MO. The difference ( $P < 0.05$ ) in live weight among the two groups began to manifest from day 30 of the study (Fig. 4.1). Total milk DMI of lambs during the study (average 59 days) was not different ( $P > 0.05$ ) for the two groups (Table 4.2) as lambs were fed the same amount of milk at the same age.

A large variation in pellet intake was observed amongst the MP lambs (Fig. 4.2). The mean pellet intake of MP lambs for the duration of the study was 125.33 g DM / day. Minimum and maximum mean pellet intakes were 32.56 g DM / day vs 150.48 g DM / day; 17.6 g DM / day vs 295.98 g DM / day and 17.6 g DM / day vs 776.16 g DM / day at 21, 35 and 56 days of age, respectively. ADG was greater ( $P < 0.01$ ) in MP than the MO group (Table 4.2). However, there was no difference ( $P > 0.05$ ) in the gain to feed ratio among the two groups. A simple linear regression was fitted to evaluate how much of the variation in LWG was explained by ME intake over the experimental period.  $LWG = -4.88 (\pm 1.15) + 0.05 (\pm 0.004) \text{ ME intake } (R^2 = 0.93; n = 15).$

Table 4.2 The effects of feeding treatments, milk only (MO) vs milk + pellets (MP) on growth performance (mean  $\pm$  SE) of lambs

| Parameter                          | Treatment          |                    | P value |
|------------------------------------|--------------------|--------------------|---------|
|                                    | MO (n = 8)         | MP (n = 7)         |         |
| Initial live weight, kg            | 6.12 $\pm$ 0.30    | 6.11 $\pm$ 0.32    | 0.97    |
| Final live weight, kg              | 13.67 $\pm$ 0.77   | 18.68 $\pm$ 0.82   | <0.01   |
| Total DMI <sup>1</sup> (milk), kg  | 10.62 $\pm$ 0.10   | 10.41 $\pm$ 0.11   | 0.18    |
| Total DMI (pellet), kg             | -                  | 7.45 $\pm$ 1.06    | -       |
| Total ME <sup>2</sup> intake, MJ   | 239.77 $\pm$ 12.65 | 327.52 $\pm$ 13.52 | <0.01   |
| Live weight gain <sup>3</sup> , kg | 7.54 $\pm$ 0.63    | 12.57 $\pm$ 0.67   | <0.01   |
| Average daily gain, g/day          | 128.37 $\pm$ 10.52 | 214.59 $\pm$ 11.24 | <0.01   |
| Gain-to-feed <sup>4</sup> ratio    | 0.71 $\pm$ 0.02    | 0.71 $\pm$ 0.02    | 0.97    |

<sup>1</sup>DMI= Dry matter intake

<sup>2</sup>ME = Metabolisable energy

<sup>3</sup>LWG = Live weight gain from day 1 to average 59 days.

<sup>4</sup>Gain-to-feed = kilogram live weight gain per kilogram DMI

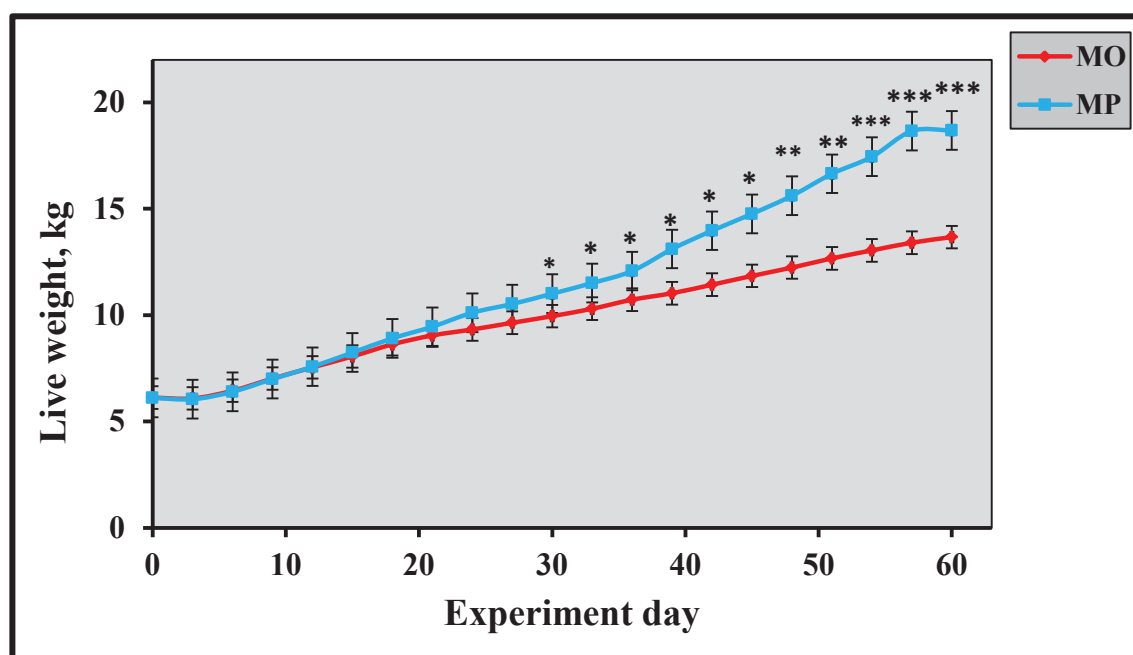


Figure 4.1 Live weights of artificially reared lambs fed milk only (MO) and milk + pellets (MP) for 59 days. Asterisk (\*) indicates significant differences in live weights of treatment groups at  $P < 0.05$  from day 30 to day 45. Asterisk (\*\*) indicates significant differences in live weights of treatment groups at  $P < 0.01$  from day 48 to day 51. Asterisk (\*\*\*) indicates significant differences in live weights of treatment groups at  $P < 0.001$  from day 54 to average 59 days. Error bars = standard error of means.

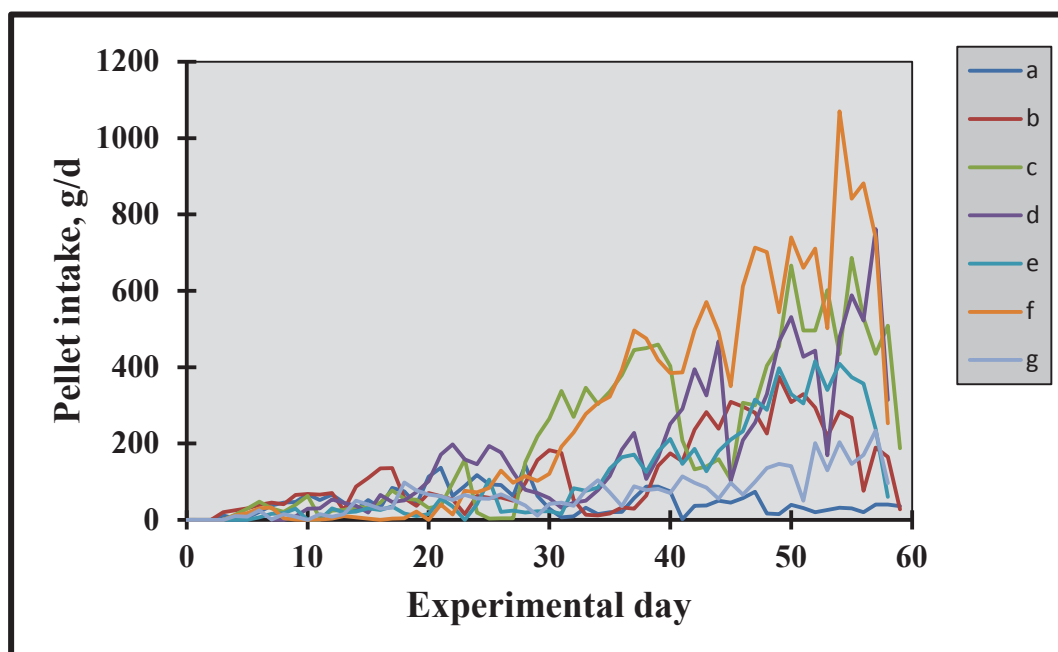


Figure 4.2 Individual lamb daily pellet intake of milk and pellet fed lambs over the study period. a to g represents individual lambs in the study.

#### 4.3.2 Carcass and organs

The addition of pellets to the diet of MP lambs significantly ( $P < 0.05$ ) increased their EBW, carcass and organ weights (Table 3). However, with the exception of the stomach weight and gut fill ( $P < 0.05$ ), differences in slaughter parameters were no longer significant ( $P > 0.05$ ) after adjusting to a common EBW (Table 4). Significant ( $P < 0.05$ ) EBW and treatment interactions were observed in the HCW, total organ weight, intestines, liver and kidney weights. In addition to the differences in weights, some subjectively scored differences were observed in the rumen, reticulum and liver among the two feeding groups (Fig 4.3, 4.4, 4.5). The liver of the MP lambs was more highly pigmented than that of the MO lambs (Fig. 4.3). The reticulum and rumen papillae of the MP lambs were also more highly pigmented than that of the MO lambs (Fig 4.4 and 4.5a, respectively).

Table 4.3 The effects of milk only (MO) vs milk and pellet (MP) feeding on the traits at slaughter (LS means  $\pm$  SE).

| Parameter                 | Treatment          |                  | P value |
|---------------------------|--------------------|------------------|---------|
|                           | MO (n=8)           | MP (n=7)         |         |
| Empty body weight, kg     | 11.93 $\pm$ 0.63   | 15.42 $\pm$ 0.67 | <0.01   |
| Hot carcass weight, kg    | 7.46 $\pm$ 0.35    | 9.35 $\pm$ 0.38  | <0.01   |
| Dressing <sup>1</sup> %   | 54.54 $\pm$ 0.47   | 50.21 $\pm$ 0.50 | <0.01   |
| Head, skin and feet, kg   | 2.75 $\pm$ 0.15    | 3.43 $\pm$ 0.16  | 0.02    |
| Total organ weight, kg    | 1.72 $\pm$ 0.15    | 2.64 $\pm$ 0.16  | 0.001   |
| Gut fill, kg              | 0.78 $\pm$ 0.15    | 2.13 $\pm$ 0.16  | <0.01   |
| Stomach <sup>2</sup> , kg | 0.22 $\pm$ 0.04    | 0.51 $\pm$ 0.04  | <0.01   |
| Intestines, kg            | 0.63 $\pm$ 0.06    | 0.91 $\pm$ 0.07  | 0.01    |
| Liver, kg                 | 187.25 $\pm$ 29.09 | 321 $\pm$ 31.097 | 0.01    |
| Kidney, kg                | 66.75 $\pm$ 6.02   | 79.43 $\pm$ 6.44 | 0.17    |

<sup>1</sup>Hot carcass weight as a percentage of final live weight.

<sup>2</sup>All four stomach compartments.

Table 4.4 The effects of milk only (MO) vs milk and pellet (MP) feeding on traits at slaughter (LS means  $\pm$  SE) using empty body weight (EBW) as a covariate

| Parameter                 | Treatment         |                   | P value <sup>1</sup> |       |                  |
|---------------------------|-------------------|-------------------|----------------------|-------|------------------|
|                           | MO (n = 8)        | MP (n = 7)        | EBW                  | Trt   | EBW $\times$ Trt |
| EBW, kg                   | 11.93 $\pm$ 0.63  | 15.42 $\pm$ 0.67  | -                    | <0.01 | -                |
| Head, skin and feet, kg   | 3.24 $\pm$ 0.09   | 3.03 $\pm$ 0.05   | <0.001               | 0.21  | 0.18             |
| HCW <sup>2</sup> , kg     | 8.64 $\pm$ 0.12   | 8.36 $\pm$ 0.07   | <0.001               | 0.75  | 0.02             |
| Gut fill, kg              | 0.92 $\pm$ 0.30   | 1.81 $\pm$ 0.16   | 0.17                 | 0.01  | 0.63             |
| Total organs, kg          | 1.80 $\pm$ 0.12   | 2.19 $\pm$ 0.07   | 0.002                | 0.20  | 0.02             |
| Stomach <sup>3</sup> , kg | 0.25 $\pm$ 0.05   | 0.41 $\pm$ 0.03   | 0.04                 | 0.02  | 0.35             |
| Intestines, kg            | 0.59 $\pm$ 0.06   | 0.73 $\pm$ 0.03   | 0.03                 | 0.82  | <0.01            |
| Liver, g                  | 174.6 $\pm$ 24.91 | 235.0 $\pm$ 13.57 | 0.03                 | 0.67  | <0.01            |
| Kidney, g                 | 36.73 $\pm$ 11.80 | 72.58 $\pm$ 6.43  | 0.06                 | 0.48  | 0.01             |

<sup>1</sup>P-value: Trt = treatment; EBW $\times$ Trt = empty body weight  $\times$  treatment interaction.

<sup>2</sup>HCW = Hot carcass weight.

<sup>3</sup>All four stomach compartments.

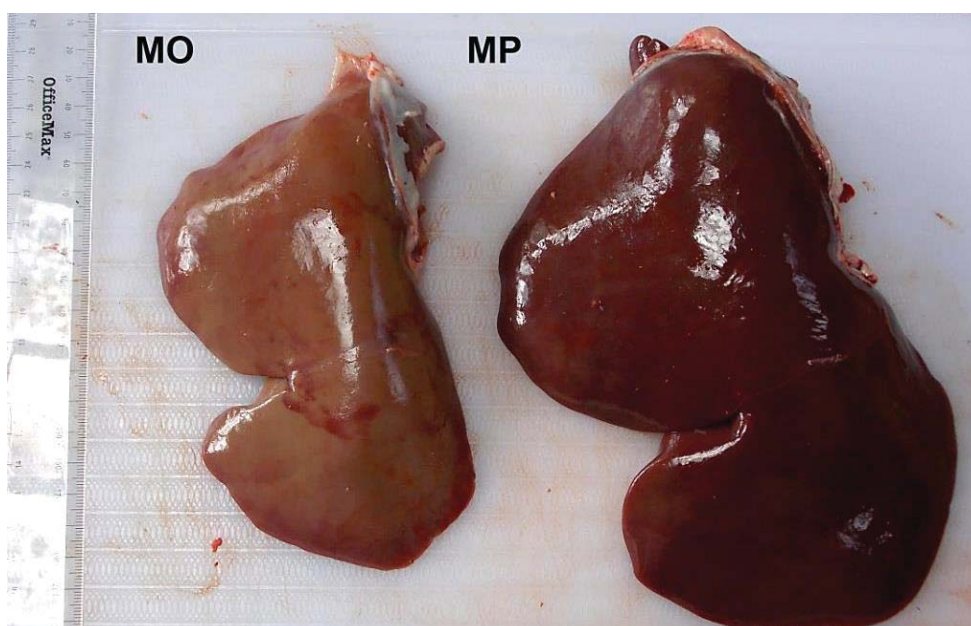


Figure 4.3 Examples of liver of milk only (MO) and milk + pellet (MP) fed lambs at average 59 days of age.

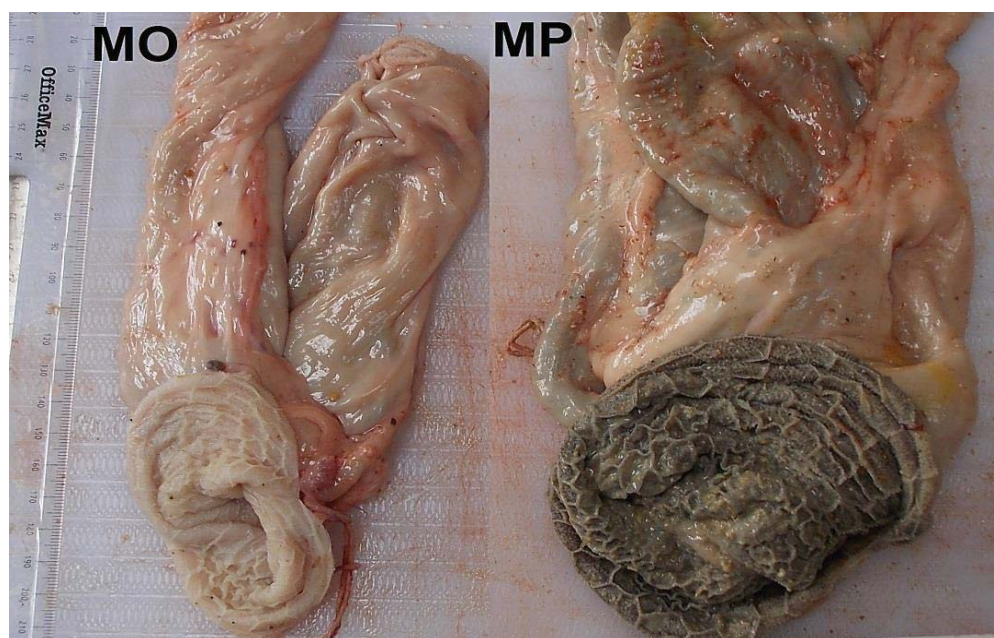


Figure 4.4 Examples of reticulum of milk only (MO) and milk + pellet (MP) fed lambs at 59 days of age.

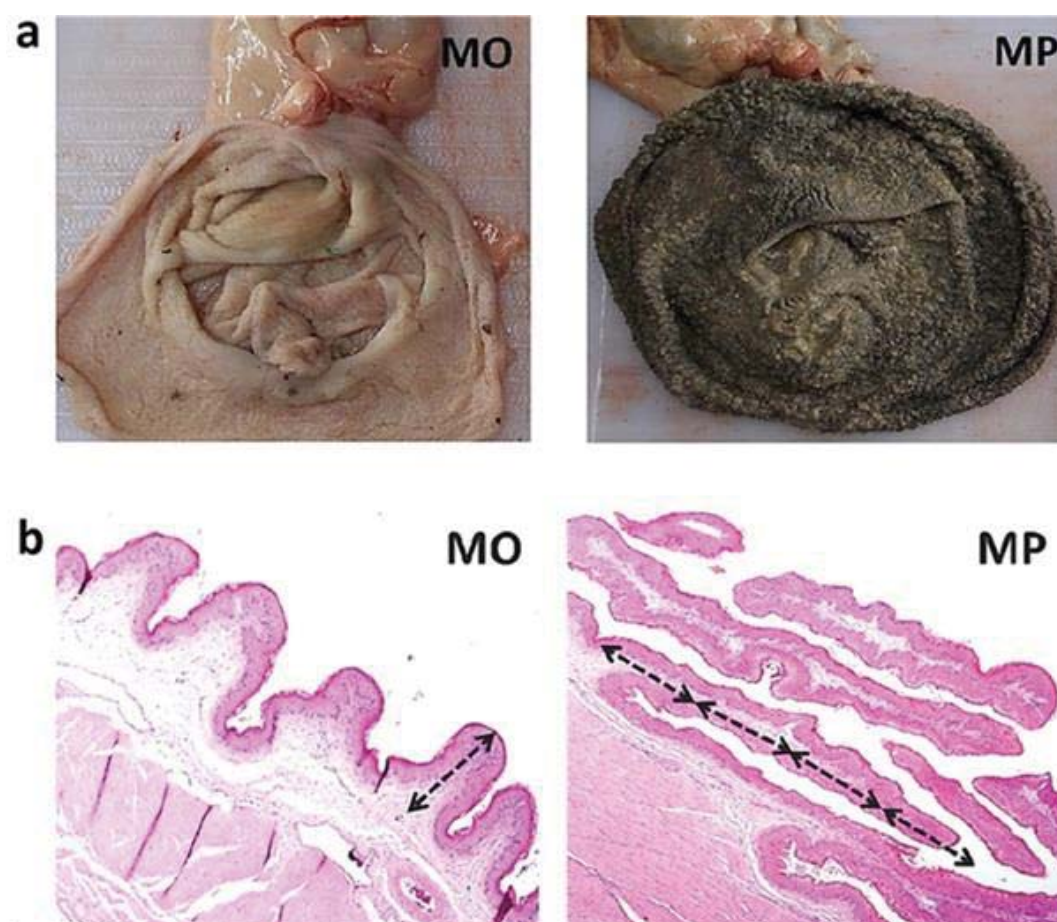


Figure 4.5 Examples of (a) Rumen development and (b) histological differences in the rumen papillae length of the milk only (MO) and milk + pellets (MP) fed lambs at average 59 days of age. Histological slides stained with haematoxylin and eosin and photos taken at 2.5x magnification using a light microscope.

The rumen of the MP lambs had longer ( $P < 0.01$ ) papillae length, circumference and a greater ( $P < 0.001$ ) papillae surface area than those from MO lambs (Table 4.5). Papillae lengths of MP lambs were almost 4 times that of the MO lambs (Fig 4.5b) but papillae width was similar ( $P > 0.05$ ) for both feeding groups (Table 4.5). A strong positive relationship ( $r^2 = 0.98$ ) was observed between the total amount of pellets consumed over the experimental period by the MP lambs and the length of their papillae (Fig 4.6).

Table 4.5 Rumen papillary development (LS means  $\pm$  SE) of milk only (MO) and milk and pellet (MP) fed lambs at average 59 days of age

| Parameter                      | Treatment            |                      | P value |
|--------------------------------|----------------------|----------------------|---------|
|                                | MO (n = 8)           | MP (n = 7)           |         |
| Length, $\mu\text{m}$          | 482.95 $\pm$ 109.33  | 1735 $\pm$ 117.5     | <0.001  |
| Width, $\mu\text{m}$           | 333.8 $\pm$ 17.18    | 339.98 $\pm$ 18.37   | 0.81    |
| Circumference, $\mu\text{m}$   | 1174.06 $\pm$ 207.79 | 3600.08 $\pm$ 220.84 | <0.001  |
| Surface area ( $\text{cm}^2$ ) | 60.61 $\pm$ 11.82    | 194.15 $\pm$ 12.64   | < 0.001 |

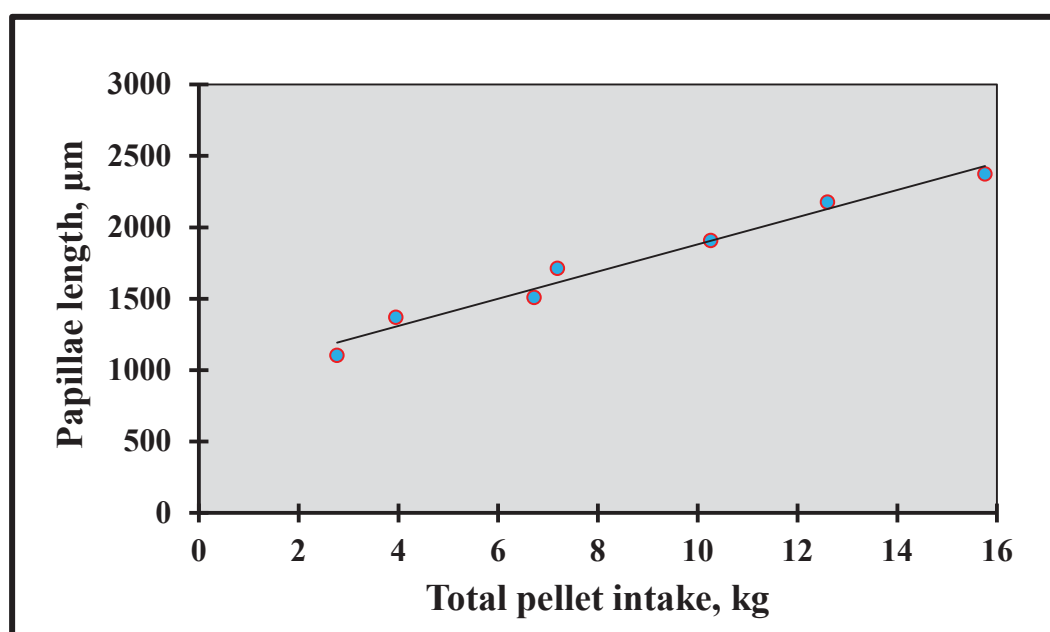


Figure 4.6 Relationship between total pellet intake over the study period and rumen papillae length of the milk and pellet fed lambs at average 59 days of age (Papillae length =  $929.9 (\pm 64.7) + 95.4 (\pm 6.8)$  pellet intake;  $r^2 = 97.5\%$ ).

#### **4.3.4 Chemical composition of carcass and organs**

Generally, amounts of water, protein, fat, ash and GE increased ( $P < 0.01$ ) as EBW increased ( $\beta = 0.26; 0.07; 0.05; 0.02$  and  $3.44$  for water, protein, fat, ash and GE, respectively) and they were higher ( $P < 0.01$ ) in the carcasses than the organs irrespective of the dietary treatments (Table 4.6). There were significant ( $P < 0.01$ ) tissue and treatment interactions amongst the chemical components with the exception of the water content. Whilst the protein and ash content of the carcass for both treatments remained the same, the organs of MO lambs contained more ( $P < 0.05$ ) protein and ash than that of MP lambs. MP lambs had higher ( $P < 0.01$ ) fat content in their carcass than in MO lambs. However, fat content in the organs was not significantly ( $P < 0.05$ ) different. Likewise, GE content in the MP carcasses was greater ( $P < 0.01$ ) than in MO carcasses, whereas the GE in the organs remained the same.

The chemical components per kilogram LWG did not differ ( $P > 0.05$ ) among the feeding groups with the exception of water content; MO lambs had greater ( $P < 0.05$ ) water content than the MP lambs (Table 4.7). The chemical composition of all the whole bodies (all body fractions together) of the lambs, however, did not differ ( $P > 0.05$ ) among the two feeding groups (data not shown).

Table 4.6 The effects of feeding treatment; milk only (MO) vs milk and pellet (MP) on the chemical composition (LS means  $\pm$  SE) of carcass and organs of the lambs using empty body weight (EBW) as a covariate

| Item             | Carcass                       |                               | Organs                        |                               | P- value <sup>1</sup> |       |      |                  |
|------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-----------------------|-------|------|------------------|
|                  | MO (n = 8)                    | MP (n = 7)                    | MO (n = 8)                    | MP (n = 7)                    | EBW                   | Tis   | Trt  | Tis $\times$ Trt |
| Water, kg        | 5.91 $\pm$ 0.09               | 6.13 $\pm$ 0.10               | 2.06 $\pm$ 0.09               | 1.94 $\pm$ 0.10               | <0.01                 | <0.01 | 0.68 | 0.11             |
| Crude protein kg | 1.50 $\pm$ 0.03 <sup>c</sup>  | 1.57 $\pm$ 0.03 <sup>c</sup>  | 0.39 $\pm$ 0.03 <sup>b</sup>  | 0.28 $\pm$ 0.03 <sup>a</sup>  | <0.01                 | <0.01 | 0.23 | 0.02             |
| Ash, kg          | 0.38 $\pm$ 0.01 <sup>c</sup>  | 0.41 $\pm$ 0.01 <sup>c</sup>  | 0.05 $\pm$ 0.01 <sup>b</sup>  | 0.01 $\pm$ 0.01 <sup>a</sup>  | <0.01                 | <0.01 | 0.84 | 0.01             |
| Fat, kg          | 0.45 $\pm$ 0.05 <sup>b</sup>  | 0.63 $\pm$ 0.05 <sup>c</sup>  | 0.18 $\pm$ 0.05 <sup>a</sup>  | 0.10 $\pm$ 0.05 <sup>a</sup>  | <0.01                 | <0.01 | 0.44 | < 0.01           |
| Gross energy, MJ | 52.39 $\pm$ 2.17 <sup>b</sup> | 60.73 $\pm$ 2.35 <sup>c</sup> | 16.40 $\pm$ 2.35 <sup>a</sup> | 10.97 $\pm$ 2.35 <sup>a</sup> | <0.01                 | <0.01 | 0.59 | 0.01             |

<sup>a,b,c</sup> values in a row with different superscripts are different from each other (LSD,  $P < 0.05$ )

<sup>1</sup>P-value: Tis = tissue; Trt = treatment; Tis $\times$ Trt = tissue  $\times$  treatment interaction

Table 4.7 The chemical components (LS means  $\pm$  SE) per kilogram live weight gain (LWG) of lambs as affected by the dietary treatment; milk only (MO) vs milk and pellet (MP).

| Parameter               | Treatment         |                   | P value |
|-------------------------|-------------------|-------------------|---------|
|                         | MO (n = 8)        | MP (n = 7)        |         |
| Water, g/kg LWG         | 593.41 $\pm$ 9.09 | 553.80 $\pm$ 9.72 | 0.01    |
| Protein, g/kg LWG       | 107.31 $\pm$ 3.07 | 102.13 $\pm$ 3.28 | 0.27    |
| Ash, g/kg LWG           | 19.10 $\pm$ 1.05  | 20.38 $\pm$ 1.12  | 0.42    |
| Fat, g/kg LWG           | 50.58 $\pm$ 5.75  | 65.85 $\pm$ 6.15  | 0.09    |
| Gross energy, MJ/kg LWG | 4.38 $\pm$ 0.26   | 4.88 $\pm$ 0.27   | 0.20    |

#### 4.3.5 Maintenance and growth energy requirements

Maintenance energy requirement of the lambs for the pooled data set was estimated using both simple and multiple regressions equations. The simple linear regression below assumed ME intake was partitioned into ME<sub>m</sub> and TER:

$$\text{Total ME intake} = 160 + 2.48 \text{ TER} (R^2 = 0.83; \text{RPE} = 8.15\%; \text{CCC} = 0.91)$$

The intercept from the above equation (160 MJ) is an estimate of the ME<sub>m</sub> over the entire experimental period. To estimate the ME<sub>m</sub> per kg LW<sup>0.75</sup> per day, 160 MJ was divided by the average metabolic LW<sup>0.75</sup> of all the lambs (6.01 kg) over the average age of 58.66 days.

$$\begin{aligned} \text{ME}_m &= \frac{160 \text{ MJ}}{(6.01 \text{ kg} \times 58.66 \text{ days})} \\ &= 0.45 \text{ MJ ME per kg LW}^{0.75} \text{ per day} \end{aligned}$$

The above regression equation showed that the efficiency of ME utilisation for growth was 0.40 (inverse of 2.48).

The multiple regression equation showing the relationship between total ME intake, total metabolic live weight ( $TLW^{0.75}$ ) and live weight gain over the experimental period was:

$$\text{Total ME intake} = 0.43 (\pm 0.07) TLW^{0.75} + 12.9 (\pm 0.58) LWG (R^2 = 0.99; \text{RPE} = 5.82\%; \text{CCC} = 0.96).$$

The above equation showed lambs in the present study used 0.43 MJ ME to maintain 1kg  $LW^{0.75}$  per day and 12.9 MJ ME to gain a 1kg of LW per day.

## **4.4 Discussion**

### **4.4.1 Growth performance**

Studies on artificial rearing have generally reported that the intake of solid feed before 21 days of age is negligible ( $< 5\text{g/day}$ ) (Owen, 1969; Walker and Hunt, 1981; Lane and Jesse, 1997) particularly when milk was offered concurrently (Walker and Hunt, 1981). In the present study, some MP lambs started nibbling at the pellets as early as 3 days of age, although milk was fed as per manufacturer's recommendations and by 9 days of age, all MP lambs were observed to be consuming pellets with intakes ranging from 13.32 g to 63.28 g DM/day. Early solid feed consumption improved the ADG of MP lambs, in agreement with the findings of Karim *et al.* (2001) and Bhatt *et al.* (2009). Improving the growth rates of lambs during the pre-weaning stage has been shown to subsequently improve post-weaning growth rates (Bhatt *et al.*, 2009; Galvani *et al.*, 2014). The current results suggest that access to solid feed improves the growth rates of pre-weaned lambs and that farmers rearing lambs artificially would likely see the benefits of providing solid feed.

The gain to feed ratio did not differ between feeding groups, which is consistent with Potchoiba *et al.* (1990) who observed no differences in two groups of goat kids fed either milk only or milk and solid feed. The gain to feed ratio of 0.71 obtained in the present study compares favourably with the ratios reported previously for lambs (0.69 to 0.73; (Chiou and Jordan, 1973b; Hodge, 1974; Greenwood *et al.*, 1998) and calves (0.64 to 0.80; (Khouri and Pickering, 1968; Diaz *et al.*, 2001) at similar stages of maturity.

A greater proportion ( $r^2 = 0.93$ ) of LWG was explained by ME intake in the present study in comparison to previous studies ( $r^2 = 0.20$  to  $0.43$ ) in lambs reared on their dams (Geenty and Dyson, 1986; van der Linden *et al.*, 2010). Doney *et al.* (1984) reported that about 84% of the variation in lamb LWG was explained by milk and pasture intake in artificially reared lambs. A possible explanation for the difference between studies may be attributed to the controlled conditions under which the present experiment and that of Doney *et al.* (1984) were conducted, allowing for more accurate estimates of milk and solid feed intake.

#### **4.4.2 Effects of dietary treatment carcass and organ development**

Weights of kidneys, liver and gastrointestinal tract (GIT) of older lambs (Johnson *et al.*, 1990) and in the GIT of rats (Ferrell *et al.*, 1986) have previously been shown to increase proportionately with greater energy intake. Contrary to these findings, weights of these organs in the present study, with the exception of the stomach, were not different between treatments after adjusting to a common EBW. The differences in stomach weights among the two groups in the present study was not unexpected as lambs fed milk and pellet had almost twice as much gut fill in their GIT as lambs fed milk only. The absence of differences in the remaining organ weights among treatment groups after adjusting to a common EBW supports the findings that growth of the

visceral organs was more closely associated with EBW than dietary treatment in young lambs (Greenwood *et al.*, 2004).

Lambs fed pellets in addition to milk, had greater rumen papillae development with respect to papillae length, circumference, and surface area. This is consistent with previous findings (Harrison *et al.*, 1960; Lane and Jesse, 1997; Coverdale *et al.*, 2004; Lesmeister and Heinrichs, 2004). The absence of difference in rumen papillae width supports the finding that papillae width is an indicator of age on rumen development rather than diet type (Lesmeister *et al.*, 2004). Papillae length increased with increasing pellet intake. Interestingly, at approximately 9 weeks, a proportion of the milk and pellet-fed lambs had papillae lengths equal to those reported by Ward (2008) in 12 weeks old lambs creep fed from 2 weeks of age. These results indicate that the addition of solid feed to milk diet very early in a lamb's life enhances rumen development which may have positive effects later in life

#### **4.4.3 Chemical composition of body tissues**

Chemical composition differed when carcass and organs were compared but no differences were present in the combined analysis, indicating only a minor effect of dietary treatments. Black (1974) reported that dietary treatment may alter an animal's chemical body composition when comparisons are made at the same age but are not present at a constant body weight. Searle *et al.* (1972) observed that the body composition of lambs during the milk feeding phase (3 weeks of age) was similar to that of lambs in the transitional stage (3 – 9 weeks of age). It is likely that MO lambs were similar in growth stage to milk phase lambs while MP lambs would be similar to the transition lambs in the study of Searle *et al.* (1972). Therefore, the lack of difference in body composition was not unexpected.

#### 4.4.4 Maintenance and growth energy requirements

Maintenance and  $ME_g$  requirements were estimated using the pooled dataset of the two feeding groups. For comparative purposes,  $ME_m$  and efficiency of ME utilisation in the present study were estimated using two regression methods. The simple regression equation (ME intake on ER) was considered as the standard for estimating  $ME_m$  and  $k_g$  (Birkett and de Lange, 2001). The accuracy of the multiple regression (ME intake on  $LW^{0.75}$  and LWG) was determined by comparing the multiple regression estimations of  $ME_m$  and  $k_g$  to the simple regression estimations as well as using the RPE and CCC values. RPE values of less than 10% can be regarded as satisfactory (Fuentes-Pila *et al.*, 2003) for  $ME_m$  or  $k_g$  prediction and a CCC value greater than 0.81 indicates an almost perfect prediction (Visser *et al.*, 2012). In the present study, values below the 10% level for RPE and above 0.81 for CCC were reached with both the simple and multiple regression models indicating that both models were accurate predictors of  $ME_m$ . However, lower RPE (5.83% vs 8.15%) and higher CCC (0.96 vs 0.91) values were obtained with the multiple regression model indicating the latter was more accurate.

Estimations of  $ME_m$  in lambs have often been limited to either very young lambs (up to 3 weeks of age) subsisting on milk only (Jagusich and Mitchell, 1971; Walker and Norton, 1971; Chiou and Jordan, 1973a) or weaned lambs greater than 20 kg LW consuming solid feed only (Mitchell and Jagusch, 1972; Thomson *et al.*, 1979; Alam *et al.*, 1991). However, the estimates for  $ME_m$  from the two regression methods 0.43 MJ/kg  $LW^{0.75}$  and 0.45 MJ/kg  $LW^{0.75}$ , obtained in the present study are slightly higher than the value of 0.39 MJ / kg  $LW^{0.75}$  obtained for 60-day old Segurenia lambs fed a milk only diet (Sanz Sampelayo *et al.*, 1995) and lower than the values of 0.50 – 0.61 MJ/kg  $LW^{0.75}$  that have been reported in housed suckling lambs (Jagusich and Mitchell, 1971;

Chiou and Jordan, 1973a; Degen and Young, 1982). Generally, housed lambs have a lower  $ME_m$  requirement than grazing lambs (Sykes and Nicol, 1983) due to minimal use of energy for activity (Luo *et al.*, 2004). The pre-mentioned studies indicate the significant variability in  $ME_m$  amongst indoor fed lambs. Variability in  $ME_m$  can be attributed to the different methods used in calculating maintenance energy, different breeds, diet, age and body weight of the lambs (CSIRO, 2007).

It is generally accepted that the ME requirement for LWG is mainly determined by the proportion of fat and protein contributing to weight gain (SCA, 1990; NRC, 2007; Deng *et al.*, 2012). This requirement is higher in growing animals that gain more fat than those that gain more protein (Luo *et al.*, 2004; Nicol and Brookes, 2007; NRC, 2007). Pre-weaned lambs generally have a greater proportion of protein than fat in their weight gain (Walker and Norton, 1971; Searle *et al.*, 1972) and therefore, their ME requirements for growth are lower than that of mature sheep. For pre-weaned milk-fed male lambs of 15 – 20 kg live weight growing at 200 g/day, ARC (1980) recommends an ME intake of 6.4 – 7.8 MJ/kg LWG which is lower than the estimated value in the present study (12.9 MJ/ kg LWG). For 25 kg weaned rams recommendations of 25 MJ ME per kg LWG have been reported (Nicol and Brookes, 2007). There is limited information on the growth energy requirements of pre-weaned lambs below 20 kg fed either milk only or milk and solid feed concurrently. Thus, additional research to determine  $ME_g$  is warranted. Nevertheless, the value obtained in the present study is similar to that reported by (Luo *et al.*, 2004) and Sahlu *et al.* (2004) for pre-weaned goats (13.4 MJ /kg LWG), suggesting that a value of about 13 MJ / kg LWG is justifiable until further work is undertaken.

#### 4.4.5 Efficiency of ME utilisation

The multiple regression model indicated that lambs used 12.9 MJ ME to gain a kilogram of live weight and the average energy retained per kg LWG estimated from the chemical body composition analysis was 4.63 MJ. Thus, the  $k_g$  value of 0.36 ( $4.63 \text{ MJ}/12.9 \text{ MJ}$ ) is similar to the  $k_g$  value of 0.40 calculated in the simple regression model of ME intake on tissue gain. These  $k_g$  values (0.36 and 0.40) are lower than values of 0.69 to 0.77 obtained in pre-weaned lambs fed milk only (Jagusich and Mitchell, 1971; Walker and Norton, 1971; Degen and Benjamin, 2005) but slightly higher than values obtained in early weaned lambs on solid feed (0.13 – 0.29) (Fennessy *et al.*, 1972; Mitchell and Jagusch, 1972; Thomson and Cammell, 1979). Combined these studies indicate that young lambs utilise solid feed with a much lower efficiency than liquid diets. Differences in efficiency of utilisation between milk and solid feed may be attributed to differences in the digestibility of the feeds (Thomson, 1972; Rattray and Joyce, 1974; Thomson and Cammell, 1979). As there was no variation in the total amount of milk consumed by lambs in the present study (due to controlled intake), it seems likely that the  $k_g$  value was influenced by the variation in pellet intake of the MP lambs which may have accounted for the lower  $k_g$  value obtained in comparison to some of the previous studies where lambs were fed milk only (Jagusich and Mitchell, 1971; Walker and Norton, 1971; Degen and Benjamin, 2005). It would be of interest to investigate the efficiency of ME utilisation for growth in young lambs offered both milk and solid feed concurrently at different levels of energy intake to determine this.

## 4.5 Conclusion

Feeding pellets in addition to milk replacer increased ADG, stomach weights and rumen development but did not change the gain to feed ratio or the chemical body composition of the lambs adjusted to the same weight. The estimated metabolisable energy requirement for maintenance ( $0.45 \text{ MJ} / \text{kg LW}^{0.75}$ ) was similar to that previously reported. However, a greater metabolisable energy was required for growth ( $12.9 \text{ MJ} / \text{kg LWG}$ ) in the present study compared to the recommendation of ARC and warrants further investigation. Early pellet consumption in pre-weaned lambs stimulated lamb rumen development at a very early age, and increased lamb growth. This suggests farmers should consider offering solid feed when artificially rearing lambs.

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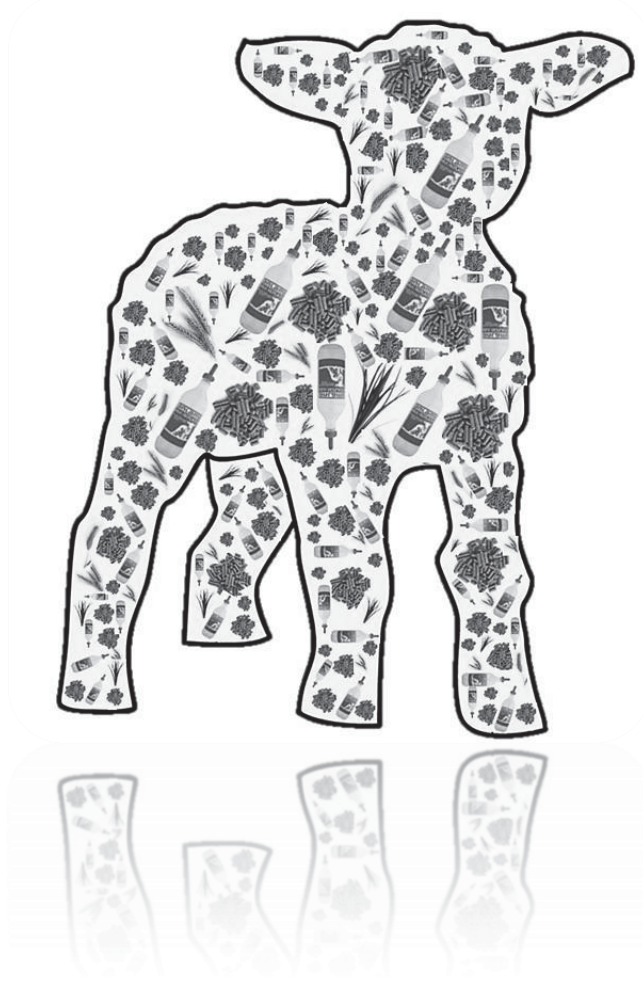




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## Chapter 5      The Effect of Different Feeding Regimens on Energy and Protein Utilisation and Partitioning For Maintenance and Growth in Artificially Reared Lambs

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Chapter based on the following publication:

**DANSO, A. S., MOREL, P. C. H., KENYON, P. R., and BLAIR, H. T.** Effect of different feeding regimens on energy and protein utilization and partitioning for maintenance and growth in pre-weaned lambs reared artificially. *Journal of Animal Science*, 94. doi:10.2527/jas.2016-0871

## FOREWORD

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The previous chapter (Chapter 4) in addition to explaining the proportion of variation explained by ME intake by lambs, the effects of energy intake on growth, chemical body composition and the utilisation of metabolisable energy for maintenance and growth of pre-weaned lambs reared artificially were also estimated. A more accurate evaluation of energy utilisation can be obtained by measuring the quantity of feed consumed together with the quantity voided in faeces and urine. This was not undertaken in Chapter 4 which limits the accuracy of ME estimated. Further, the nutrients retained within the empty body weights of lambs at the start of the experiment was calculated using other baseline data as no lambs were sacrificed at the start of the study. This could also have implications for the accuracy of estimates made. Additionally, feeding lambs to a similar age, made it difficult to establish if differences in body composition were a result of the treatment effect or differences in live weight. Thus, statistical analysis was performed using the covariate approach. Therefore, the experiment reported in Chapter 5 was designed to help address the potential limitations and to provide new information.

## ABSTRACT

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Estimation of metabolisable energy (ME) requirement for maintenance ( $ME_m$ ) and growth ( $ME_g$ ) in pre-weaned lambs has been limited in previous studies to milk-only fed lambs. This study aimed to determine energy and nitrogen (N) metabolisability of milk and pellets when fed together, in order to compare the growth and chemical body composition of lambs fed varying levels of pellets in addition to milk, and to estimate  $ME_m$ ,  $ME_g$ , and the CP:ME requirements for growth. The study included 32 twin-born Romney-cross ram lambs. Four lambs were slaughtered at 24 hours post-partum to estimate initial body composition and the remaining 28 were assigned to one of four treatment groups of seven. Group one was fed milk replacer (MR) only; group two was fed MR and allowed *ad libitum* access to pellets; groups three and four were offered 30% and 60% respectively of the average pellet intake of the *ad libitum* group the previous day whilst being fed MR. Milk replacer was fed as a proportion of the lamb's live weight (LW). Lambs from each treatment were placed in metabolic cages at 17 kg LW for 4 days to allow for total faecal and urine collection. All lambs were slaughtered at 18 kg LW. Average daily gains (ADG), ADG:ME ratio, stomach and liver weights, and rumen papillae lengths increased ( $P < 0.05$ ) with increasing pellet intake. Increasing daily ME intake increased ( $P < 0.05$ ) both daily energy and protein deposition but had no effect ( $P > 0.05$ ) on fat deposition. However, the total chemical body composition at 18 kg was unaffected ( $P > 0.05$ ) by dietary treatment. Digestibility of energy and N decreased ( $P < 0.05$ ) with increasing ME intake. Percent energy and N retained for growth were 96% vs 71% and 72% vs 30% for milk and pellets, respectively. The  $ME_m$  and  $ME_g$  values obtained were  $0.40 \text{ ME/kg LW}^{0.75} \cdot \text{d}^{-1}$  and  $13.8 \text{ MJ / kg ADG}$ , respectively. The CP:ME ratios for MR and pellet were  $11.1 \text{ g MJ}^{-1}$  and  $15.7 \text{ g MJ}^{-1}$ , respectively. However, a simulation model suggested that lambs require a CP:ME ratio

of 13.1 at 5 kg and 10.9 at 18 kg LW, indicating that protein intake may be limiting to lamb growth in early life and in excess by 18 kg LW. In conclusion, increasing pellet intake was associated with decreased N retention. The inclusion of pellets, however, improved the efficiency of ME utilization for growth in pre-weaned lambs and was beneficial for rumen development. The  $ME_g$  was higher than previously recommended values and the CP:ME intake of lambs does not match their requirements which may warrant further studies.

## 5.1 Introduction

The profitability of any farm animal production system is dependent on its inputs and outputs (Arthur *et al.*, 2004). Feed, and particularly energy intake, is the single most important input and is the major component of production costs (Ramsey *et al.*, 1998). To limit costs, it is important to understand the factors that contribute to energy use and partitioning in animals. Energy utilisation and partitioning have been studied in calves (Gerrits *et al.*, 1996; Diaz *et al.*, 2001; Blome *et al.*, 2003), sheep (Ratnayake *et al.*, 1974; Ratnayake and Joyce, 1976) and goats (Sanz Sampelayo *et al.*, 1995; Sanz Sampelayo *et al.*, 2003; Luo *et al.*, 2004). However, very few studies have been carried out in growing lambs especially during the pre-weaning period (Greenwood *et al.*, 1998). To the best of the authors' knowledge, studies in this area occurred more than 30 years ago (Gardner *et al.*, 1964; Norton *et al.*, 1970; Walker and Norton, 1971; Hodge, 1974; Ratnayake *et al.*, 1974). In New Zealand, lamb pre-weaning growth rates have improved by 50 g / day since the 1980's from 150 g /day to 200 g /day (Morris and Kenyon, 2014), suggesting that energy utilisation from the diet for maintenance and growth may be altered to accommodate such growth rates.

Nutrient requirements and utilisation by animals vary with breed, diet, management and the environment (Galvani *et al.*, 2008). Solid feed consumption in young lambs has been observed to commence as early as three days of age, making up about 42% of their total dry matter intake in the first two months of life (Danso *et al.*, 2014). However, recommendations of nutrient requirements for growing lambs pre-weaning have generally focused on milk-only fed lambs (Jagusich and Mitchell, 1971; Chiou and Jordan, 1973). Therefore, the aims of the present study were: 1) to determine the energy and nitrogen balance of milk and pellets when fed together, 2) to compare the growth

and chemical body composition of lambs as affected by increasing levels of energy intake, and 3) to estimate maintenance and growth energy requirements and the protein to energy requirements for growth in lambs.

## **5.2 Materials and methods**

The study was conducted from August to December, 2014, at Massey University, 5 km South of Palmerston North, New Zealand. The experimental protocol was approved by the Massey University Animal Ethics Committee (MUAEC protocol 14/64).

### **5.2.1 *Experimental design, animals and management***

Thirty-two sets of Romney mixed-sex twin lambs were selected for the study at 24 hours post-partum. One male lamb from each set was then separated from the dam. Four lambs were slaughtered at separation to provide baseline (BL) data and the remaining 28 were moved indoors and hand-reared. All lambs were kept in single pens with floor space measuring 1.94m<sup>2</sup>. All pens had rubber mats. The average minimum and maximum room temperature recorded over the study period were 15°C and 23°C, respectively.

All lambs were fed milk replacer (MR) by bottle feeding. Liquid MR (Milligans Feed Ltd, Oamaru, New Zealand) was prepared daily with 39°C tap water in a ratio of 1 part MR to 4 parts water. Lambs were fed four times daily and the quantity of MR fed was adjusted as lambs grew. The quantity of MR powder fed to lambs was to provide 1.5 times maintenance using the following equation (Danso et al., 2014):

$$\text{Milk replacer/day (g)} = 66.48 + 14.3 \text{ live weight (kg)}$$

The 28 lambs were allocated to four treatment groups. The first group (MO; n=7) received MR only and had no access to pellets, the remaining three groups (n=7 per group) received the same level of MR in addition to varying amounts of concentrate pelleted feed to encourage variation in ME intake: The MP<sub>ad</sub> group was offered *ad libitum* access to pellets whilst the MP<sub>30</sub> and MP<sub>60</sub> groups were restricted to 30% and 60% of the average pellet intake of the MP<sub>ad</sub> group on the previous day, respectively. Performance Pellets (Reliance Feeds, Canterbury, New Zealand) were offered were offered in feeders to MP<sub>ad</sub>, MP<sub>30</sub>, and MP<sub>60</sub> lambs from the start of the study until slaughter at 18 kg live weight (LW).

Milk intake and pellet intake were recorded as daily amount offered minus refusal. Lambs were weighed twice a week, on Mondays and Thursdays before the first feeding of the day and the MR allowance was adjusted accordingly.

### **5.2.2 Total faecal and urine collection**

At 17 kg LW four lambs from each treatment were put in metabolic crates for four days until slaughter at 18 kg LW. Feeding of lambs continued as usual. Total faeces and urine excreted were collected and weighed daily. Each day, 0.2 g of Potassium dichromate (K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>) was put into the urine collection bottle to inhibit growth of bacteria and enzymatic processes that cause loss of nitrogen (N) (Diaz *et al.*, 2001). Daily faecal samples collected per lamb were freeze-dried and pooled over the four-day collection period. The daily urine collection was filtered through cotton wool and subsampled. The four subsamples were then pooled per lamb. All samples were stored at -20°C for further analysis.

### **5.2.3 Slaughter**

All lambs were slaughtered when they reached the target LW of 18 kg regardless of age. Prior to slaughter, lambs were weighed after being fasted overnight. They were slaughtered by captive bolt and exsanguination, skinned, eviscerated and carcass parameters were measured. Weights of the head, skin and feet, hot carcass and organs were recorded. The stomach and intestines were weighed before and after removal of contents to determine gut fill. Weights of the liver, kidneys, and spleen were also recorded. The carcass and organs were then stored at -20°C until further analysis.

### **5.2.4 Body tissues sampling**

Body components were separated into four fractions: carcass, all internal organs, head, and skin. The fractions were each cut into small blocks and minced separately in a commercial butcher's mincer, once through a 10-mm grinding plate and once through a 6-mm grinding plate and finally in a kitchen mincer through a 3-mm grinding plate. Subsamples of the ground body components were collected into plastic containers, weighed and stored at -20°C until further analysis

### **5.2.5 Proximate Analysis**

All diet, body tissue, faecal and urine samples were analysed according to AOAC, (1990) for N by the Leco total combustion method (AOAC method 968.06), dry matter (DM) using a convection oven at 105°C (AOAC methods 930.15 and 925.10), ash was determined in a furnace at 550°C (AOAC method 942.05), organic matter (OM) following the AOAC method 942.05 and gross energy (GE) was determined using bomb calorimeter (Leco, AC 350, Leco Corporation, St Joseph, MI, USA).

Urine samples were assayed for N and GE. Fats in the pellets and in body tissues were determined by the Soxtec extraction method (AOAC method 991.36; AOAC (1990)) while fat in milk was determined using the Mojonnier extraction method (AOAC 954.02; AOAC (1990)). Neutral detergent fibre (NDF), acid detergent fibre (ADF) and lignin in both the pellet and faecal samples were determined using the Tecator Fibretec System (AOAC method 2002.04) following the method described by Robertson and Van Soest (1981). The composition of MR and pellets are presented in Table 5.1.

Table 5.1 Chemical analysis of milk replacer and pellets as fed to lambs

| Chemical component         | Milk replacer | Pellets <sup>1</sup> |
|----------------------------|---------------|----------------------|
| Dry matter, %              | 96.9          | 88.2                 |
| Ash, %                     | 5             | 8.9                  |
| Crude protein, %           | 24.4          | 16.9                 |
| Fat, %                     | 27            | 2.8                  |
| Lactose <sup>2</sup> , %   | 38            | -                    |
| Minerals <sup>2</sup> , %  | 6             | -                    |
| Gross energy, kJ/g         | 22.7          | 15.2                 |
| Neutral detergent fibre, % | -             | 11.8                 |
| Acid detergent fibre, %    | -             | 4.3                  |
| Lignin, %                  | -             | 0.9                  |

<sup>1</sup>Composed of barley, soya bean meal, canola, peas, wheat, maize, oats, molasses, vegetable oil, grass seed meal, minerals, vitamins, prebiotics and essential oils

<sup>2</sup>Values presented as stated by the manufacturer; GE: Gross energy; NDF: Neutral detergent fibre; ADF: Acid detergent fibre.

### 5.2.6 Histological studies

Two square tissue samples measuring 1 cm<sup>2</sup> were taken from the left side cranial ventral sac (Lesmeister *et al.*, 2004) of the rumen of each lamb within 30 minutes of slaughter. The tissues were placed in Tissues-Loc cassettes and fixed immediately into a 10% formalin (buffered) solution for 48 hours. The samples were then cut into two fingers

measuring 1 cm × 0.5 cm each. The tissues were put into 10% NB formalin and stored overnight. Each tissue was embedded into paraffin wax and then cut into two sections, each measuring 3µm thick. The sections were placed on slides and stained with haematoxylin and eosin and kept for histological examination. The slides were observed under a light microscope. Rumen papillae (n= 20 per lamb) length, width and circumference were measured at 2.5x magnification. Papillae length was defined as the distance from the tip to the base of the papillae. The width of the tip, middle, and base of the papillae were measured and the average of the three measurements was recorded as the width of the papillae. The papillae circumference was determined by measuring the contour line of each papilla using ImageJ software (Rasband, 1997). Rumen papilla was considered to be cylindrical in shape with one closed end, thus the lateral papillae surface area was calculated as:

$$\text{Surface area of papillae (cm}^2\text{)} = 2\pi rL + \pi r^2 \text{ (Hill } et al., 2005\text{)}$$

Where r is the radius in cm and L is the length in cm

### **5.2.7 Calculations**

Average daily gain (ADG) of each lamb was calculated as live weight gain (LWG) over the study period divided by the number of experimental days per lamb. The gain-to-feed ratio was calculated as the kilogram LWG divided by the kilogram dry matter intake (DMI).

Empty body weight (EBW) of the baseline group and at the end of the study was calculated as the sum of the weight of the whole body components: head, skin and feet, carcass and digesta-free organs. The initial EBW of each lamb was calculated as 88.2%

of the initial LW of each lamb as derived from the BL slaughter group. The amount of DM, GE, protein, fat, ash, and water in the initial EBW were each calculated by multiplying the average percentage chemical composition in the EBW of the BL group by the initial EBW of each lamb. The composition of gain for each lamb at the slaughter (18 kg LW) was calculated as the difference between the amounts of DM, GE, protein, fat, ash, and water in the empty bodies of the lambs at slaughter and the initial EBW body composition. Crude protein for body tissue samples was calculated as  $N \times 6.25$ .

Gross energy and N intake were calculated as milk and pellet intake multiplied by the nutrient content of each feed. Apparent digestibilities of energy and N were determined by subtracting the energy and N excreted in faeces from the amount of energy and N consumed, respectively. Metabolisable energy (ME) and retained N (RN) were determined by subtracting energy and N excreted in urine from the digested nutrients.

Metabolisable energy intake and digestible N intake for each treatment were computed using values obtained in the digestibility study period. Ørskov and Benzoin (1969) showed that any liquid sucked from a bottle, regardless of stage of maturity, passed entirely to the abomasum and intestines. Therefore, digestibilities of energy and N in pellets were estimated with the assumption that all lambs digested and metabolised milk energy and N similar to the MO group and hence any additional energy or N in the faeces and urine was a result of the pellet intake.

The ME and CP intake from all treatment groups, as well as the metabolic LW ( $LW^{0.75}$  / d) and ADG data to 18 kg LW were used to derive parameters for the daily maintenance (a) and growth (b) energy and protein requirements using the multiple regression equation:

$$\text{ME (or CP) intake} \cdot \text{d}^{-1} = a \times \text{LW}^{0.75} \cdot \text{d}^{-1} + b \times \text{ADG}$$

The efficiency of ME utilisation for growth ( $k_g$ ) was estimated by regressing daily ME intake on energy retention (ER) per day using the equation (Birkett and de Lange, 2001):

$$\text{ME intake} \cdot \text{d}^{-1} = \alpha + \beta \times \text{ER} \cdot \text{d}^{-1}$$

Where  $\alpha$  = intercept and  $\frac{1}{\beta} = k_g$

The efficiency of protein utilisation for growth was estimated by regressing daily CP intake on protein deposition (Pd) per day using the equation:

$$\text{CP intake} \cdot \text{d}^{-1} = \alpha + \beta \times \text{Pd} \cdot \text{d}^{-1}$$

Where  $\alpha$  = intercept and  $\frac{1}{\beta}$  = efficiency of utilization of CP intake for Pd

#### **5.2.8 Statistical analysis**

The growth performance, carcass and organ weights, and body composition were analysed using the generalised linear model procedure (Proc GLM) in SAS (2013) with fixed effect of the feeding group. Differences were identified using the least significant difference (LSD) mean comparison test when the F-value for the feeding effect was significant ( $P < 0.05$ ).

Energy and nitrogen balance data were analysed using Proc GLM with fixed effect of the feeding group. Differences were identified using the least significant difference (LSD) mean comparison test when the F-value for the feeding effect was significant ( $P$

$< 0.05$ ). Due to the unequal number of lambs in each treatment group the residual standard deviation (RSD), (square root of the mean square error) values were presented.

A repeated measure analysis was performed on the rumen papillae dimensions using Proc MIXED in SAS (2013) with fixed effect of feeding group and lamb nested in the feeding group as a random effect. The slides were included in the model as repeated measures. Differences were identified using the LSD mean comparison test when the F-value for the feeding effect was significant ( $P < 0.05$ ).

The linear regression analyses to determine the ME and CP requirements for maintenance and growth and the efficiency of ME and CP utilisation for growth for the pooled data set were conducted using Proc REG procedure in SAS (2013). Relative prediction error (RPE) (Fuentes-Pila *et al.*, 1996; Fuentes-Pila *et al.*, 2003) and the concordance correlation coefficient (CCC) (Lin, 1989; Nickerson, 1997) were used to evaluate the goodness of fit of the equation developed.

## **5.3 Results**

### **5.3.1 Energy and nitrogen balance**

Five lambs from the MP<sub>ad</sub> group had exceeded the 17 kg LW by the time the balance study started and therefore only two lambs from that group were used. Gross energy intake from MR during the digestibility study did not differ ( $P > 0.05$ ) among treatment groups (Table 5.2). Pellet energy intake did not differ ( $P > 0.05$ ) between MP<sub>60</sub> and MP<sub>ad</sub> but both were greater ( $P < 0.05$ ) than MP<sub>30</sub>. Digestible energy (DE) decreased ( $P < 0.001$ ) with increasing GE intake. Urinary energy excreted did not differ ( $P > 0.05$ ) amongst the pellet-fed lambs, but all the pellet groups were greater ( $P < 0.05$ ) than the

MO-fed lambs. The efficiency with which GE was metabolised was lower ( $P < 0.05$ ) in all pellet-fed lambs than the MO-fed lambs.

Table 5.2 Energy and nitrogen balances (LS means) in artificially reared lambs offered incremental levels of pellets (0% [MO], 30% [MP<sub>30</sub>], 60% [MP<sub>60</sub>], and *ad libitum* [MP<sub>ad</sub>]) in addition to milk replacer over a four-day period at 17 kg live weight.

| Parameter                          | Treatment          |                     |                     |                     | RSD  | P value |
|------------------------------------|--------------------|---------------------|---------------------|---------------------|------|---------|
|                                    | MO                 | MP <sub>30</sub>    | MP <sub>60</sub>    | MP <sub>ad</sub>    |      |         |
| n                                  | 4                  | 4                   | 4                   | 2                   |      |         |
| Energy balance                     |                    |                     |                     |                     |      |         |
| Total gross energy (GE) intake, MJ | 28.72 <sup>a</sup> | 33.10 <sup>b</sup>  | 37.11 <sup>c</sup>  | 39.32 <sup>c</sup>  | 1.86 | <0.01   |
| GE intake (milk), MJ               | 28.72              | 28.26               | 27.92               | 27.48               | 0.77 | 0.30    |
| GE intake (pellet), MJ             | -                  | 4.84 <sup>b</sup>   | 9.19 <sup>a</sup>   | 11.86 <sup>a</sup>  | 1.59 | 0.01    |
| Faecal energy (FE), MJ             | 0.40 <sup>a</sup>  | 1.48 <sup>ab</sup>  | 3.23 <sup>c</sup>   | 2.88 <sup>bc</sup>  | 0.89 | 0.01    |
| Digestible energy(DE), MJ          | 28.32 <sup>a</sup> | 31.62 <sup>b</sup>  | 33.88 <sup>c</sup>  | 36.45 <sup>c</sup>  | 1.36 | <0.01   |
| DE, % of GE intake                 | 98.62 <sup>c</sup> | 95.49 <sup>bc</sup> | 91.32 <sup>a</sup>  | 92.99 <sup>ab</sup> | 2.13 | 0.004   |
| Urinary energy (UE), MJ            | 0.65 <sup>a</sup>  | 0.76 <sup>ab</sup>  | 0.98 <sup>b</sup>   | 1.02 <sup>b</sup>   | 0.14 | 0.02    |
| Metabolisable energy (ME), MJ      | 27.67 <sup>a</sup> | 30.86 <sup>b</sup>  | 32.89 <sup>c</sup>  | 35.42 <sup>d</sup>  | 1.24 | <0.01   |
| ME, % of GE intake                 | 96.37 <sup>c</sup> | 93.19 <sup>b</sup>  | 88.67 <sup>a</sup>  | 90.41 <sup>ab</sup> | 2.17 | 0.003   |
| ME, % of DE                        | 97.71              | 97.59               | 97.10               | 97.21               | 0.33 | 0.09    |
| Nitrogen balance                   |                    |                     |                     |                     |      |         |
| Total nitrogen (N) intake, g       | 49.34 <sup>a</sup> | 57.15 <sup>b</sup>  | 64.29 <sup>c</sup>  | 68.25 <sup>c</sup>  | 3.28 | <0.01   |
| N intake (milk), g                 | 49.34              | 48.56               | 47.97               | 47.19               | 1.33 | 0.31    |
| N intake (pellet), g               | -                  | 8.56 <sup>a</sup>   | 16.32 <sup>b</sup>  | 21.06 <sup>b</sup>  | 2.82 | <0.01   |
| Faecal N, g                        | 1.11 <sup>a</sup>  | 3.32 <sup>a</sup>   | 6.11 <sup>b</sup>   | 6.24 <sup>b</sup>   | 1.45 | 0.002   |
| Digestible N (DN), g               | 48.22 <sup>a</sup> | 53.84 <sup>b</sup>  | 58.18 <sup>c</sup>  | 62.0 <sup>c</sup>   | 2.42 | <0.01   |
| DN, % of N intake                  | 97.76 <sup>c</sup> | 94.18 <sup>b</sup>  | 90.56 <sup>a</sup>  | 91.1 <sup>ab</sup>  | 1.93 | 0.002   |
| Urinary N (UN), g                  | 12.78 <sup>a</sup> | 16.84 <sup>ab</sup> | 19.20 <sup>b</sup>  | 22.02 <sup>b</sup>  | 3.24 | 0.03    |
| Retained N (RN), g                 | 35.44 <sup>a</sup> | 37.00 <sup>ab</sup> | 38.98 <sup>bc</sup> | 39.99 <sup>c</sup>  | 1.50 | 0.01    |
| RN, % of N intake                  | 71.81 <sup>b</sup> | 64.81 <sup>a</sup>  | 60.63 <sup>a</sup>  | 59.32 <sup>a</sup>  | 4.22 | 0.01    |
| RN, % of DN                        | 73.47              | 68.86               | 67.02               | 64.94               | 4.45 | 0.15    |

<sup>1</sup>Treatment: MO = no pellet offered; MP<sub>30</sub>: pellets offered were restricted to 30% of *ad libitum* intake; MP<sub>60</sub> = pellets offered were restricted to 60% of *ad libitum* intake; MP<sub>ad</sub> = pellets offered *ad libitum*;

<sup>2</sup>RSD = residual standard deviation (square root of the mean square error).

<sup>a,b,c</sup> means in a row with different superscripts are different from each other (LSD,  $P < 0.05$ ).

Nitrogen intake increased with increasing pellet consumption (Table 5.2). Faecal N and urinary N excreted were greater ( $P < 0.05$ ) in MP<sub>60</sub> and MP<sub>ad</sub> lambs than in MO and MP<sub>30</sub>. Retained N relative to digested N did not differ ( $P > 0.05$ ) amongst the treatment groups although the RN to N intake ratio was greater ( $P = 0.01$ ) in MO lambs than the pellet-fed lambs

### 5.3.2 Intakes and growth

The initial and final live weights and LWG did not differ ( $P > 0.05$ ) among treatment groups but MP<sub>ad</sub> lambs reached slaughter weight faster ( $P < 0.01$ ) than the MP<sub>30</sub>, MP<sub>60</sub> and MO lambs (Table 5.3). The ADG was greatest ( $P < 0.001$ ) for MP<sub>ad</sub> lambs and least ( $P < 0.001$ ) for MO lambs. No differences were observed in the total DMI among the treatment groups. As expected, daily DMI and daily ME intake were greater ( $P < 0.001$ ) for MP<sub>ad</sub> lambs and lower ( $P < 0.001$ ) for MO lambs. Crude protein intake per day increased ( $P < 0.001$ ) with increasing ME intake per day. The CP:ME ratio was greatest ( $P < 0.001$ ) for MP<sub>ad</sub> lambs and least ( $P < 0.001$ ) for MO lambs. The gain:feed ratio did not differ ( $P > 0.05$ ) among treatment groups, but the ADG per MJ ME intake was greatest ( $P < 0.001$ ) for MP<sub>ad</sub> lambs and least ( $P < 0.001$ ) for MO lambs. For every 1 MJ of ME intake the ADG of MP<sub>ad</sub> lambs increased by 39 g which was 7 g greater than the ADG of MO lambs and 3 g greater than the ADG of MP<sub>30</sub> and MP<sub>60</sub> lambs.

Table 5.3 Intakes and growth (LS means) of artificially reared lambs offered incremental levels of pellets (0% [MO], 30% [MP<sub>30</sub>], 60% [MP<sub>60</sub>], and *ad libitum* [MP<sub>ad</sub>]) in addition to milk replacer to 18 kg live weight.

| Parameter                           | Treatment <sup>1</sup> |                      |                      |                     |                 | P-value |
|-------------------------------------|------------------------|----------------------|----------------------|---------------------|-----------------|---------|
|                                     | MO                     | MP <sub>30</sub>     | MP <sub>60</sub>     | MP <sub>ad</sub>    | SE <sup>2</sup> |         |
| Initial live weight, kg             | 4.97                   | 5.11                 | 5.05                 | 5.06                | 0.25            | 0.98    |
| Age at slaughter, d                 | 93.00 <sup>c</sup>     | 80.42 <sup>b</sup>   | 76.43 <sup>b</sup>   | 66.57 <sup>a</sup>  | 1.89            | <0.001  |
| Final live weight, kg               | 18.17                  | 18.70                | 18.50                | 18.86               | 0.18            | 0.06    |
| Live weight gain (LWG), kg          | 13.19                  | 13.63                | 13.45                | 13.80               | 0.27            | 0.45    |
| Average daily gain (ADG), g         | 143.59 <sup>a</sup>    | 171.63 <sup>b</sup>  | 178.73 <sup>b</sup>  | 211.31 <sup>c</sup> | 3.71            | <0.001  |
| Total Milk intake, kg               | 93.03 <sup>c</sup>     | 81.14 <sup>b</sup>   | 74.79 <sup>b</sup>   | 64.62 <sup>a</sup>  | 1.63            | <0.001  |
| Total Pellet intake, kg             | -                      | 2.76 <sup>a</sup>    | 4.35 <sup>b</sup>    | 6.64 <sup>c</sup>   | 0.51            | <0.001  |
| Total Dry matter intake (DMI), kg   | 18.03                  | 18.16                | 18.33                | 18.38               | 0.49            | 0.95    |
| DMI/d, g                            | 196.16 <sup>a</sup>    | 228.83 <sup>b</sup>  | 243.66 <sup>b</sup>  | 281.27 <sup>c</sup> | 6.76            | <0.001  |
| Total ME intake, MJ                 | 406.74 <sup>c</sup>    | 384.57 <sup>bc</sup> | 373.97 <sup>ab</sup> | 354.21 <sup>a</sup> | 8.04            | 0.001   |
| ME intake / d, MJ                   | 4.43 <sup>a</sup>      | 4.84 <sup>b</sup>    | 4.97 <sup>b</sup>    | 5.42 <sup>c</sup>   | 0.10            | <0.001  |
| Total crude protein (CP) intake, kg | 4.54                   | 4.42                 | 4.38                 | 4.27                | 0.11            | 0.36    |
| CP intake / d, g                    | 49.34 <sup>a</sup>     | 55.71 <sup>b</sup>   | 58.23 <sup>b</sup>   | 65.33 <sup>c</sup>  | 1.36            | <0.001  |
| Gain:feed ratio, kg/kg              | 0.73                   | 0.75                 | 0.73                 | 0.77                | 0.02            | 0.66    |
| ADG:ME intake / d ratio, g/MJ       | 32.46 <sup>a</sup>     | 35.51 <sup>b</sup>   | 36.12 <sup>b</sup>   | 39.24 <sup>c</sup>  | 0.72            | <0.001  |
| CP:ME ratio                         | 11.15 <sup>c</sup>     | 11.51 <sup>b</sup>   | 11.71 <sup>b</sup>   | 12.05 <sup>a</sup>  | 0.06            | <0.001  |

<sup>1</sup>Treatment: MO = no pellet offered; MP<sub>30</sub>: pellets offered were restricted to 30% of *ad libitum* intake; MP<sub>60</sub> = pellets offered were restricted to 60% of *ad libitum* intake; MP<sub>ad</sub> = pellets offered *ad libitum*

<sup>2</sup>SE = standard error of means.

<sup>a,b,c</sup> Means in the same row with no superscript letters after them or with a common superscript letter following them are not significantly different (LSD, P < 0.05)

### 5.3.3 Milk and pellet utilisation

The mean values of milk and pellet N and energy utilisation, respectively across the four groups are presented in Figures 5.1 and 5.2. Both Figures (5.1 and 5.2) are based on the assumption that the nutrients in milk are digested and metabolised similarly across the treatment groups and hence any additional nutrient in the faeces or urine was a result of pellet intake.

For every 100 g of N consumed from milk, approximately 98% was digested of which, 72% was retained for growth (Fig. 5.1). For every 100 g of N consumed from pellets, approximately 73% was digested, and only 30% was retained for growth.

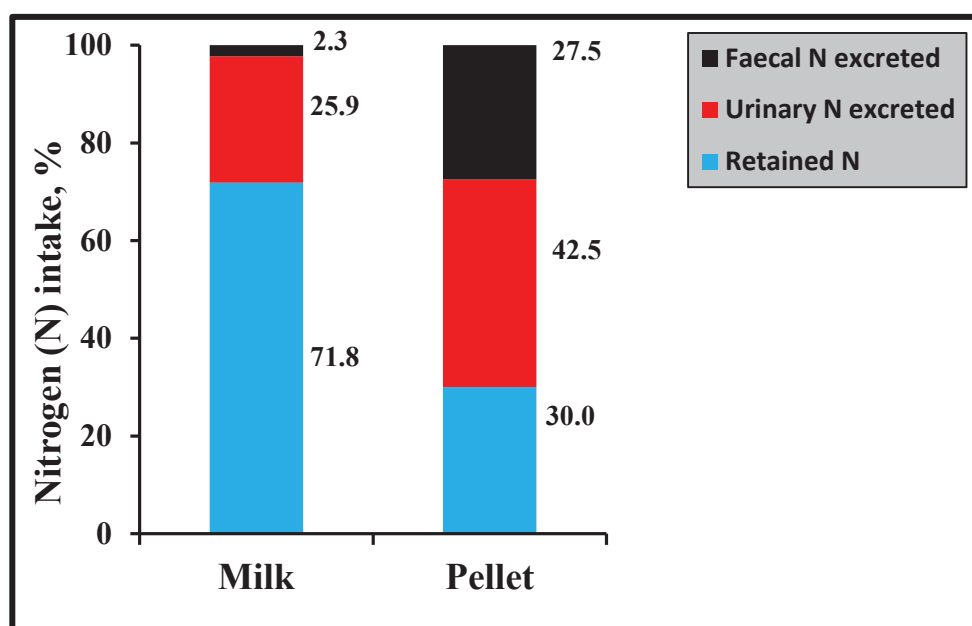


Figure 5.1 Nitrogen (N; g) utilisation from milk and pellets consumed by artificially reared lambs offered incremental levels of pellets (0% [MO], 30% [MP<sub>30</sub>], 60% [MP<sub>60</sub>], and *ad libitum* [MP<sub>ad</sub>]) in addition to milk replacer over four days at 17 kg live weight.

For every 100 MJ of GE ingested from milk, approximately 98% was digested of which 96% was retained for growth (Fig 5.2). For every 100 MJ of pellet GE consumed, 74 MJ was digested and 71 MJ retained for growth (Fig. 5.2).

Based on Figures 5.1 and 5.2, the CP:ME ratio of MR containing 244 g CP /kg DM and 21.9 MJ ME /kg DM is 11.14 and that of pellets containing 169 g CP /kg DM and 10.8 MJ ME /kg DM is 15.64.

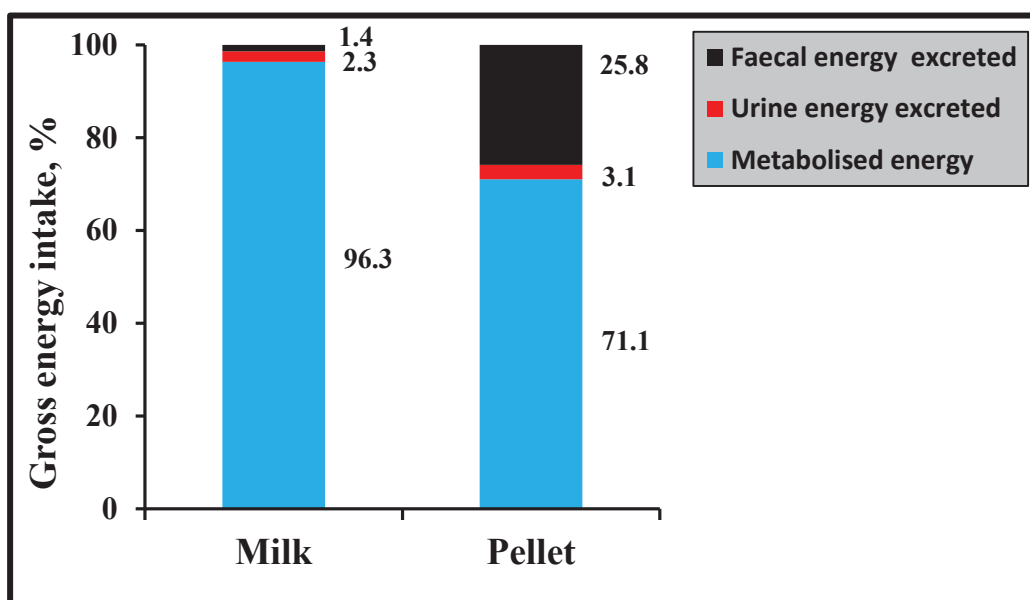


Figure 5.2. Gross energy (GE; MJ) utilisation from milk and pellets consumed by artificially reared lambs offered incremental levels of pellets (0% [MO], 30% [MP<sub>30</sub>], 60% [MP<sub>60</sub>], and *ad libitum* [MP<sub>ad</sub>]) in addition to milk replacer over four days at 17 kg live weight.

### 5.3.4 Slaughter traits

Dietary treatment did not affect the empty body weights of lambs (Table 5.4). The dressing percentages were, however, greater ( $P = 0.01$ ) for MO and MP<sub>30</sub> lambs than those of the MP<sub>60</sub> and MP<sub>ad</sub> lambs. Liver, stomach, and intestinal weights were greatest ( $P < 0.05$ ) in MP<sub>ad</sub> and least ( $P < 0.05$ ) in MO lambs. No differences ( $P > 0.05$ ) were observed in head, skin and feet, spleen, kidney, and all other internal organs weights between treatments groups.

Table 5.4 Weights of carcass and visceral organs (LS means) of artificially reared lambs offered incremental levels of pellets (0% [MO], 30% [MP<sub>30</sub>], 60% [MP<sub>60</sub>], and *ad libitum* [MP<sub>ad</sub>]) in addition to milk replacer from age one to 18 kg live weight

| Parameter                            | Treatment <sup>1</sup> |                     |                     |                    | SE <sup>2</sup> | P value |
|--------------------------------------|------------------------|---------------------|---------------------|--------------------|-----------------|---------|
|                                      | MO                     | MP <sub>30</sub>    | MP <sub>60</sub>    | MP <sub>ad</sub>   |                 |         |
| Empty body weight, kg                | 16.05                  | 16.35               | 15.88               | 15.88              | 0.18            | 0.25    |
| Dressing <sup>1</sup> , %            | 52.77 <sup>b</sup>     | 52.34 <sup>b</sup>  | 50.09 <sup>a</sup>  | 49.76 <sup>a</sup> | 0.70            | 0.01    |
| Head, kg                             | 1.33                   | 1.29                | 1.26                | 1.23               | 0.03            | 0.08    |
| Skin and feet, kg                    | 2.78                   | 2.72                | 2.68                | 2.53               | 0.07            | 0.10    |
| Hot carcass weight <sup>3</sup> , kg | 9.59                   | 9.80                | 9.26                | 9.38               | 0.14            | 0.06    |
| Total visceral weight, kg            | 2.35 <sup>a</sup>      | 2.53 <sup>ab</sup>  | 2.66 <sup>bc</sup>  | 2.73 <sup>c</sup>  | 0.06            | 0.001   |
| Liver, g                             | 259.1 <sup>a</sup>     | 269.7 <sup>ab</sup> | 292.2 <sup>bc</sup> | 313.3 <sup>c</sup> | 8.75            | 0.001   |
| Spleen, g                            | 35.85                  | 35.14               | 29.42               | 34.14              | 1.88            | 0.10    |
| Kidney, g                            | 75.71                  | 65.57               | 69.00               | 67.57              | 3.18            | 0.15    |
| Stomach <sup>4</sup> , g             | 229.4 <sup>a</sup>     | 279.7 <sup>b</sup>  | 333.7 <sup>c</sup>  | 347.1 <sup>c</sup> | 13.63           | <0.01   |
| Intestines, g                        | 698.7 <sup>a</sup>     | 843.0 <sup>b</sup>  | 954.0 <sup>bc</sup> | 964.6 <sup>c</sup> | 41.48           | <0.01   |
| Gut fill, kg                         | 0.62 <sup>a</sup>      | 1.19 <sup>b</sup>   | 1.38 <sup>b</sup>   | 1.81 <sup>c</sup>  | 0.18            | <0.01   |
| All other organs, kg                 | 1.06                   | 1.03                | 0.99                | 1.01               | 0.02            | 0.16    |

<sup>1</sup>Treatment: MO = no pellet offered; MP<sub>30</sub>: pellets offered were restricted to 30% of *ad libitum* intake; MP<sub>60</sub> = pellets offered were restricted to 60% of *ad libitum* intake; MP<sub>ad</sub> = pellets offered *ad libitum*

<sup>2</sup>SE = standard error of means.

<sup>3</sup>Hot carcass weight as a percentage of final live weight

<sup>4</sup>All four stomach compartments

<sup>a,b,c</sup> Means in the same row with no superscript letters after them or with a common superscript letter following them are not significantly different (LSD,  $P < 0.05$ ).

### 5.3.5 Histological observations

The rumen of the MP<sub>ad</sub> and MP<sub>60</sub> lambs had longer ( $P < 0.05$ ) papillae length, circumference and a greater ( $P < 0.05$ ) papillae surface area than those from MO and MP<sub>30</sub> lambs (Table 5.5). The width of papillae, however, did not differ ( $P > 0.05$ ) between treatment groups. A positive linear relationship ( $r^2 = 0.43$ ) was observed between the total amount of pellets consumed over the experimental period by the pellet fed lambs and the length of their papillae (Fig 5.3). For every kilogram of pellet consumed, papillae length increased by 93  $\mu\text{m}$  and this was presented in a simple regression equation as:

Papillae length =  $741.8 (\pm 124.2) + 93.1 (\pm 24.56)$  pellet intake ( $r^2 = 43\%$ ; RPE = 19.59; CCC = 0.60).

Table 5.5 Rumen papillary development (LS means) of artificially reared lambs offered incremental levels of pellets (0% [MO], 30% [MP<sub>30</sub>], 60% [MP<sub>60</sub>], and *ad libitum* [MP<sub>ad</sub>]) in addition to milk replacer to 18 kg live weight

| Parameter                                  | Treatment <sup>1</sup> |                      |                      |                      | SE <sup>2</sup> | P value |
|--------------------------------------------|------------------------|----------------------|----------------------|----------------------|-----------------|---------|
|                                            | MO                     | MP <sub>30</sub>     | MP <sub>60</sub>     | MP <sub>ad</sub>     |                 |         |
| Length, $\mu\text{m}$                      | 827.71 <sup>a</sup>    | 949.37 <sup>a</sup>  | 1317.59 <sup>b</sup> | 1238.55 <sup>b</sup> | 96.99           | 0.004   |
| Width, $\mu\text{m}$                       | 399.04                 | 356.86               | 392.39               | 375.51               | 26.20           | 0.67    |
| Circumference <sup>3</sup> , $\mu\text{m}$ | 1880.34 <sup>a</sup>   | 2164.40 <sup>a</sup> | 2961.56 <sup>b</sup> | 2758.57 <sup>b</sup> | 202.68          | 0.003   |
| Surface area (cm <sup>2</sup> )            | 116.68 <sup>a</sup>    | 117.79 <sup>a</sup>  | 169.49 <sup>b</sup>  | 155.81 <sup>b</sup>  | 11.86           | 0.01    |

<sup>1</sup>Treatment: MO = no pellet offered; MP<sub>30</sub>: pellets offered were restricted to 30% of *ad libitum* intake; MP<sub>60</sub> = pellets offered were restricted to 60% of *ad libitum* intake; MP<sub>ad</sub> = pellets offered *ad libitum*

<sup>2</sup>SE = standard error of means.

<sup>a,b</sup> means in a row with different superscripts are different from each other (LSD,  $P < 0.05$ )

<sup>3</sup>Circumference was determined by measuring the contour line of each papilla

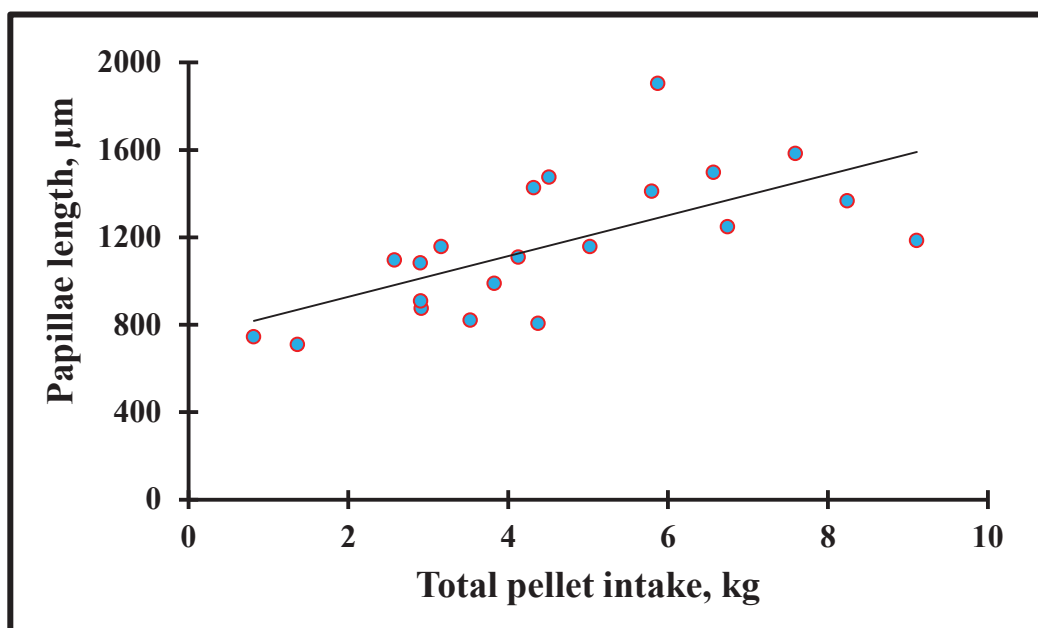


Figure 5.3 Relationship between total pellet intake and rumen papillae length of artificially reared lambs offered four levels of pellets (0% [MO], 30% [MP<sub>30</sub>], 60% [MP<sub>60</sub>], and *ad libitum* [MP<sub>ad</sub>]) in addition to milk replacer to 18 kg live weight Papillae length = 741.8 (± 124.2) + 93.1 (± 24.56) pellet intake ( $r^2 = 43\%$ )

### 5.3.6 Body composition

The total amount of DM, protein, fat, ash, water, OM and GE in the empty bodies of the lambs did not differ ( $P > 0.05$ ) between treatment groups (Table 5.6). As a percentage of the EBW, the percentages of protein, fat, ash, water, OM and GE in the empty bodies of the lambs did not differ ( $P > 0.05$ ) between treatment groups with the exception of the OM content which was greatest ( $P < 0.05$ ) in MO and least ( $P < 0.05$ ) in the MP<sub>ad</sub>. In comparison to the baseline (BL) group, the concentration of DM, protein, fat, and OM of lambs slaughtered at 18 kg relative to their empty bodies on average increased by about 10%, 4%, 8%, and 13% points, respectively, whereas water and ash content decreased by approximately 11% and 1% points, respectively.

Table 5.6. Chemical body composition of artificially reared lambs offered incremental levels of pellets (0% [MO], 30% [MP<sub>30</sub>], 60% [MP<sub>60</sub>], and *ad libitum* [MP<sub>ad</sub>]) in addition to milk replacer at 18 kg live weight

| Parameter             | Baseline group <sup>1</sup> | Treatment <sup>2</sup> |                     |                     |                    | SE <sup>3</sup> | P value |
|-----------------------|-----------------------------|------------------------|---------------------|---------------------|--------------------|-----------------|---------|
|                       |                             | MO                     | MP <sub>30</sub>    | MP <sub>60</sub>    | MP <sub>ad</sub>   |                 |         |
| n                     | 4                           | 7                      | 7                   | 7                   | 7                  |                 |         |
| EBW <sup>4</sup> , kg | 4.17 ± 0.4                  | 16.05                  | 16.35               | 15.88               | 15.88              | 0.18            | 0.26    |
| DM, kg                | 1.14 ± 0.11                 | 5.75                   | 5.58                | 5.41                | 5.33               | 0.13            | 0.13    |
| Water, kg             | 3.58 ± 0.4                  | 10.36                  | 10.82               | 10.54               | 10.60              | 0.13            | 0.10    |
| Crude protein, kg     | 0.76 ± 0.1                  | 3.37                   | 3.30                | 3.24                | 3.24               | 0.07            | 0.50    |
| Fat, kg               | 0.12 ± 0.04                 | 1.81                   | 1.70                | 1.67                | 1.62               | 0.09            | 0.49    |
| Ash, kg               | 0.23 ± 0.03                 | 0.56                   | 0.58                | 0.56                | 0.57               | 0.02            | 0.90    |
| Organic matter, kg    | 0.91 ± 0.09                 | 5.20                   | 5.00                | 4.84                | 4.76               | 0.12            | 0.07    |
| GE <sup>5</sup> , MJ  | 22.82 ± 2.22                | 148.7                  | 142.8               | 138.3               | 135.1              | 4.14            | 0.14    |
| Component, % of EBW   |                             |                        |                     |                     |                    |                 |         |
| DM                    | 24.19 ± 0.74                | 35.68                  | 34.00               | 33.91               | 33.45              | 0.61            | 0.08    |
| Water                 | 75.81 ± 0.75                | 64.32                  | 66.00               | 66.09               | 66.55              | 0.61            | 0.08    |
| Crude protein         | 16.15 ± 1.23                | 20.92                  | 20.12               | 20.30               | 20.35              | 0.32            | 0.33    |
| Fat                   | 2.63 ± 0.99                 | 11.19                  | 10.39               | 10.48               | 10.16              | 0.49            | 0.50    |
| Ash                   | 4.85 ± 0.43                 | 3.44                   | 3.53                | 3.55                | 3.60               | 0.14            | 0.87    |
| Organic Matter        | 19.34 ± 0.74                | 32.23 <sup>b</sup>     | 30.46 <sup>ab</sup> | 30.37 <sup>ab</sup> | 29.85 <sup>a</sup> | 0.55            | 0.03    |
| GE, MJ/kg EBW         | 4.84 ± 0.34                 | 9.21                   | 8.70                | 8.67                | 8.47               | 0.2             | 0.08    |

<sup>1</sup>Values are LS means ± SE

<sup>2</sup>Treatment: MO = no pellet offered; MP<sub>30</sub> = pellets offered were restricted to 30% of *ad libitum* intake; MP<sub>60</sub> = pellets offered were restricted to 60% of *ad libitum* intake; MP<sub>ad</sub> = pellets offered *ad libitum*

<sup>3</sup>SE = standard error.

<sup>4</sup>EBW = Empty body weight (minus blood weight);

<sup>5</sup>GE = gross energy;

<sup>a,b</sup>Means in the same row with no superscript letters after them or with a common superscript letter following them are not significantly different (LSD, P < 0.05)

The amounts of DM, protein, OM, and GE per kg LWG were greatest (P < 0.05) in MO whilst, water, ash and fat contents gained did not differ (P > 0.05) among the treatment groups (Table 5.7). Energy retained per day (ER / d) was greatest (P < 0.01) in MP<sub>ad</sub> lambs and least (P < 0.01) in MO lambs (Table 5.7). Protein deposited per day (Pd/d) followed a similar trend. Fat deposited per day (Fd/d) however did not differ (P > 0.05) among treatment groups.

Table 5.7 The chemical composition per kilogram live weight gain (LWG) (LS means) of artificially reared lambs offered incremental levels of pellets (0% [MO], 30% [MP<sub>30</sub>], 60% [MP<sub>60</sub>], and *ad libitum* [MP<sub>ad</sub>]) in addition to milk replacer from age one to 18 kg live weight

| Parameter                   | Treatment          |                     |                     |                     | SE <sup>2</sup> | P value |
|-----------------------------|--------------------|---------------------|---------------------|---------------------|-----------------|---------|
|                             | MO                 | MP <sub>30</sub>    | MP <sub>60</sub>    | MP <sub>ad</sub>    |                 |         |
| Water, g/kg LWG             | 533.9              | 544.1               | 532.2               | 5234.0              | 9.97            | 0.57    |
| Dry matter, g/kg LWG        | 355.8 <sup>b</sup> | 308.4 <sup>a</sup>  | 322.3 <sup>ab</sup> | 329.0 <sup>ab</sup> | 8.02            | <0.01   |
| Protein, g/kg LWG           | 202.0 <sup>b</sup> | 188.7 <sup>ab</sup> | 187.5 <sup>ab</sup> | 182.8 <sup>a</sup>  | 4.36            | 0.03    |
| Fat, g/kg LWG               | 128.1              | 116.2               | 115.4               | 109.2               | 5.78            | 0.16    |
| Ash, g/kg LWG               | 25.98              | 26.47               | 25.97               | 25.82               | 1.72            | 0.99    |
| OM, g/kg LWG                | 329.8 <sup>b</sup> | 302.5 <sup>ab</sup> | 296.3 <sup>a</sup>  | 282.6 <sup>a</sup>  | 7.44            | 0.001   |
| GE <sup>3</sup> , MJ/kg LWG | 9.65 <sup>b</sup>  | 8.86 <sup>ab</sup>  | 8.69 <sup>ab</sup>  | 8.23 <sup>a</sup>   | 0.26            | 0.01    |
| Nutrient gain / day         |                    |                     |                     |                     |                 |         |
| Protein deposited, g        | 29.00 <sup>a</sup> | 32.37 <sup>ab</sup> | 33.47 <sup>b</sup>  | 38.67 <sup>c</sup>  | 1.00            | <0.01   |
| Fat deposited, g            | 18.46              | 19.99               | 20.69               | 23.13               | 1.22            | 0.08    |
| Energy retained, MJ         | 1.39 <sup>a</sup>  | 1.52 <sup>ab</sup>  | 1.55 <sup>ab</sup>  | 1.74 <sup>b</sup>   | 0.06            | 0.01    |

<sup>1</sup>Treatment: MO = no pellet offered; MP<sub>30</sub> = pellets offered were restricted to 30% of *ad libitum* intake; MP<sub>60</sub> = pellets offered were restricted to 60% of *ad libitum* intake; MP<sub>ad</sub> = pellets offered *ad libitum*

<sup>2</sup>SE = standard error.

<sup>3</sup>GE = gross energy;

<sup>a,b,c</sup>means in a row with different superscripts are different from each other (LSD,  $P < 0.05$ )

### 5.3.7 Energy and requirements for maintenance and growth

The total chemical body composition of lambs did not differ except for organic matter ( $P > 0.05$ ) among the feeding groups. Therefore, the partitioning of ME intake for maintenance (ME<sub>m</sub>) and growth (ME<sub>g</sub>) were calculated from the pooled data set. The ME<sub>m</sub> and ME<sub>g</sub> values were estimated by regressing  $LW^{0.75} \cdot d^{-1}$  and ADG on ME intake  $\cdot d^{-1}$ . The multiple regression equation for estimating ME<sub>m</sub> and ME<sub>g</sub> requirement is given below:

$$ME \text{ intake} \cdot d^{-1} = 0.40 (\pm 0.05) LW^{0.75} \cdot d^{-1} + 13.8 (\pm 1.67) ADG (R^2 = 0.99; RPE = 4.21\%; CCC = 0.88).$$

The above equation indicates that lambs in the present study utilised 0.40 MJ/kg  $LW^{0.75} \cdot d^{-1}$  for maintenance and 13.8 MJ/d to gain a kilogram of ADG. The relationship between ME intake and energy retention (ER) is presented by the equation:

$$ME \text{ intake} \cdot d^{-1} = 2.01 (\pm 0.41) + 1.89 (\pm 0.26) ER \cdot d^{-1} (r^2 = 0.67; RPE = 5.29\%; CCC = 0.80).$$

The efficiency of utilisation of ME for growth ( $k_g$ ) derived from the above equation is 0.53 (i.e.  $1/1.89$ ).

### **5.3.8 Protein requirements for maintenance and growth**

The multiple regression equation for estimating  $CP_m$  and  $CP_g$  requirement is given below:

$$CP \text{ intake} \cdot d^{-1} = 2.74 (\pm 0.68) LW^{0.75} \cdot d^{-1} + 0.23 (\pm 0.02) ADG (R^2 = 0.99; RPE = 5.25\%; CCC = 0.89)$$

The above equation indicates that lambs in the present study utilised 2.74 g CP / kg  $LW^{0.75} \cdot d^{-1}$  for maintenance and 0.23 g CP to gain a gram of ADG.

The relationship between daily CP intake and Pd is represented by the equation:

$$CP = 8.56 (\pm 4.99) + 1.47 (\pm 0.15) Pd \cdot d^{-1} (r^2 = 0.79; RPE = 5.50\%; CCC = 0.88).$$

The efficiency of utilisation of CP intake for protein deposition ( $k_p$ ) derived from the above equation is 0.68 (i.e.  $1/1.47$ ).

The CP and ME requirements calculated above were used to model the CP:ME requirements of growing lambs from 5 kg LW to 18 kg LW (Fig 5.4). The model based

on data from this trial showed that for a lamb growing at 180 g/d, the CP:ME ratio needed to meet its growth requirement decreases in a curvilinear fashion from a value of 13.1 at 5 kg LW to 10.9 at 18 kg LW. Whereas the actual CP:ME intake of lambs across all groups in the present study increased linearly as LW increased from 11.2 at 5 kg LW to 11.7 at 18 kg LW.

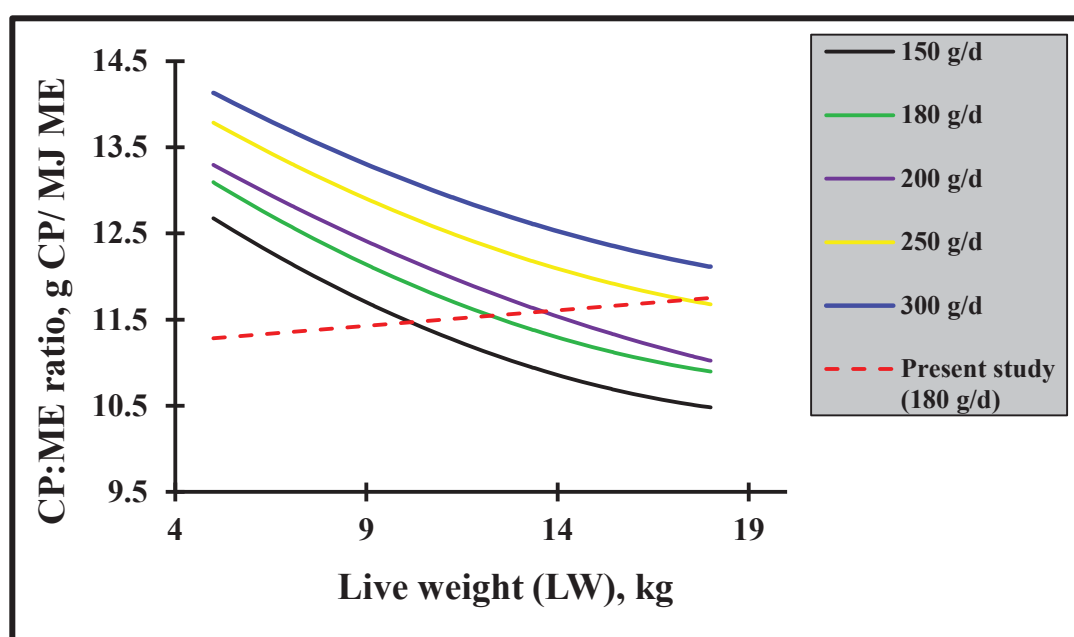


Figure 5.4 A theoretical simulation model (solid lines) showing the crude protein (CP) to metabolisable energy (ME) requirements of lambs from 5 kg LW to 18 kg LW growing at 150 to 300 g /d. Short dashes represents actual CP:ME intake of artificially reared lambs offered four levels of pellets (*ad libitum* [MP<sub>ad</sub>] or restricted to 0% [MO], 30% [MP<sub>30</sub>] and 60% [MP<sub>60</sub>], of the *ad libitum* intake) in addition to milk replacer from 5 kg LW to 18 kg LW growing at 180 g /d.

## 5.4 Discussion

This study aimed to 1) determine the energy and N balance of milk and pellets when they are fed together, 2) to compare the chemical body composition at a set weight of lambs as affected by increasing levels of energy intake, and 3) to estimate the

maintenance and growth energy requirements and the CP:ME requirements for growth in lambs fed varying levels of pellets in addition to milk.

#### **5.4.1 Energy and Nitrogen balance**

Any liquid sucked from a bottle passed entirely to the abomasum and intestines regardless of stage of maturity (Ørskov and Benzin, 1969). Therefore, the present study assumed that the digestion and metabolisability of the nutrients in milk for the MP<sub>30</sub>, MP<sub>60</sub> and MP<sub>ad</sub> treatment groups were similar to the MO group. Thus, any additional nutrient excreted in the faeces and urine resulted from pellet intake.

Previous studies have shown that for lambs fed exclusively milk, digestibility and metabolisability ranges were between 98 % to 99 % and 92 % to 96 %, respectively (Hodge, 1965; Jagusch *et al.*, 1971; Walker and Norton, 1971) while for those for solid feed only were 42 % to 72.2 % and 35 % to 56 %, respectively (Jagusch *et al.*, 1971; Kamalzadeh and Shabani, 2007; Deng *et al.*, 2012; Xu *et al.*, 2015). In the present study, digestibility and metabolisability values for pellets (74.2% and 71.1%, respectively) were lower than that for milk (98.6% and 96.3%, respectively) but greater than the range of values reported previously for lambs fed solid feed only. Lambs in the current study received pellets from one day of age and were therefore likely to be well adapted to the pellets before the balance study conducted at 17 kg LW. Thus, the present result suggests that the provision of solid feeds to lambs very early in life enables them to adapt their metabolism more quickly, and thus improving pellet energy utilisation.

The inclusion of pellets increased faecal N output in the present study. A high faecal N excretion has been observed in young ruminants offered solid feed in addition to milk

(Labussiere *et al.*, 2009). However, faecal N excretion was unaffected by high levels of milk intake in calves fed increasing amounts of milk (Silva *et al.*, 2015). Although increased faecal N excretion can be attributed to the physical characteristics of the diet, it may also reflect an increase in metabolic faecal N that typically occurs with increased growth rates rather than a reduction in protein digestibility (Lofgreen and Kleiber, 1953; Hodge, 1965). Lambs offered pellets in addition to milk had higher growth rates than their counterparts fed milk only, which may have accounted for the high faecal N losses.

Only 30% of N intake from pellets was retained for growth, compared to 70% retained from milk. This resulted in a relatively low dietary N retention in the pellet-fed lambs. This observation is consistent with previous studies which have reported low efficiencies of N utilisation when solid feeds were included in the ration of pre-weaned calves (Hill *et al.*, 2008; Labussiere *et al.*, 2009). Contrary to these findings, Berends *et al.* (2012) reported an increased efficiency of N retention in veal calves of similar maturity to the lambs in the present study when fed solid feed containing 9 % CP in addition to being fed MR (i.e., for each gram of N ingested from solid feed, 0.77 g N was retained). The greater N retention was caused by the recycling of urea N stimulated by feeding low-protein solid feed (Berends *et al.*, 2012). The pellets offered to lambs in the present study and the solid feeds offered to calves in the studies of Hill *et al.* (2008) and Labussiere *et al.* (2009) contained much higher CP contents (16% to 20%) which may have contributed to the lower N retention.

#### **5.4.2 Intakes and growth**

The addition of pellets increased daily DMI and consequently, increased the daily energy and protein consumption of lambs. As expected, this increased pre-weaning growth rates, which concurs with previous findings in lambs (Ward, 2008; Bhatt *et al.*,

2009) and calves (Cardoso *et al.*, 2015; Silva *et al.*, 2015). The total DMI to a fixed LW (18 kg) did not differ among treatment groups resulting in similar gain-to-feed ratios among the treatment groups. However, the more pellets consumed by the lambs, the greater the ADG per unit ME intake and the lesser total ME intake required to reach the 18 kg LW. This was likely due to the constant overhead cost of maintenance spread over more grams of gain which diluted the maintenance costs (Purchas, 1986). As a result, MP<sub>ad</sub> lambs grew faster and more efficiently than lambs from the other treatment groups with MO lambs being the least efficient.

The stomach, liver weight and the total visceral weights were greater for MP<sub>60</sub> and MP<sub>ad</sub> treatment groups. Increasing solid feed intake increased the weights of the gastrointestinal tissues such as the stomach in pre-weaned lambs (Danso *et al.*, 2014). Therefore, the lower dressing percentage for the MP<sub>60</sub> and MP<sub>ad</sub> in comparison to MO and MP<sub>30</sub> groups was not unexpected, as lambs were fed to similar final LW. Rumen papillae development was also greater for the MP<sub>60</sub> and MP<sub>ad</sub> treatment groups. Both rumen and liver undergo physical and metabolic developments following the initiation of solid feed intake (Hamada *et al.*, 1976; Baldwin, 2000; Baldwin *et al.*, 2004). The greater stomach and liver weight, and enhanced rumen development in response to greater pellet consumption indicated that the quantity of solid feed consumed during the pre-weaning period is an important factor in the effective stimulation of rumen and liver development. It is recommended that pellets should be made available to artificially reared lambs or lambs who do not have access to herbage to help with early rumen development, consequently allowing the earlier weaning of the lambs.

### **5.4.3 Body composition**

Daily protein deposited and energy retained increased linearly with increasing ME intake in lambs. However, the total protein and energy content in the empty bodies of the lambs did not differ between group, indicating that dietary treatment had no impact on the body composition when lambs were fed to a similar live weight, agreeing with the findings of Black (1974). Supporting Silva *et al.* (2015), the addition of solid feed to milk increased N retention and growth performance but had no effect on the total protein deposited in the empty bodies of lambs at a set weight. This may be related to the additional N intake from the pellets. The efficiency of N utilisation from an extra dietary N source is usually lower than the N efficiency of the base diet (milk), thereby decreasing the efficiency of protein deposition (Silva *et al.*, 2015).

Rumen and liver development in young ruminants are associated with high energetic costs (Gill *et al.*, 1989; Burrin *et al.*, 1990; McBride and Kelly, 1990) accounting for up to 50% of the total animal energy expenditure during that period (Canas *et al.*, 1982; Ferrell, 1988; Seal and Reynolds, 1993). Therefore, at least some of the additional energy intake in the pellet-fed lambs may have been used for rumen and liver development, as indicated crudely by greater size of these organs, rather than contributing to increased total energy content in the lamb bodies. The present study indicates that little change occurs in total chemical body composition of lambs during the transitional period of growth due to the increased energetic costs associated with rumen and liver developments.

#### **5.4.4 ME requirements for maintenance and growth**

Metabolisable energy requirement of lambs is usually defined as digestible energy less the urinary energy and methane (Black, 1971). However, for the purpose of the present study, it represented the energy available in the body of lambs to meet the needs of maintenance and growth, and was defined as digestible energy less the energy excreted in urine. The value obtained for  $ME_m$  ( $0.41 \text{ ME/kg LW}^{0.75} \cdot \text{d}^{-1}$ ) was similar to the ARC recommended value for maintenance (0.40; ARC (1980)) and the value of 0.39 of Sanz Sampelayo *et al.* (1995). The value obtained for  $ME_g$  ( $13.8 \text{ MJ / kg ADG}$ ) was comparable to the  $12.9 \text{ MJ / kg LWG}$  and  $13 \text{ MJ/ kg LWG}$  obtained for lambs (Chapter 4) and kid goats respectively, given milk and solid feeds concurrently (Luo *et al.*, 2004; Sahlu *et al.*, 2004) but greater than the ARC (1980) recommended value of 6.4 – 7.8 MJ/kg LWG.

#### **5.4.5 Efficiency of ME utilisation for growth ( $k_g$ )**

The efficiency of ME utilisation for growth ( $k_g$ ) was estimated using two regression models. The  $k_g$  value of 0.53 was determined by regressing ME intake on energy retention (ER), whereas a  $k_g$  value of 0.64 was derived by dividing the mean ER per kg LWG (8.86 MJ) obtained from the comparative slaughter method by the  $ME_g$  ( $13.8 \text{ MJ / kg LWG}$ ). Both  $k_g$  values are comparable to the estimates of 0.69 to 0.77 obtained for lambs fed exclusively on milk (Jagusich and Mitchell, 1971; Walker and Norton, 1971; Degen and Benjamin, 2005) but higher than the value of 0.40 obtained in Chapter 4 and for 8 to 14 week-old lambs fed exclusively on solid feed (0.13-0.29; (Fennessy *et al.*, 1972; Mitchell and Jagusich, 1972; Thomson and Cammell, 1979). A possible explanation for the higher efficiency in the present study is the proportion of fat in LWG. Lambs in the current study deposited more fat per kg LWG (average 116.4 g/kg

LWG) in comparison to that deposited in the Suffolk lambs (average 58.2 g/kg LWG) used in Chapter 4. It is known that an increase in fat deposition in tissue gain improves the  $k_g$  value (Rattray and Joyce, 1974; 1976) which may explain the higher value. More research is therefore needed to determine if different levels of fat deposition in pre-weaned lambs affects the  $k_g$  value.

#### **5.4.6 Protein to energy requirements**

The energy and nitrogen balances showed that the CP:ME ratio of MR and pellet used in the present study was 11.1 and 15.7, respectively. However, the simulation model indicated that lambs require a CP:ME ratio of 13.1 at 5 kg and 10.9 at 18 kg LW. Similarly, Black *et al.* (1973) showed that lambs crude protein to net energy requirements of lambs decreased in a linear fashion from a value of 12.2 at 5 kg LW to a value of 9 at 18 kg LW. The actual CP:ME intake of lambs in the present study, however, increased as LW increased which may demonstrate an increasing reliance on solid feed as lambs grew. Combined, these few data may indicate that protein intake relative to energy intake may be a limiting factor to lamb growth in early life while being in excess later on when solid feed becomes the main source of nutrient intake, thereby wasting protein. However, further studies are warranted to elucidate these findings.

### **5.5 Conclusions**

Increased levels of pellets in the diet of pre-weaned lambs and, therefore, greater daily ME intake was associated with increased daily gains, organ weights and the efficiency of gain (ratio of ADG:ME intake / day). Increased daily ME intake did not alter the total chemical body composition of the lambs fed to a fixed live weight which may partly be

explained by the increased energetic costs associated with rumen and liver developments. Metabolisable energy requirement for maintenance ( $0.40 \text{ MJ} / \text{kg LW}^{0.75}$ ) was similar to that previously reported. A greater metabolisable energy was required for growth ( $13.8 \text{ MJ} / \text{kg LWG}$ ) in the present study compared to previous recommendations and warrants further investigation. The inclusion of pellets in the diet improved the efficiency of ME utilisation for growth in pre-weaned lambs and was beneficial for the physical development of the rumen. It is therefore recommended that pellets be made available to artificially reared or lambs who do not have access to herbage to allow for early weaning of the lambs. The gross efficiency of N retained for growth was lower for pellets than milk. Therefore, addition of pellets to the milk ration of pre-weaned lamb decreased the overall N utilisation. The CP:ME ratio of the intake of MR and pellet was 11.1 and 15.6, respectively. However, lambs require a CP:ME ratio of 13.1 at 5 kg and 10.9 at 18 kg LW indicating that protein intake of milk replacer or milk may be limiting to lamb growth in early life while being in excess in later life which may warrant further studies.

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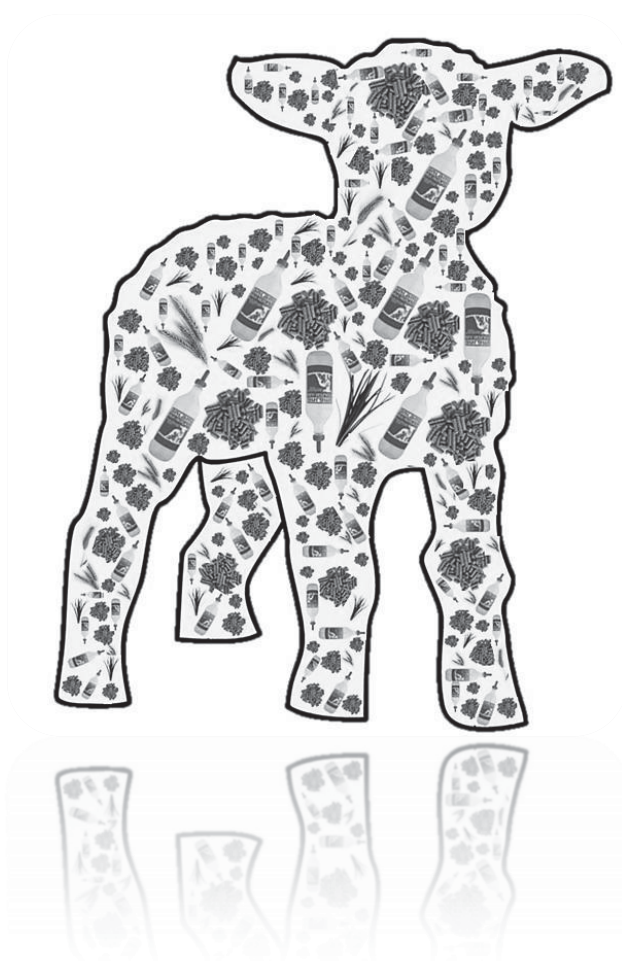




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## Chapter 6      Effects of Dietary Protein to Energy Ratios on Growth, Body Composition and Nutrient Utilisation in Lambs Reared Artificially with Milk and Pellets

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## ABSTRACT

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A growth simulation model has shown that the crude protein (CP) to metabolisable energy (ME) intake ratio of lambs reared artificially does not ideally match their requirements which could be limiting growth in early life. To determine the effects of different CP:ME ratios via milk and pellets intake on the growth, body composition and nutrient utilisation, 28 twin-born male lambs were allocated to a randomised 2 x 2 factorial design with seven lambs per treatment from age one day postpartum until 18kg LW. Treatments consisted of two protein levels in milk replacer (MR) (normal protein milk [NM; 24% CP] and high-protein milk [HM; 31.2% CP]) fed as a proportion of the lamb's live weight (LW) and *ad libitum* access to two protein levels in pellets (low-protein pellets [LP; 13.6%] and high-protein pellets [HP; 19.5%]); resulting in four groups (NMLP, NMHP, HMLP, HMHP). Five lambs from each treatment were placed in metabolic cages at 9 kg and 16 kg LW for 4 days to allow for total faecal and urine collection. All lambs were slaughtered at 18 kg LW. The weights and chemical composition of the carcass, organs, head and skin were determined. Lambs fed HM had greater ( $P < 0.01$ ) crude protein (CP) intake, daily gain, gain to feed ratio and greater liver and kidney weights than lambs fed NM. Pellet type had no effect ( $P > 0.05$ ) on any of the intake or growth measurements. Lambs fed HM had higher ( $P < 0.05$ ) total protein content in their blood than those fed NM at 18 kg LW. Deposition of protein (PD) and water in the carcass, organs, and empty bodies were greater ( $P < 0.01$ ) in HM than in NM lambs. Fat deposited and energy retained in carcasses, organs, and empty bodies were greater ( $P < 0.05$ ) in NM than in HM lambs, demonstrating that MR composition can markedly affect the chemical body composition in pre-weaned lambs. Pellet type had no effect ( $P > 0.05$ ) on the nutrient deposition in the body tissues. The

response of PD to CP intake was curvilinear and a maximum of 44 g /d was reached at CP intake of 98.8 g / d. In conclusion, increasing the CP level of milk replacer from 24% to 31% was a more efficient way of improving growth rates and protein deposition in pre-weaned lambs than increasing the CP level of their pellet feeds.

## 6.1 Introduction

One of the aims of the New Zealand sheep industry is to improve live weight gain (LWG) of pre-weaned lambs and consequently, the total weaning weight of lambs per ewe (Morris and Kenyon, 2014). However, improving LWG in animals does not necessarily result in increase in lean tissue deposition as the LWG may be deposited as fat (Stobo *et al.*, 1966). Sheep farmers in New Zealand are paid premiums for the production of lean lamb carcasses (Schreurs, 2012). Therefore, emphasis may switch from maximising daily gain to increasing the lean tissue growth in the near future. Further, lean animals are more efficient than fat animals from a nutritional perspective as less feed energy is required to deposit 1 g of lean tissue compared with 1 g of adipose tissue (van Milgen and Noblet, 2003).

Altering dietary composition is one approach to improve the efficiency of gain in pre-weaned ruminants. Increasing crude protein (CP) content of milk replacers (MR), and thus the corresponding protein:energy ratio can increase weight gain and protein deposition in lambs (Jagusch *et al.*, 1970; Norton *et al.*, 1970) and calves (Diaz *et al.*, 2001; Blome *et al.*, 2003). Likewise, offering low-protein solid feed to veal calves reduces urinary N excretion and improves N utilisation for protein gain (Berends *et al.*, 2012). However, there is no information on the effect of altering both MR and solid feed (pellet) composition on growth, body composition, and N utilisation in lambs pre-weaning.

A simple growth simulation model based on equations derived in a recent study (Chapter 5) showed that the CP to metabolisable energy (ME) ratio (CP:ME) needed to meet the lamb's growth requirement decreased in a curvilinear fashion from a value of

13.1 at 5 kg live weight (LW) to 10 at 30 kg LW. Given that the CP: ME ratio in ewe's milk is about 11 (Paten *et al.*, 2013) and 16 to 20 in New Zealand pastures (Brookes and Nicol, 2007), the CP:ME ratios do not ideally match the young lambs nutritional requirements, which could limit growth in early life while wasting protein in late lactation. It is, therefore, hypothesised that increasing the CP content of MR in the first 4 weeks of life will help improve lamb growth whilst offering low-protein solid feed concurrently will also help reduce urinary N excretion until weaning. The aim of this study was to feed pre-weaned lambs milk and pellet diets containing different protein:energy ratios and determine their effects on growth, body composition and nutrient utilisation during artificial rearing.

## **6.2 Materials and methods**

The study was conducted at Massey University 5 km South of Palmerston North, New Zealand during the months of September to December 2015. The study and animal handling procedures were approved by the Massey University Animal Ethics Committee (MUAEC protocol 15/54).

### **6.2.1 Experimental design, animals and management**

Thirty-two sets of mixed-sex Romney twin lambs were selected for the study. Lambs were allowed to suckle from the dam for one day post-partum before one male lamb was removed. Four lambs were slaughtered at separation to provide baseline data and the remaining 28 male lambs were moved indoors, kept in single pens and hand-reared. The 28 lambs were randomly allocated to a 2 x 2 factorial design with seven lambs per treatment. Treatments consisted of two protein levels in MR (normal protein milk (NM; Milligans Feed Ltd, Oamaru, New Zealand) and high-protein milk (HM)) and two

protein levels in pellets (low-protein pellets (LP) and high-protein pellets (HP)); resulting in four potential groups (NMLP, NMHP, HMLP, HMHP). The HM consisted of 80% NM and 20% milk protein concentrate ([MPC]; Fonterra, New Zealand) to give a milk replacer containing 31.2% crude protein. The chemical composition of the milk replacers and pellets are presented in Table 6.1. The ME contents of both milk replacers were identical, and the ME content of both pellets were also similar.

Liquid MR was prepared daily with warm water at 30°C at a ratio of 1 part MR to 4 parts water to give a 20 % dry matter intake (DMI) per kg milk as fed. The quantity of MR and the frequency of feeding were adjusted as lambs grew and aged. Lambs were fed milk five times daily totalling 15% of their LW in the first 14 days. From day 15, feeding was reduced to four times totalling 10% of their LW until the end of the study. This resulted in an average initial amount of milk powder being 175 g/day at 5 kg LW which gradually increased to 320 g/day at 18 kg LW. The quantity of milk fed per kg LW was reduced to encourage greater pellet consumption. Pellets were offered *ad libitum* to all lambs from day one until the end of the study. Milk intake and pellet intake were recorded as the daily amount offered, minus the amount refused. Lambs were weighed twice a week, on Mondays and Thursdays, before the first feed of the day.

### **6.2.2 Blood sampling and analysis**

Blood was collected from each lamb by jugular venipuncture on the day of separation from their dam, at 14 kg and 18 kg LW 1 h after the second milk feeding of the day. Blood samples were collected using 5-mL serum sampling tubes (Vacutainer; Becton, Dickinson and Company) and placed immediately on ice for 1 h to allow samples to coagulate. Tubes were then centrifuged in a Heraeus Labofuge 200 Centrifuge (Thermo

Scientific Heraeus) at  $3000 \times g$  for 15 min at room temperature to obtain serum. Aliquots of the serum were then transferred into Eppendorf tubes and analyzed within 24 h of collection. Serum was analyzed for non-esterified fatty acids (NEFA) (kit number 279-75401; Wako Pure Chemical Industries, Ltd., Osaka, Japan), total protein (Roche Diagnostics Ltd., Mannheim, Germany), urea N (Roche Diagnostics Ltd., Mannheim, Germany) and glucose (Roche Diagnostics Ltd., Mannheim, Germany).

Table 6.1 Analytical chemical composition of milk replacers and pellets as fed to artificially reared lambs.

| Chemical composition       | Diet <sup>1</sup> |      |                 |                 |                 |
|----------------------------|-------------------|------|-----------------|-----------------|-----------------|
|                            | NM                | MPC  | HM <sup>2</sup> | LP <sup>3</sup> | HP <sup>3</sup> |
| Dry matter, %              | 97.7              | 96.4 | 97.4            | 87.3            | 87.5            |
| Ash, %                     | 5.4               | 7.4  | 5.8             | 3.4             | 3.9             |
| Crude protein, %           | 24.0              | 60.2 | 31.2            | 13.6            | 19.5            |
| Fat, %                     | 27.2              | 1.2  | 22              | 2.4             | 2.4             |
| Lactose, %                 | 38.0              | 25.7 | 35.54           | -               | -               |
| Minerals, g / 100 g        |                   |      |                 |                 |                 |
| Calcium                    | 0.82              | 1.76 | 1.01            | 0.14            | 0.16            |
| Magnesium,                 | 0.11              | 0.10 | 0.11            | 0.23            | 0.25            |
| Potassium                  | 1.02              | 0.93 | 1.0             | 0.87            | 1.18            |
| Sodium                     | 0.25              | 0.20 | 0.24            | 0.02            | 0.01            |
| Phosphorus                 | 0.64              | 1.22 | 0.76            | 0.46            | 0.53            |
| Sulphur                    | 0.27              | 0.50 | 0.32            | 0.17            | 0.21            |
| Chloride                   | 0.62              | 0.39 | 0.57            | 0.19            | 0.15            |
| Gross energy, kJ/g         | 22.8              | 19.3 | 22.1            | 16.4            | 16.5            |
| Neutral Detergent fibre, % | -                 | -    | -               | 21.6            | 20.1            |
| Acid detergent fibre, %    | -                 | -    | -               | 6.6             | 6.1             |
| Lignin, %                  | -                 | -    | -               | 1.6             | 1.4             |

<sup>1</sup>Diet: NM = Normal-protein milk; MPC = Milk protein concentrate; HM = High-protein milk; LP = Low-protein pellet; HP = High-protein pellet.

<sup>2</sup>Composed of 80% normal protein milk and 20% MPC

<sup>3</sup>Composed of wheat middling, soya bean, molasses and barley.

### **6.2.3 Total faecal and urine collection**

A nutrient balance study was conducted to determine energy and nitrogen (N) utilisation. A total of 20 lambs ( $n = 5$  per treatment group) were put in metabolic cages at 9 kg (period one) and 16 kg (period two) live weight for four days to allow for total urine and faecal collections. The same lambs were used in both periods. Faeces were collected in aluminium pans and the quantity excreted was measured daily over the four-day period. Faeces collected per lamb over the four day period were pooled within lamb and sub-sampled for each period of the digestibility study. They were then freeze-dried, ground, and stored until further analysis. Faecal samples were analysed for contents of N, gross energy (GE), Neutral Detergent fibre (NDF) and Acid detergent fibre (ADF).

The funnel tray underneath the crate allowed for urine collection into a plastic bucket. Each day, 0.2 g of Potassium dichromate ( $K_2Cr_2O_7$ ) was put into the collection bucket to inhibit growth of bacteria and enzymatic processes that cause loss of nitrogen (Diaz *et al.*, 2001). The volume of urine collected per lamb was measured daily and 10 ml sub-sample was pooled (for each period of the digestibility study) over the four-day period. The pooled sub-samples were frozen and stored at  $-20^{\circ}C$  for further analysis. Urine samples were analysed for GE and N.

### **6.2.4 Slaughter**

Lambs were weighed after being fasted overnight. They were then slaughtered by captive bolt stunning and exsanguination, skinned and eviscerated. The carcass and non-carcass parameters were measured. Weights of the head, skin and feet, hot carcass and organs were recorded. The stomach and intestines were weighed before and after

removal of contents to determine gut fill. Weights of the liver, kidney, and spleen were also recorded. The carcass and organs were then stored at  $-20^{\circ}\text{C}$  until further analysis.

#### **6.2.5 *Body tissues sampling***

Body components were separated into four fractions: 1) carcass; 2) visceral organs, 3) head and 4) skin. The fractions were cut into small blocks and minced separately in a commercial butcher's mincer, once through a 10mm grinding plate and twice through a 3mm grinding plate. Prior to mincing, the frozen body components were weighed again to account for moisture losses. Subsamples of the ground tissues from the carcass and organs were collected into plastic containers, weighed and stored at  $-20^{\circ}\text{C}$  until further analysis

#### **6.2.6 *Proximate Analysis of diet and body tissues***

Samples of dietary and body tissues were analysed for nitrogen (N) using the Leco total combustion method (Dumas method; AOAC method 968.06), gross energy (GE) by bomb calorimetry (Leco, AC 350, Leco Corporation, St Joseph, MI, USA) and dry matter (DM) using a convection oven at  $105^{\circ}\text{C}$  (AOAC methods 930.15 and 925.10). The ash content was measured by placing samples in a furnace at  $550^{\circ}\text{C}$  (AOAC method 942.05) and lactose was determined using the enzymatic method (Boehringer Mannheim/R-Biofarm Enzyme kit for Lactose/D-Galactose – enzymatic digestion colourimetric was determined at 340nm).

Fat concentration in pellets and body tissues were determined by the Soxtec extraction method (AOAC method 991.36) while milk fat was determined using the Mojonnier extraction method (AOAC 954.02). Neutral detergent fibre (NDF), acid detergent fibre (ADF) and lignin were determined using the Tecator Fibretec System following the

method described by Robertson and Van Soest (1981) (AOAC method 2002.04). Mineral composition of the diets was analysed using the Inductively Coupled Plasma Emission Spectrometry (ICP) method. Fats, ash, N and DM were all determined following the AOAC (1990) method.

#### **6.2.7 Histological studies**

Two square rumen tissue samples measuring 1cm x 1cm were taken from the left side cranial ventral sac (Lesmeister *et al.*, 2004) of the rumen of each animal within 30 minutes of slaughter. The tissues were placed in Tissues-Loc cassettes and fixed immediately in a 10% formalin (buffered) solution for 48 hours. The samples were then cut into two fingers measuring 1 cm x 0.5 cm each. The tissues were put into 10% NB formalin and stored overnight. Each tissue was embedded in paraffin wax and then cut into two sections, each measuring 3µm thick. The sections were placed on two slides and stained with haematoxylin and eosin and kept for histological examination. The slides were observed under a light microscope. Forty (twenty per slide) rumen papillae lengths and widths were measured at 2.5x magnification. Papillae length was defined as the distance from the tip to the base of the papillae. The width of the tip, middle, and base of the papillae were measured and the average of the three measurements was recorded as the width of the papillae. The papillae circumference was determined by measuring the contour line of each papilla using ImageJ software (Rasband, 1997). Rumen papilla was taken to be cylindrical in shape with one closed end, thus the lateral papillae lateral surface area was calculated as:

$$\text{Surface area of papillae (cm}^2\text{)} = 2\pi rL + \pi r^2 \text{ (Hill } et al., 2005\text{)}$$

Where  $r$  is the radius in cm and  $L$  is the length in cm

### **6.2.8 Calculations**

Milk and pellet intake were determined as the daily amount offered less amount refused. Nitrogen (N) intake was calculated as milk and pellet intake multiplied by the N concentration of each feed. Digestible N (DN) was calculated as N intake less N excreted in faeces. Retained N (RN) was determined by subtracting urinary N excreted from DN.

Crude protein content was calculated as  $N \times 6.38$  for milk and  $N \times 6.25$  for pellets. Gross energy (GE) intake was calculated as milk and pellet intake multiplied by the gross energy content of each feed. Digestible energy (DE) was determined by subtracting energy excreted in faeces from GE intake. Metabolisable energy (ME) intake was determined by subtracting energy excreted in urine from DE. Total ME intake was calculated as the sum of ME in milk and pellets.

Average daily gain (ADG) of each lamb was calculated as LWG over the study period divided by the number of experimental days per lamb. The gain to feed ratio was calculated as both the kilogram LWG divided by the kilogram dry matter intake (DMI) and g ADG divided by the MJ ME intake/d.

Empty body weight (EBW) of the baseline group and at the end of the study was calculated as the sum of the weight of the whole body components: head, skin and feet, carcass and digesta-free organs (but not including blood weight). The chemical composition of the lambs at the start of the study and the chemical composition of gain were calculated as described in Chapter 5. Crude protein for body tissue samples was calculated as  $N \times 6.25$ .

### **6.2.9 Statistical analysis**

Growth performance, carcass and organ weights and body composition were analysed using the generalised linear  $2 \times 2$  factorial model procedure (Proc GLM) in SAS (2013) with fixed effects of milk type and pellet type and the interaction of milk and pellet type. Differences were identified using the least significant difference mean comparison test when the F-value for the diet effect was significant ( $P < 0.05$ ).

A repeated measure analysis was performed on the rumen papillae dimensions using Proc Mixed in SAS (2013) with fixed effects of milk type and pellet type and their interaction and, lamb nested in the feeding group as a random effect. A slide effect was included in the model as a repeated measure. Differences were identified using the least significant difference mean comparison test when the F-value for the diet effect was significant ( $P < 0.05$ ).

Blood metabolite analyses were carried out using repeated measure mixed model (Proc Mixed; SAS 2013). The two protein levels for milk and pellet were included in the model as fixed effects, and lamb nested in the milk and pellet interaction as a random effect. The weight of lamb at blood collection (5 kg, 14 kg and 18 kg LW) was included in the model as a repeated measure. Differences were identified using the least significant difference mean comparison test when the F-value for the diet effect was significant ( $P < 0.05$ ).

Energy and nitrogen balance data were analysed using the Mixed procedure of SAS (2013). Milk and pellet types were included in the model as fixed effects, and lamb nested in the milk by pellet interaction as a random effect, and the weight of the lambs during the balance study (9 kg and 16 kg LW) included in the model as a repeated

measure. Differences were identified using the least significant difference mean comparison test when the F-value for the diet effect was significant ( $P < 0.05$ ).

## **6.3 Results**

### **6.3.1 *Nitrogen and energy balance***

There were significant milk and weight interactions observed in total N intake, total milk N intake, digestible N and urinary N excretion (Table 6.2). All four instances (total N intake, total milk N intake, digestible N and urinary N excretion) were by scale, thus, the differences observed were greater ( $P < 0.05$ ) at 16 kg LW than at 9 kg LW. At both LWs, lambs fed HM had greater ( $P < 0.05$ ) total N intake, total milk N intake, digestible N and urinary N excretion than those fed NM. The interaction was caused by the total N, total milk N and digestible N intakes of HM being approximately 20% greater than NM and the urinary N excretion being 35% greater than the NM at both LWs.

Faecal N excretion was not affected ( $P > 0.05$ ) by the type of milk nor pellet consumed (Table 6.2). Urinary N excretion was greater ( $P < 0.001$ ) for HM fed lambs than NM lambs but it was unaffected ( $P > 0.05$ ) by the type of pellet offered.

The GE intake from milk was greater ( $P < 0.05$ ) for NM than HM group (Table 6.2). Lambs offered HP consumed more pellets during the balance study than their counterparts offered the LP. Consequently, the total GE intake was greater ( $P < 0.05$ ) in lambs offered HP than those offered LP. There was no effect ( $P > 0.05$ ) of milk type on the total GE consumed. The type of milk or pellet consumed did not affect ( $P > 0.05$ ) the amount of faecal energy excreted. Urinary energy excreted was greater in the HM than NM group.

Table 6.2 Effect of dietary treatment on nitrogen (N) and energy balances<sup>1</sup> (LS means) of lambs reared artificially on two protein levels in milk replacer and two protein levels in pellets at 9 kg and 16 kg live weights over a four-day period.

|                                  |       | Treatment <sup>2</sup> |       |                |      |                    |                    |                    |                    |      |       | SE <sup>3</sup> |       | P- value <sup>4</sup> |       |       |        |       |      |      |
|----------------------------------|-------|------------------------|-------|----------------|------|--------------------|--------------------|--------------------|--------------------|------|-------|-----------------|-------|-----------------------|-------|-------|--------|-------|------|------|
|                                  |       | Milk protein           |       | Pellet protein |      | SE                 |                    | Milk × Weight      |                    |      |       |                 |       |                       |       |       |        |       |      |      |
|                                  |       | NM                     | HM    | LP             | HP   |                    |                    | NM                 | HM                 | 9kg  | 16kg  | NM              | HM    | 16kg                  | 10    | Milk  | Pellet | Wt    | M×P  | M×Wt |
| n                                | 10    | 10                     | 10    | 10             | 10   | 10                 | 10                 | 10                 | 10                 | 10   | 10    | 10              | 10    | 10                    |       |       |        |       |      |      |
| Nitrogen balance                 |       |                        |       |                |      |                    |                    |                    |                    |      |       |                 |       |                       |       |       |        |       |      |      |
| Total N intake, g                | 46.42 | 58.00                  | 50.62 | 53.81          | 0.92 | 35.09 <sup>a</sup> | 44.04 <sup>b</sup> | 57.75 <sup>c</sup> | 71.98 <sup>d</sup> | 1.12 | <0.01 | 0.03            | <0.01 | 0.98                  | 0.03  | <0.01 | 0.95   | <0.01 | 0.81 | 0.03 |
| N intake (milk), g               | 37.63 | 48.98                  | 43.05 | 43.56          | 0.34 | 30.42 <sup>a</sup> | 39.90 <sup>b</sup> | 44.84 <sup>c</sup> | 58.06 <sup>d</sup> | 0.48 | <0.01 | 0.31            | <0.01 | 0.95                  | <0.01 | 0.81  | 0.03   | <0.01 | 0.81 | 0.03 |
| N intake (pellet), g             | 8.79  | 9.03                   | 7.57  | 10.25          | 0.99 | 4.67               | 4.14               | 12.91              | 13.92              | 1.12 | 0.86  | 0.07            | <0.01 | 0.96                  | 0.50  | <0.01 | 0.96   | <0.01 | 0.96 | 0.50 |
| Faecal N, g                      | 3.89  | 4.75                   | 3.95  | 4.69           | 0.40 | 2.28               | 2.60               | 5.50               | 6.91               | 0.39 | 0.14  | 0.20            | <0.01 | 0.69                  | 0.18  | <0.01 | 0.69   | <0.01 | 0.69 | 0.18 |
| Digestible N (DN), g             | 42.53 | 53.25                  | 46.67 | 49.11          | 0.73 | 32.81 <sup>a</sup> | 41.44 <sup>b</sup> | 52.24 <sup>c</sup> | 65.07 <sup>d</sup> | 0.90 | <0.01 | 0.03            | <0.01 | 0.81                  | 0.03  | <0.01 | 0.81   | <0.01 | 0.81 | 0.03 |
| Urinary N, g                     | 14.58 | 22.29                  | 17.88 | 18.99          | 0.84 | 9.46 <sup>a</sup>  | 14.69 <sup>b</sup> | 19.71 <sup>c</sup> | 29.88 <sup>d</sup> | 0.76 | <0.01 | 0.37            | <0.01 | 0.91                  | 0.01  | <0.01 | 0.91   | <0.01 | 0.91 | 0.01 |
| RN <sup>5</sup> , g              | 27.94 | 30.97                  | 28.78 | 30.13          | 0.99 | 23.35              | 26.74              | 32.53              | 35.19              | 1.13 | 0.05  | 0.35            | <0.01 | 0.79                  | 0.75  | <0.01 | 0.79   | <0.01 | 0.79 | 0.75 |
| Energy balance                   |       |                        |       |                |      |                    |                    |                    |                    |      |       |                 |       |                       |       |       |        |       |      |      |
| Total GE <sup>6</sup> intake, MJ | 29.15 | 28.76                  | 27.80 | 30.11          | 0.69 | 21.75              | 21.02              | 36.56              | 36.49              | 0.82 | 0.69  | 0.03            | <0.01 | 0.95                  | 0.69  | <0.01 | 0.95   | <0.01 | 0.95 | 0.69 |
| GE intake (milk), MJ             | 22.58 | 22.00                  | 22.16 | 22.42          | 0.17 | 18.25              | 17.92              | 26.90              | 26.08              | 0.24 | 0.03  | 0.29            | <0.01 | 0.93                  | 0.32  | <0.01 | 0.93   | <0.01 | 0.93 | 0.32 |
| GE intake (pellet), MJ           | 6.57  | 6.75                   | 5.64  | 7.69           | 0.74 | 3.50               | 3.10               | 9.65               | 10.41              | 0.84 | 0.86  | 0.07            | <0.01 | 0.97                  | 0.50  | <0.01 | 0.97   | <0.01 | 0.97 | 0.50 |
| Faecal                           | 2.17  | 2.45                   | 2.09  | 2.53           | 0.27 | 0.99               | 1.05               | 3.35               | 3.84               | 0.27 | 0.47  | 0.27            | <0.01 | 0.82                  | 0.44  | <0.01 | 0.82   | <0.01 | 0.82 | 0.44 |



There was a milk and weight interaction effect on the retained protein (RP): to ME ratio (Table 6.2). The RP:ME ratio was greater ( $P < 0.05$ ) at 9 kg LW than at 16 kg LW for both milk types. At similar weights, lambs fed HM had greater ( $P < 0.05$ ) RP:ME ratio than those fed NM. However, there was no difference ( $P > 0.05$ ) in RP:ME ratio between NM fed lambs at 9 kg LW and HM fed lambs at 16 kg LW. The interaction was caused by the HM being 1.5 greater than NM at 9 kg LW while this difference was only 0.7 at 16 kg. The CP:ME ratio of milk were greater ( $P < 0.001$ ) for HM than NM but did not differ ( $P > 0.05$ ) between the pellet type.

A multiple linear regression with the N intake from milk and the N intake from pellet was fitted to the measured DN intake data. The regression coefficients represent the digestibility coefficients for milk N and pellet N (Eq 6.1), and a similar approach was used for retained N (RN) (Eq.6.2). A multiple linear regression with the GE intake from milk and the GE intake from pellet was fitted to the measured ME intake data. The regression coefficients represent the digestibility coefficients for milk energy and pellet energy (Eq 6.3), and similar approach was used for the ME (Eq.6)

$$DN = 0.97 (\pm 0.01) NI_{NM} + 0.96 (\pm 0.01) NI_{HM} + 0.67 (\pm 0.01) NI_{LP} + 0.68 (\pm 0.03) NI_{HP} \\ (R^2 = 0.99) \quad (6.1)$$

$$RN = 0.70 (\pm 0.04) NI_{NM} + 0.59 (\pm 0.03) NI_{HM} + 0.14 (\pm 0.16) NI_{LP} + 0.19 (\pm 0.13) NI_{HP} \\ (R^2 = 0.99) \quad (6.2)$$

$$DE = 0.98 (\pm 0.02) GEI_{NM} + 0.97 (\pm 0.02) GEI_{HM} + 0.71 (\pm 0.05) GEI_{LP} + 0.74 (\pm 0.04) \\ GEI_{HP} (R^2 = 0.99) \quad (6.3)$$

$$ME = 0.96 (\pm 0.02) GEI_{NM} + 0.94 (\pm 0.02) GEI_{HM} + 0.68 (\pm 0.06) GEI_{LP} + 0.71 (\pm 0.04) \\ GEI_{HP} (R^2 = 0.99) \quad (6.4)$$

### **6.3.2 Intake and growth performance**

No milk by pellet interactions were detected ( $P > 0.05$ ) for any of the intake or growth parameters measured. Therefore, only the means of the main effects are presented (Table 6.3). The initial and final LW of the lambs did not differ ( $P > 0.05$ ) between treatment groups. Total milk intake was higher ( $P < 0.01$ ) for lambs that received the normal protein milk (NM) than for those that received the high-protein milk (HM). Total pellet intake was unaffected ( $P > 0.05$ ) by pellet type.

Total crude protein (CP) intake, total CP milk intake and CP intake per day were greater ( $P < 0.001$ ) in HM than NM fed lambs. Lambs offered high protein pellet (HP) consumed more ( $P < 0.04$ ) CP per day than their counterparts offered low protein pellet (LP). However, the total CP intake did not differ ( $P > 0.05$ ) between the pellet groups. Total metabolisable energy (ME) intake and ME intake from milk were greater ( $P < 0.001$ ) for the NM than HM fed lambs but unaffected ( $P > 0.05$ ) by pellet type. The type of milk or pellet consumed did not affect ( $P > 0.05$ ) ME intake /d.

The ADG for HM lambs was greater ( $P < 0.001$ ) than NM lambs (Table 6.3). Therefore, HM lambs reached slaughter weight faster ( $P = 0.01$ ) than the NM lambs. There was no difference ( $P > 0.05$ ) in age at slaughter for the pellet groups. The gain to feed ratio was greater ( $P = 0.01$ ) for lambs fed HM than their counterparts fed the NM. The CP:ME ratio was affected by diet type with the ratio for HM lambs being greater ( $P < 0.05$ ) than NM lambs and that of HP being greater ( $P < 0.05$ ) than LP lambs.

Table 6.3 Intake and growth (LS means) of artificial reared lambs fed two protein levels in milk replacer and two protein levels in pellets to 18 kg live weight

| Item                                | Treatment <sup>1</sup> |       |                |       | SE <sup>2</sup> | P- value |        |
|-------------------------------------|------------------------|-------|----------------|-------|-----------------|----------|--------|
|                                     | Milk protein           |       | Pellet protein |       |                 | Milk     | Pellet |
|                                     | NM                     | HM    | LP             | HP    |                 |          |        |
| n                                   | 14                     | 14    | 14             | 14    |                 |          |        |
| Initial live weight, kg             | 5.33                   | 5.51  | 5.46           | 5.38  | 0.21            | 0.53     | 0.80   |
| Final live weight, kg               | 18.77                  | 18.89 | 18.99          | 18.67 | 0.15            | 0.59     | 0.14   |
| Age at slaughter, d                 | 60.07                  | 53.00 | 58.00          | 55.07 | 1.68            | 0.01     | 0.23   |
| Live weight gain (LWG), kg          | 13.45                  | 13.38 | 13.54          | 13.29 | 0.23            | 0.85     | 0.46   |
| Average daily gain, g               | 228.7                  | 260.2 | 239.5          | 249.4 | 5.83            | <0.001   | 0.24   |
| Total milk intake, kg               | 67.34                  | 60.00 | 65.76          | 61.59 | 1.61            | <0.01    | 0.08   |
| Total pellet intake, kg             | 5.01                   | 5.24  | 5.16           | 5.08  | 0.35            | 0.64     | 0.87   |
| Total dry matter intake (DMI), kg   | 17.53                  | 16.27 | 17.34          | 16.46 | 0.47            | 0.07     | 0.20   |
| Total milk DMI, kg                  | 13.16                  | 11.69 | 12.83          | 12.02 | 0.31            | <0.01    | 0.08   |
| Total pellet DMI, kg                | 4.37                   | 4.57  | 4.51           | 4.44  | 0.30            | 0.64     | 0.87   |
| DMI/d, g                            | 297.4                  | 314.1 | 305.2          | 306.2 | 5.32            | 0.04     | 0.89   |
| Gain: feed ratio, kg LWG/kg DMI     | 0.77                   | 0.83  | 0.78           | 0.81  | 0.01            | 0.01     | 0.17   |
| Total crude protein (CP) intake, kg | 4.07                   | 4.61  | 4.30           | 4.37  | 0.12            | <0.01    | 0.73   |
| Total milk CP intake, kg            | 3.23                   | 3.75  | 3.60           | 3.38  | 0.09            | <0.001   | 0.10   |
|                                     |                        |       |                |       |                 |          | 0.74   |

|                                            |       |       |       |       |      |        |        |      |
|--------------------------------------------|-------|-------|-------|-------|------|--------|--------|------|
| Total pellet CP intake, kg                 | 0.84  | 0.86  | 0.71  | 0.99  | 0.06 | 0.75   | 0.002  | 0.90 |
| CP intake / d, g                           | 69.16 | 89.11 | 76.48 | 81.80 | 1.41 | <0.001 | <0.01  | 0.83 |
| Total metabolisable energy (ME) intake, MJ | 355.6 | 317.8 | 345.6 | 327.8 | 8.54 | <0.01  | 0.15   | 0.70 |
| Total milk ME intake, MJ                   | 295.7 | 255.4 | 284.6 | 266.5 | 6.93 | <0.001 | 0.08   | 0.54 |
| Total pellet ME intake, MJ                 | 59.86 | 62.42 | 60.95 | 61.32 | 4.12 | 0.78   | 0.80   | 0.88 |
| ME intake / d, MJ                          | 5.66  | 5.62  | 5.66  | 5.62  | 0.10 | 0.78   | 0.80   | 0.88 |
| CP: ME intake ratio, g CP / MJ ME          | 11.46 | 14.52 | 12.58 | 13.41 | 0.04 | <0.001 | <0.001 | 0.20 |

<sup>1</sup>Treatment: NM = Normal-protein milk (24.0% CP); HM = High-protein milk (31.2% CP); LP = Low-protein pellet (13.6% CP); HP = High-protein pellet (19.5% CP).

<sup>2</sup>SE: standard error

<sup>3</sup>M×P: milk×pellet interaction

### **6.3.3 Blood metabolites**

There were no interactions ( $P > 0.05$ ) between diet type and weight at blood collection on any of the measured blood serum metabolites. All serum blood metabolites decreased ( $P < 0.05$ ) from 5kg LW to later weights, but there were no differences ( $P > 0.05$ ) in blood metabolites at 14 kg and 18 kg (Table 6.4). Serum glucose and blood urea N (BUN) were higher ( $P < 0.05$ ) in lambs offered HP pellets than their counterparts offered LP pellets. The quantities of total protein and BUN were higher ( $P < 0.05$ ) in lambs that consumed HM than their counterparts that consumed NM. NEFA was not affected ( $P > 0.05$ ) by the type of milk or pellet fed to lambs

### **6.3.4 Slaughter parameters**

There were no interactions between milk and pellet type ( $P > 0.05$ ) for any of the slaughter parameters measured. There was no effect ( $P > 0.05$ ) of pellet type on any slaughter parameters with the exception of the weights of intestines and other organs (heart, lungs, etc.) being greater ( $P > 0.05$ ) for LP than HP lambs (Table 6.5). Lambs fed HM had greater ( $P < 0.05$ ) liver and kidney weights than those fed NM, whereas the weight of the head was greater ( $P < 0.05$ ) for NM fed lambs than the HM fed lambs.

### **6.3.5 Histological studies**

There were no effects ( $P > 0.05$ ) of milk or pellet type on any of the measured histological parameters (Table 6.6).

Table 6.4 Effect of dietary treatment on blood serum metabolites (LS means)<sup>1</sup> of lambs reared artificially on two protein levels in milk replacer and two protein levels in pellets from up to 18 kg live weight.

| Treatment <sup>2</sup> |              | Pellet protein |       |       |       | SE <sup>4</sup> | Weight <sup>3</sup> |                    |                    | SE   | P-value |        | M×P <sup>5</sup> |        |
|------------------------|--------------|----------------|-------|-------|-------|-----------------|---------------------|--------------------|--------------------|------|---------|--------|------------------|--------|
| Blood metabolite       | Milk protein | NM             | HM    | LP    | HP    | SE <sup>4</sup> | 5kg                 | 14 kg              | 18 kg              | SE   | Milk    | Pellet |                  | Weight |
| n                      |              | 14             | 14    | 14    | 14    |                 | 28                  | 28                 | 28                 |      |         |        |                  |        |
| Glucose, mmol/L        |              | 5.78           | 5.93  | 5.69  | 6.01  | 0.09            | 6.63 <sup>b</sup>   | 5.55 <sup>a</sup>  | 5.40 <sup>a</sup>  | 0.12 | 0.25    | 0.02   | <0.001           | 0.90   |
| Total protein, g/L     |              | 54.85          | 57.95 | 56.12 | 56.69 | 0.80            | 65.75 <sup>b</sup>  | 52.07 <sup>a</sup> | 51.39 <sup>a</sup> | 0.95 | 0.01    | 0.62   | <0.001           | 0.28   |
| Urea N, mmol/L         |              | 6.32           | 7.41  | 6.39  | 7.34  | 0.27            | 9.78 <sup>b</sup>   | 5.66 <sup>a</sup>  | 5.16 <sup>a</sup>  | 0.27 | 0.01    | 0.02   | <0.001           | 0.32   |
| NEFA, mmol/L           |              | 0.29           | 0.29  | 0.30  | 0.29  | 0.02            | 0.52 <sup>b</sup>   | 0.19 <sup>a</sup>  | 0.17 <sup>a</sup>  | 0.03 | 1.00    | 0.74   | <0.001           | 0.17   |

<sup>a,b</sup> Means in a row under weight with different superscripts are different from each other (LSD,  $P < 0.05$ ).

<sup>1</sup> Blood serum metabolite values over the study period

<sup>2</sup> Treatment: NM = Normal protein milk (24.0% CP); HM = High-protein milk (31.2% CP); LP = Low-protein milk (13.6% CP); HP = High-protein pellet (19.5% CP);

<sup>3</sup> Blood serum metabolite values across treatment groups.

<sup>4</sup> SE: standard error

<sup>5</sup> M×P: milk × pellet interaction

Table 6.5 Effect of dietary treatment on slaughter parameters (LS means) of lambs reared artificially on two protein levels in milk replacer and two protein levels in pellets to 18 kg live weight

| n                         | Treatment <sup>1</sup> |       | Pellet protein |       | SE <sup>2</sup> | P- value |        |                  |
|---------------------------|------------------------|-------|----------------|-------|-----------------|----------|--------|------------------|
|                           | Milk protein           |       | LP             | HP    |                 | Milk     | Pellet | M×P <sup>3</sup> |
|                           | NM                     | HM    |                |       |                 |          |        |                  |
|                           | 14                     | 14    | 14             | 14    |                 |          |        |                  |
| EBW <sup>4</sup> , kg     | 15.70                  | 15.49 | 15.78          | 15.41 | 0.14            | 0.29     | 0.07   | 0.50             |
| Dressing <sup>5</sup> , % | 50.26                  | 49.35 | 49.79          | 49.81 | 0.53            | 0.24     | 0.98   | 0.87             |
| Head, kg                  | 1.23                   | 1.13  | 1.19           | 1.17  | 0.02            | <0.01    | 0.46   | 0.67             |
| Skin and feet, kg         | 2.40                   | 2.49  | 2.48           | 2.42  | 0.04            | 0.16     | 0.31   | 0.54             |
| Hot carcass weight, kg    | 9.32                   | 9.43  | 9.45           | 9.30  | 0.10            | 0.47     | 0.29   | 0.89             |
| Total visceral weight, kg | 2.64                   | 2.55  | 2.66           | 2.53  | 0.05            | 0.21     | 0.09   | 0.37             |
| Liver, g                  | 318.0                  | 344.7 | 328.4          | 334.4 | 8.92            | 0.04     | 0.63   | 0.99             |
| Spleen, g                 | 36.14                  | 37.78 | 37.93          | 36.0  | 1.49            | 0.44     | 0.37   | 0.27             |
| Kidney, g                 | 69.64                  | 78.28 | 73.42          | 74.50 | 1.71            | <0.01    | 0.66   | 0.43             |
| Stomach <sup>6</sup> , g  | 341.2                  | 347.9 | 343.7          | 345.4 | 12.9            | 0.72     | 0.93   | 0.56             |
| Intestines, g             | 961.0                  | 937.4 | 998.9          | 899.6 | 26.9            | 0.54     | 0.02   | 0.26             |
| Gut fill, kg              | 1.88                   | 2.17  | 1.99           | 2.06  | 0.11            | 0.08     | 0.65   | 0.65             |
| All other organs, g       | 751.2                  | 700.1 | 757.4          | 694.4 | 18.2            | 0.06     | 0.02   | 0.31             |

<sup>1</sup>Treatment: NM = Normal protein milk (24.0% CP); HM = High-protein milk (31.2% CP); LP = Low-protein pellet (13.6% CP); HP = High-protein pellet (19.5% CP).

<sup>2</sup>SE: standard error.

<sup>3</sup>M×P: milk × pellet interaction

<sup>4</sup>EBW: empty body weight.

<sup>5</sup>Hot carcass weight as a percentage of final live weight

<sup>6</sup>All four stomach compartments

Table 6.6 Effect of dietary treatment on rumen papillary development (LS means) in lambs reared artificially on two protein levels in milk replacer and two protein levels in pellets up to 18 kg live weight

| Item                            | Treatment <sup>1</sup> |       | Pellet protein |       | SE <sup>2</sup> | P- value |        |                  |
|---------------------------------|------------------------|-------|----------------|-------|-----------------|----------|--------|------------------|
|                                 | Milk protein           |       |                |       |                 | Milk     | Pellet | M×P <sup>3</sup> |
|                                 | NM                     | HM    | LP             | HP    |                 |          |        |                  |
| n                               | 14                     | 14    | 14             | 14    |                 |          |        |                  |
| Length, μm                      | 1257                   | 1248  | 1238           | 1267  | 38.5            | 0.87     | 0.61   | 0.62             |
| Width, μm                       | 320.3                  | 336.4 | 323.7          | 333.0 | 12.1            | 0.36     | 0.58   | 0.60             |
| Circumference, μm               | 2758                   | 2764  | 2724           | 2797  | 80.8            | 0.96     | 0.52   | 0.61             |
| Surface area (cm <sup>2</sup> ) | 136.6                  | 143.2 | 136.7          | 143.2 | 7.77            | 0.55     | 0.56   | 0.50             |

<sup>1</sup>Treatment: NM = Normal protein milk (24.0% CP); HM = High-protein milk (31.2% CP); LP = Low-protein pellet (13.6% CP); HP = High-protein pellet (19.5% CP).

<sup>2</sup>SE: standard error.

<sup>3</sup>M×P: milk×pellet interaction.

### **6.3.6 Body composition**

There were no interactions ( $P > 0.05$ ) between milk type and pellet type on the chemical body composition of lambs (Table 6.7). Pellet type did not affect ( $P > 0.05$ ) any of the measured traits. The DM, water, protein, fat and GE content of the carcass, organs and empty bodies of the lambs were affected ( $P < 0.05$ ) by the type of milk consumed. Ash content of the various tissues was unaffected ( $P > 0.05$ ) by neither milk nor pellet type. Lambs fed HM had a greater ( $P < 0.05$ ) protein and water content in their carcasses, organs and empty bodies than their counterparts fed the NM. The NM fed lambs had a greater DM, fat and GE content in their carcasses, organs and empty bodies than the HM fed lambs. The chemical composition of the head and skin did not differ ( $P > 0.05$ ) between milk groups with the exception of the fat and GE content of the head being greater ( $P < 0.01$ ) in lambs fed NM.

There were no interactions ( $P > 0.05$ ) between milk type and pellet type on the rates of daily nutrient deposition (Table 6.8). The type of milk fed affected the rate of daily nutrient deposition in body tissues of lambs. Deposition of protein and water in the carcass, organs, skin and empty bodies of lambs were greater ( $P < 0.05$ ) in the HM treatment group than in the NM treatment group. Daily fat deposition and energy retention in the carcass, organs, head and empty bodies were greater ( $P < 0.05$ ) for NM fed lambs than the HM fed lambs. Lambs fed the HM had a greater ( $P < 0.05$ ) ash content in their empty bodies than those fed the NM. Pellet type had no effect ( $P > 0.05$ ) on the daily nutrient deposition in the various body tissues with the exception of greater ( $P < 0.05$ ) protein deposition in the heads of the HP than those of LP.

Table 6.7 Effect of dietary treatment on chemical composition of carcass, organs, head, skin and empty bodies (LS means) of the baseline slaughter group and lambs reared artificially on two protein levels in milk replacer and two protein levels in pellets at 18 kg live weight

| n           | Baseline EBW <sup>1</sup><br>(mean ± SD <sup>2</sup> ) | Treatment <sup>3</sup> |       |       |       | Pellet protein |        | SE <sup>4</sup> | P- value |        |                  |
|-------------|--------------------------------------------------------|------------------------|-------|-------|-------|----------------|--------|-----------------|----------|--------|------------------|
|             |                                                        | Milk protein           |       | HM    |       | LP             | HP     |                 | Milk     | Pellet | M×P <sup>5</sup> |
|             |                                                        | NM                     | 14    | 14    | 14    |                |        |                 |          |        |                  |
| Carcass     |                                                        |                        |       |       |       |                |        |                 |          |        |                  |
| DM, g/kg    | 250.8 ± 10.7                                           | 342.0                  | 311.8 | 323.1 | 330.7 | 4.25           | <0.001 | 0.22            | 0.79     |        |                  |
| Ash, g/kg   | 53.0 ± 4.12                                            | 37.70                  | 40.45 | 38.57 | 39.58 | 1.82           | 0.30   | 0.70            | 0.87     |        |                  |
| CP, g/kg    | 165.4 ± 7.57                                           | 183.8                  | 189.6 | 185.3 | 188.1 | 1.63           | 0.02   | 0.25            | 0.66     |        |                  |
| Fat, g/kg   | 14.68 ± 14.1                                           | 114.1                  | 77.16 | 93.27 | 98.01 | 3.86           | <0.001 | 0.39            | 0.99     |        |                  |
| Water, g/kg | 749.2 ± 10.7                                           | 658.0                  | 688.2 | 676.9 | 669.3 | 4.25           | <0.001 | 0.22            | 0.79     |        |                  |
| GE, MJ/kg   | 4.84 ± 0.41                                            | 8.64                   | 7.42  | 8.04  | 8.02  | 0.10           | <0.001 | 0.90            | 1.00     |        |                  |
| Organs      |                                                        |                        |       |       |       |                |        |                 |          |        |                  |
| DM, g/kg    | 222.0 ± 11.4                                           | 269.8                  | 229.9 | 247.8 | 251.9 | 4.68           | <0.001 | 0.54            | 0.52     |        |                  |
| Ash, g/kg   | 12.62 ± 0.63                                           | 11.73                  | 12.23 | 12.04 | 11.92 | 0.25           | 0.17   | 0.74            | 0.22     |        |                  |
| CP, g/kg    | 154.1 ± 8.20                                           | 136.3                  | 146.4 | 141.0 | 141.7 | 1.28           | <0.001 | 0.70            | 0.87     |        |                  |
| Fat, g/kg   | 22.91 ± 4.52                                           | 96.64                  | 42.87 | 64.86 | 74.64 | 6.69           | <0.001 | 0.31            | 0.35     |        |                  |
| Water, g/kg | 778.0 ± 11.4                                           | 730.2                  | 770.1 | 752.2 | 748.1 | 4.68           | <0.001 | 0.53            | 0.52     |        |                  |
| GE, MJ/kg   | 5.62 ± 0.35                                            | 7.82                   | 6.25  | 6.94  | 7.13  | 0.18           | <0.001 | 0.48            | 0.91     |        |                  |
| Head        |                                                        |                        |       |       |       |                |        |                 |          |        |                  |
| DM, g/kg    | 251.3 ± 14.9                                           | 329.2                  | 320.9 | 328.4 | 321.6 | 3.17           | 0.08   | 0.14            | 0.98     |        |                  |
| Ash, g/kg   | 63.35 ± 10.0                                           | 61.96                  | 65.93 | 66.26 | 61.63 | 2.59           | 0.28   | 0.22            | 0.61     |        |                  |
| CP, g/kg    | 165.5 ± 3.25                                           | 171.8                  | 172.6 | 169.2 | 175.1 | 2.55           | 0.84   | 0.11            | 0.93     |        |                  |
| Fat, g/kg   | 23.08 ± 3.22                                           | 94.22                  | 79.74 | 89.42 | 84.54 | 1.92           | <0.001 | 0.08            | 0.10     |        |                  |
| Water, g/kg | 748.7 ± 14.9                                           | 670.8                  | 679.1 | 671.6 | 678.4 | 3.17           | 0.07   | 0.14            | 0.98     |        |                  |
| GE, MJ/kg   | 4.85 ± 0.16                                            | 7.90                   | 7.35  | 7.62  | 7.64  | 0.08           | <0.001 | 0.91            | 0.64     |        |                  |
| Skin        |                                                        |                        |       |       |       |                |        |                 |          |        |                  |

|             |              |       |       |       |       |      |        |      |      |
|-------------|--------------|-------|-------|-------|-------|------|--------|------|------|
| DM, g/kg    | 285.0 ± 30.4 | 312.6 | 329.4 | 332.8 | 309.1 | 9.09 | 0.20   | 0.08 | 0.44 |
| Ash, g/kg   | 14.42 ± 1.92 | 13.42 | 14.53 | 14.24 | 13.70 | 0.67 | 0.25   | 0.57 | 0.89 |
| CP, g/kg    | 257.6 ± 29.8 | 288.2 | 298.9 | 305.2 | 281.9 | 8.20 | 0.36   | 0.06 | 0.48 |
| Fat, g/kg   | 21.24 ± 2.80 | 28.33 | 30.97 | 27.59 | 31.72 | 2.39 | 0.44   | 0.23 | 0.92 |
| Water, g/kg | 715.0 ± 30.4 | 687.4 | 670.6 | 667.2 | 690.9 | 9.09 | 0.20   | 0.08 | 0.44 |
| GE, MJ/kg   | 6.53 ± 0.75  | 7.75  | 8.05  | 8.17  | 7.64  | 0.22 | 0.34   | 0.10 | 0.50 |
| Empty body  |              |       |       |       |       |      |        |      |      |
| DM, g/kg    | 253.1 ± 10.9 | 324.3 | 301.7 | 312.4 | 313.6 | 2.99 | <0.001 | 0.76 | 0.42 |
| Ash, g/kg   | 39.24 ± 2.64 | 31.55 | 33.48 | 32.35 | 32.68 | 1.15 | 0.24   | 0.84 | 0.72 |
| CP, g/kg    | 182.7 ± 7.17 | 190.8 | 198.8 | 195.5 | 194.1 | 1.38 | <0.001 | 0.49 | 0.69 |
| Fat, g/kg   | 18.27 ± 7.68 | 96.43 | 64.20 | 77.85 | 82.77 | 2.85 | <0.001 | 0.23 | 0.62 |
| Water, g/kg | 746.9 ± 10.9 | 675.7 | 698.3 | 687.6 | 686.4 | 2.99 | <0.001 | 0.76 | 0.42 |
| GE, MJ/kg   | 5.33 ± 0.30  | 8.31  | 7.32  | 7.85  | 7.79  | 0.08 | <0.001 | 0.61 | 0.74 |

<sup>1</sup>EBW: empty body weight<sup>2</sup>SD: standard deviation.<sup>3</sup>Treatment: NM = Normal protein milk (24.0% CP); HM = High-protein milk (31.2% CP); LP = Low-protein pellet (13.6% CP); HP = High-protein pellet (19.5% CP).<sup>4</sup>SE: standard error.<sup>5</sup>M×P: milk × pellet interaction.

Table 6.8 Effect of dietary treatment on the dry matter (DM), ash, protein, fat, water and energy deposition rates in carcass, organs, head, skin and empty bodies (LS means) of lambs reared artificially on two protein levels in milk replacer and two protein levels in pellets up to 18 kg live weight.

| n            | Treatment <sup>1</sup> |       |                |       | SE <sup>2</sup> | P- value |        |                  |
|--------------|------------------------|-------|----------------|-------|-----------------|----------|--------|------------------|
|              | Milk protein           |       | Pellet protein |       |                 | Milk     | Pellet | M×P <sup>3</sup> |
|              | NM                     | HM    | LP             | HP    |                 |          |        |                  |
|              | 14                     | 14    | 14             | 14    |                 |          |        |                  |
| Carcass      |                        |       |                |       |                 |          |        |                  |
| DM, g/day    | 44.14                  | 44.23 | 42.66          | 45.71 | 1.25            | 0.96     | 0.10   | 0.72             |
| Ash, g/day   | 3.85                   | 4.78  | 4.47           | 4.16  | 0.36            | 0.08     | 0.54   | 0.81             |
| CP, g/day    | 22.53                  | 26.26 | 23.71          | 25.07 | 0.68            | 0.001    | 0.17   | 0.91             |
| Fat, g/day   | 17.53                  | 13.21 | 14.60          | 16.14 | 0.68            | <0.001   | 0.12   | 0.83             |
| Water, g/day | 74.31                  | 88.16 | 80.56          | 81.91 | 2.37            | <0.001   | 0.69   | 0.82             |
| GE, MJ/d     | 1.18                   | 1.11  | 1.12           | 1.16  | 0.03            | 0.11     | 0.31   | 0.73             |
| Organs       |                        |       |                |       |                 |          |        |                  |
| DM, g/day    | 8.95                   | 7.80  | 8.30           | 8.45  | 0.33            | 0.02     | 0.74   | 0.86             |
| Ash, g/day   | 0.35                   | 0.40  | 0.38           | 0.38  | 0.02            | 0.05     | 0.98   | 0.62             |
| CP, g/day    | 3.97                   | 4.75  | 4.38           | 4.35  | 0.15            | 0.001    | 0.92   | 0.73             |
| Fat, g/day   | 3.96                   | 1.75  | 2.61           | 3.10  | 0.29            | <0.001   | 0.25   | 0.33             |
| Water, g/day | 21.94                  | 25.66 | 23.96          | 23.64 | 0.90            | 0.01     | 0.79   | 0.53             |
| GE, MJ/d     | 0.27                   | 0.22  | 0.24           | 0.25  | 0.01            | <0.01    | 0.58   | 0.88             |
| Head         |                        |       |                |       |                 |          |        |                  |
| DM, g/day    | 4.68                   | 4.45  | 4.56           | 4.56  | 0.13            | 0.21     | 0.99   | 0.91             |
| Ash, g/day   | 0.74                   | 0.80  | 0.81           | 0.73  | 0.06            | 0.44     | 0.31   | 0.84             |
| CP, g/day    | 2.14                   | 2.08  | 2.00           | 2.22  | 0.06            | 0.55     | 0.02   | 0.68             |
| Fat, g/day   | 1.77                   | 1.51  | 1.66           | 1.62  | 0.07            | 0.02     | 0.75   | 0.33             |
| Water, g/day | 7.51                   | 7.19  | 7.15           | 7.55  | 0.25            | 0.37     | 0.27   | 0.79             |
| GE, MJ/d     | 0.12                   | 0.11  | 0.12           | 0.11  | 0.004           | 0.04     | 0.35   | 0.74             |

|              |       |       |       |       |      |        |      |      |  |
|--------------|-------|-------|-------|-------|------|--------|------|------|--|
| Skin         |       |       |       |       |      |        |      |      |  |
| DM, g/day    | 7.97  | 10.26 | 9.59  | 8.65  | 0.47 | 0.002  | 0.17 | 0.64 |  |
| Ash, g/day   | 0.31  | 0.41  | 0.37  | 0.35  | 0.03 | 0.03   | 0.59 | 0.92 |  |
| CP, g/day    | 7.42  | 9.32  | 8.84  | 7.92  | 0.41 | 0.004  | 0.13 | 0.66 |  |
| Fat, g/day   | 0.80  | 1.07  | 0.83  | 1.04  | 0.11 | 0.09   | 0.18 | 0.86 |  |
| Water, g/day | 16.03 | 18.48 | 16.44 | 18.07 | 0.75 | 0.03   | 0.14 | 0.32 |  |
| GE, MJ/d     | 0.21  | 0.26  | 0.24  | 0.22  | 0.01 | 0.004  | 0.25 | 0.72 |  |
| Empty body   |       |       |       |       |      |        |      |      |  |
| DM, g/day    | 65.75 | 66.73 | 65.11 | 67.37 | 1.59 | 0.66   | 0.32 | 0.65 |  |
| Ash, g/day   | 5.24  | 6.39  | 5.72  | 5.92  | 0.37 | 0.04   | 0.70 | 0.77 |  |
| CP, g/day    | 36.07 | 42.43 | 38.94 | 39.56 | 0.86 | <0.001 | 0.61 | 0.83 |  |
| Fat, g/day   | 24.06 | 17.53 | 19.69 | 21.90 | 0.85 | <0.001 | 0.08 | 0.57 |  |
| Water, g/day | 119.8 | 139.5 | 128.1 | 131.2 | 3.17 | <0.001 | 0.49 | 0.82 |  |
| GE, MJ/d     | 1.78  | 1.70  | 1.72  | 1.76  | 0.04 | 0.18   | 0.51 | 0.77 |  |

<sup>1</sup>Treatment: NM = Normal protein milk (24.0% CP); HM = High-protein milk (31.2% CP); LP = Low-protein pellet (13.6% CP); HP = High-protein pellet (19.5% CP).

<sup>2</sup>SE: standard error.

<sup>3</sup>M×P: milk × pellet interaction.

The relationship between the CP:ME intake ratio, protein and fat deposition rates per day is shown in Figure 6.1.

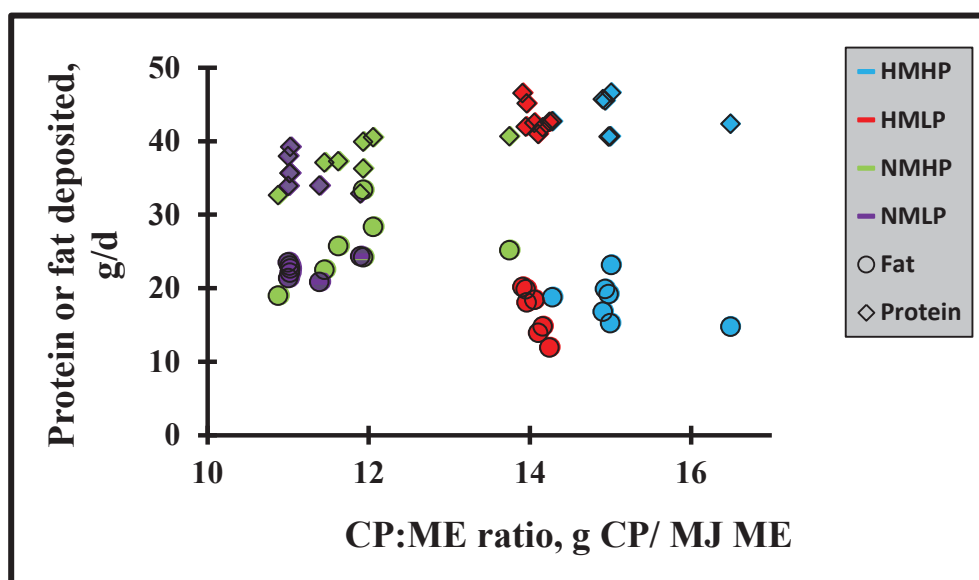


Figure 6.1 Graphic representation of the CP:ME intake ratio against the rate of protein and fat deposition in artificially reared lambs offered two protein levels in milk replacer (24% CP [NM]; 31.2% CP [HM] and two protein levels in pellets (13.5% CP [LP]; 19.5% CP[HP]) up to 18 kg live weight; resulting in four treatment groups (NMLP, NMHP, HMLP and HMHP).

The best model describing the relationship between daily CP intake and daily protein deposition (PD/d) included the intercept and the quadratic effect of CP intake (Eq. 6.5, Fig 6.2):

$$PD / d = - 39.27 (\pm 20.63) + 1.70 \text{ CPI} / d (\pm 0.53) - 0.0086 \text{ CPI} / d^2 (\pm 0.003) \quad (6.5)$$

$$(R^2 = 0.84)$$

The response of protein deposition (PD) to protein intake was curvilinear (Fig. 6.2). Based on Equation 6.5, it can be calculated that the maximum potential protein deposition ( $PD_{max}$ ) of 44.7 g per day is attained at a CP intake of 98.8 g per day.

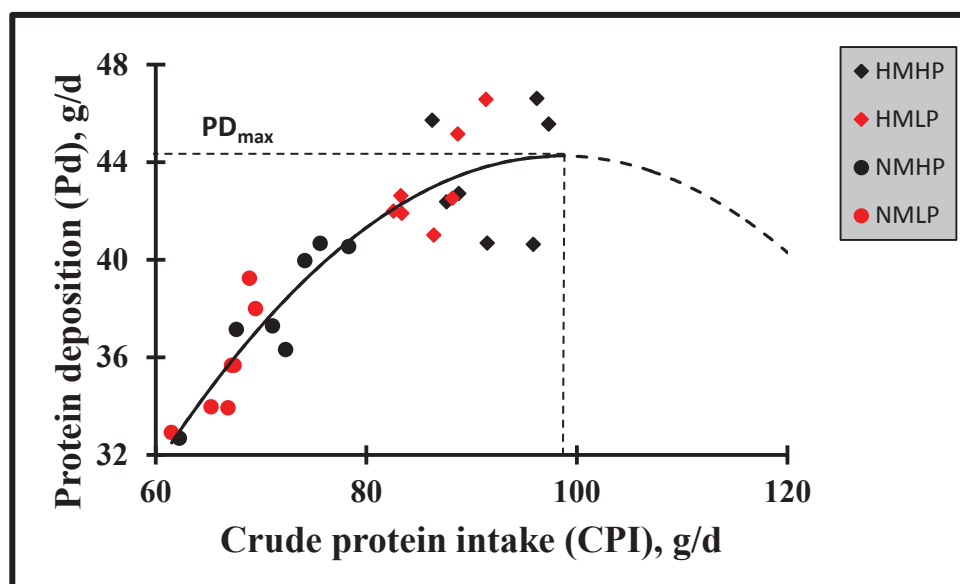


Figure 6.2 Relationship between daily protein intake and rate of protein deposition in artificially reared lambs offered two protein levels in milk replacer (24% CP [NM]; 31.2% CP [HM] and two protein levels in pellets (13.5% CP [LP]; 19.5% CP[HP]) up to 18 kg live weight; resulting in four treatment groups (NMLP, NMHP, HMLP and HMHP).  $PD_{max}$  = Maximum observed protein deposition.

## 6.4 Discussion

### 6.4.1 Nitrogen balance

The higher CP intake from the HM increased urinary N excretion but, did not affect faecal N excretion. These results support previous findings in lambs (Gabler and Heinrichs, 2003; Marini and Van Amburgh, 2003). Usually, when feed intake is not restricted, faecal N excretions do not differ regardless of the N content of the diet (Siddons *et al.*, 1985; Marini and Van Amburgh, 2003; Marini *et al.*, 2004). The higher urinary excretion resulted in minor differences in N retention between the HM and NM

treatment groups. Chapter 5 indicated that pre-weaned lambs growing at 240 g/d require a CP:ME intake ratio of about 13.7 at 5 kg LW. However, the CP:ME ratio offered to the HM fed lambs in the present study was about 14.3. Therefore, the high urinary N excretion of the HM fed lambs is likely due to the CP:ME intake of HM fed lambs being higher than requirements. Thus, the greater digestible N consumed could not be assimilated with the level of the dietary energy available. Dietary energy intake affects N excretion in urine and ultimately the overall efficiency of N utilisation (Kebreab *et al.*, 2002).

The type of pellet offered had no effect on N retention and the protein to energy ratios in the balance study. In contrast to this finding, Berends *et al.* (2012) and (Berends *et al.*, 2014) found that provision of LP in addition to milk replacer (MR) was associated with increased N retention in veal calves. Two explanations are plausible for the differences between the calf studies (Berends *et al.*, 2012; Berends *et al.*, 2014) and the present experiment. Firstly, in order to create uniformity in pellet composition, the CP content in LP used in the present study could not be made any lower than 135 g / kg DM. This CP content was nearly 50% higher than the 88g /kg DM in the pellet diets of Berends *et al.* (2012). Therefore, it is possible that the LP used in the present study was not low enough to stimulate urea recycling resulting in no difference in N retentions between the pellet types. Secondly, the MR used in the present study contained higher CP (24.4% for NM and 31.2% for HM) than the 21.2% CP in the MR used in the calf studies (Berends *et al.*, 2012; Berends *et al.*, 2014). Further, studies are therefore needed to elucidate the interaction between low-protein solid feed and milk replacer in young lambs. Regardless, the findings in the present study suggest that the CP content of the LP was sufficient to meet the protein requirements of the lambs.

### **6.4.2 Intake and growth**

Lambs were fed both NM and HM replacer as proportion of their live weights. Consequently, the greater ADG of lambs fed HM is a result of the higher CP:ME content of the milk. A 28% increase in the CP content of HM was associated with a 14% increase in ADG over the study period resulting in the HM lamb reaching the target LW weight earlier (approximately 7 days) than NM lambs. Similarly, increases in ADG in response to high CP milk have been demonstrated in lambs (Jagusich *et al.*, 1970; Norton *et al.*, 1970) and calves (Diaz *et al.*, 2001; Blome *et al.*, 2003) during the pre-weaning period. Furthermore, the efficiency of gain (gain to feed ratio) was greater for lambs fed the HM than those fed NM. These findings emphasise the importance of CP content in the milk diets of lambs in the first eight weeks of life.

An increase in the CP content of the pellets was neither reflected in the growth of the lambs nor in the efficiency of gain. This may either indicate that the LP pellet was sufficient to meet the growth requirement of lambs pre-weaning or the potential benefit of altering pellet composition might occur only when the greater proportion of the lambs' nutrient intake is from pellets. These findings suggest that increasing the CP content of milk in the first eight weeks of life appears to be a more efficient way of improving growth rates in pre-weaned lambs than increasing the CP content of solid feeds.

### **6.4.3 Slaughter parameters**

Liver and kidney weights were greater for lambs fed HM than NM replacer, whereas the intestinal weight and the other organs (combined weights of heart, lungs etc) were greater in lambs offered LP than HP pellets. Liver and kidney weights increase in

response to protein intake whilst intestinal weight increases with increasing energy intake (Bikker *et al.*, 1994). Urea is the main end product from protein breakdown and is synthesised in the liver and excreted by the kidney in urine (Zervas and Zijlstra, 2002). Therefore, the increased liver, kidney and intestinal weights (food processing organs) are indicative of increased metabolic activity (Bikker *et al.*, 1994; Bikker *et al.*, 1995). The responses of metabolically active food processing organs to nutrient intake have been previously reported in calves (Blome *et al.*, 2003; Hill *et al.*, 2008) and pigs (Rao and McCracken, 1992; Bikker *et al.*, 1995) fed diets containing high CP and high energy content, respectively. Combined, these studies suggest that dietary composition influences the growth of different organs.

#### **6.4.4 Body composition**

The type of milk replacer fed to lambs influenced the chemical composition of the various body tissues. Reduction in fat deposition and energy retention and an increase in water and protein content were observed in lambs fed HM replacer. The rate of protein deposition in gain has the greatest impact on ADG due to its association with water deposition as lean tissue (Roy, 1980). Therefore, the higher daily protein and water deposition rates (lean tissue deposition) of the HM lambs may explain their greater ADG. Further, high protein deposition may also indicate that protein intake of lambs fed HM was sufficient to meet their maintenance and growth requirements.

The balance study demonstrated that there was no difference in the N retained by lambs fed NM or HM replacer. However, a decrease in body protein deposition of NM fed lambs was observed. These results concur with those of Jagusch *et al.* (1970) and Norton *et al.* (1970) when they varied the CP content of MR from 61g/kg DM to 285g/kg DM and reported differences in the protein and fat content (high protein and

low fat) in the empty bodies of milk-only fed lambs as CP intake increased. The lower protein deposition in NM fed lambs was likely related to the increased fat deposition, which may have diluted the protein content in gain (Labussiere *et al.*, 2008). The higher fat and energy content may also indicate that lambs fed NM were provided with less available protein in relation to energy for tissue protein synthesis and growth which resulted in more energy deposited as fat (Manso, 1998).

Searle *et al.* (1972) reported that rumen development in response to solid feed intake typically limits the extent to which chemical body composition can be altered in lambs at the transitional stage of growth (3 – 9 weeks of age). However, the present study demonstrated that the body composition of lambs in the transitional stage of growth can be altered when lambs are fed milk diets differing in protein content regardless of rumen development. The chemical body composition of lambs was however, unaffected by type of pellets offered in the present study. The current study demonstrates that feeding a milk replacer with CP content of 24%, which is the “normal” rate fed to lambs, increases the quantity of energy available for adipose tissue deposition, ultimately resulting in fatter carcasses. However, it is unknown if this difference in fat deposition would still exist at a heavier slaughter live weight which might incur a financial penalty for farmers.

The highest protein deposition rate attained by the HM replacer fed lambs was approximately 42.4 g / d which was very close to the  $PD_{max}$  of 44.7 g /d indicated by the multiple regression model. A  $PD_{max}$  of 44 g /d and 45 g/ d have previously been achieved in older lambs (from 24 kg to 45 kg LW) offered pelleted diets containing CP:ME ratios of 12.8 and 15.6, respectively (Kyriazakis and Oldham, 1993). There appears to be no more recent published reports on the  $PD_{max}$  for lambs with which to

make a direct comparison. However, this finding indicates nutrient intake should be in excess of the CP:ME requirements for lambs to attain their maximum protein deposition rate. Moreover, being able to quantify the maximum protein deposition for different sheep genotypes in New Zealand will allow for a better estimation of their nutrient requirements and thus the development of feeding strategies which will maximise lamb growth and potentially farm profitability.

#### **6.4.5 Blood metabolites**

Blood urea N (BUN) concentration can be a useful indicator of protein status in animals (Vosooghi-poostindoz *et al.*, 2014) and is also an indicator of N utilisation in ruminants (Kohn *et al.*, 2005). In this study, increasing dietary protein increased the BUN of HM replacer and HP pellet fed lambs indicating differences in protein levels, similar to the findings to that of Vosooghi-poostindoz *et al.* (2014). High BUN concentration in growing lambs and calves could also indicate an increased absorption of ruminal ammonia (Hatfield *et al.*, 1998) which results from the extensive ruminal degradation of dietary protein (Quigley and Bernard, 1992; Sharifabadi and Naserian, 2014; Santos *et al.*, 2015). The high BUN concentration in lambs offered HP was likely due to increased ruminal fermentation of dietary protein and absorption of ammonia from the rumen (Quigley *et al.*, 2006). This result indicates that serum concentration of BUN is sensitive in detecting differences in protein metabolism between diets.

Lambs offered HP pellet had higher blood glucose concentration than their counterparts offered LP. The higher blood glucose concentration observed in lambs fed HP might be due to their higher digestible protein, thus resulting in increased precursor availability for gluconeogenesis (generation of glucose from non-carbohydrate sources) (Schmidt and Keith, 1983). Intestinal glucose absorption is insufficient to meet the glucose needs

of ruminants (Schmidt and Keith, 1983). Therefore, gluconeogenesis is a major source of glucose in ruminants accounting for approximately 75% of their total glucose needs (Donkin and Hammon, 2005). Nevertheless, the blood glucose concentrations of lambs in the present study were in the range reported previously for suckling lambs (Kenyon *et al.*, 2011) which indicates that the supply of glucose required by body tissues to function was adequate.

Total serum protein concentration in HM fed lambs was greater than their counterparts fed NM. Serum proteins are synthesised in the liver (Kaneko, 1997). Thus, the difference in total protein concentration in blood further confirms that liver function differed between the two milk treatment groups.

Overall, all blood metabolites concentrations decreased as lambs grew older and LW increased which may represent a response to rumen development associated with solid feed intake (Norouzian and Valizadeh, 2014).

## **6.5 Conclusion**

Increasing the CP content of milk replacer, resulting in a higher CP:ME ratio of intake, increased average daily gain and increased gain to feed ratio in pre-weaned lambs. Further, it increased the protein content and decreased the fat content in the empty bodies of lambs at 18 kg live weight. However, altering the composition of pellets did not affect any of the measured performance traits in the present study. The results of the present study do not support the hypothesis that feeding low-protein pellets can reduce urinary N excretion and thereby increase N retention. The highest protein deposition rate of 42 g/d was attained in lambs fed high-protein milk along with pellets which was close to the estimated  $PD_{max}$  value of 45 g/d previously reported in weaned lambs.

Overall, the results of the present study demonstrate that altering protein content of milk replacer can markedly affect growth and chemical body composition in pre-weaned lambs and maximum potential protein deposition rates can be reached during the pre-weaning period when lambs are fed in excess of their CP:ME requirements.

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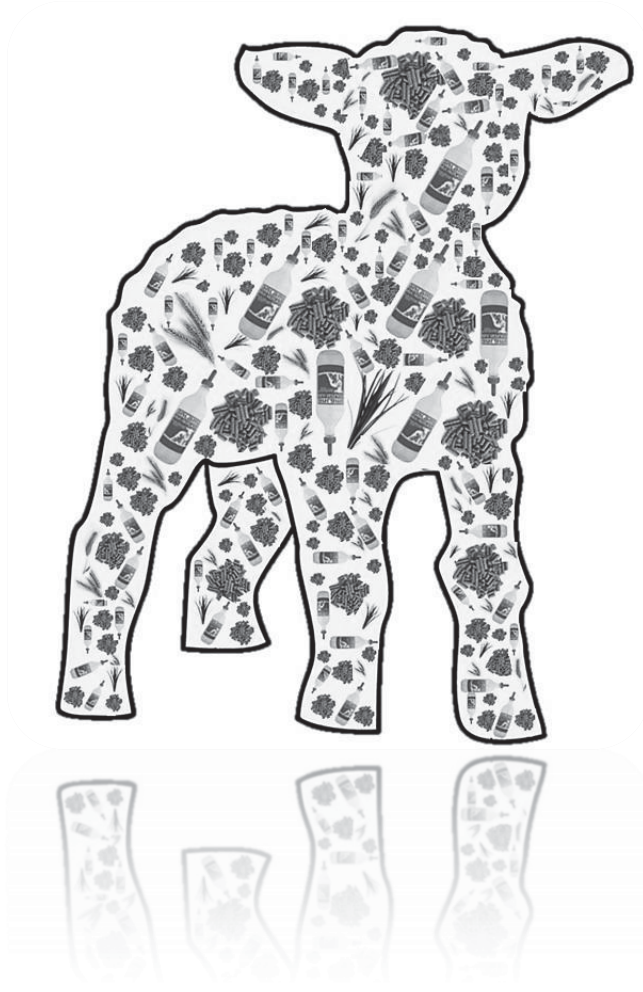
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## Chapter 7      Predicting Feed Intake of Pre-Weaned Lambs from Faecal and Dietary Chemical Composition

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Chapter based on the following presentation at the 67<sup>th</sup> EAAP Conference:

**DANSO, A. S., MOREL, P. C. H., KENYON, P. R., and BLAIR, H. T.** Predicting intake from faecal chemical composition in pre-weaned lambs consuming milk and pellets. 67<sup>th</sup> Annual Meeting of the European Federation of Animal Science, Belfast UK, 29<sup>th</sup> Aug – 2<sup>nd</sup> Sept 2016. Book of Abstracts p 522.

## ABSTRACT

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Predicting feed intake is difficult in suckling lambs consuming both milk and pasture. Feed intake values are typically predicted from values derived from those fed either milk only or solid feed only. The aim of the present study was to investigate if the dry matter (DM), organic matter (OM) and metabolisable energy (ME) intakes of lambs fed a combination of milk and pellets under controlled conditions can be predicted with sufficient accuracy from dietary and faecal chemical composition. A total of 34 lambs with live weight (LW) ranges of 9 kg to 18 kg were used in the study. Fifty-four faecal samples with detailed information about their chemical composition and the chemical composition of the milk and pellets consumed by pre-weaned lambs from two digestibility trials were used in this study. The lambs were bottle-fed milk replacer with or without access to pellets and kept in metabolic cages for four days. Records of feed intake, LW of lambs and the bulked total faecal collection for each lamb were used to develop the prediction equations. Pellet DMI (g/d) was predicted ( $R^2=0.75$ ; RPE = 39.28%; CCC= 0.83) from the neutral detergent fibre (NDF) concentration in faeces and pellets, pellets DM (%) and LW (kg). Milk DMI (g/d) was predicted ( $R^2 = 0.96$ ; RPE = 3.48%; CCC= 0.98) from faecal Nitrogen concentration and LW. Milk and pellet DMI and their ME content were combined to predict DMI/d ( $R^2 = 0.89$ ; RPE = 9.93%; CCC= 0.93), and ME intake/d ( $R^2=0.93$ ; RPE = 6.53%; CCC= 0.96). The equations developed were validated against 40 spot faecal samples randomly selected from the lambs. The DM, OM and ME intakes were predicted with high accuracy and precision ( $R^2 = 0.79$ , 0.80 and 0.87; RPE = 15.09 %, 14.90 % and 9.48 %; CCC = 0.88, 0.88 and 0.93 for DM, OM and ME intakes, respectively). The results indicated that the equations developed can be used with enough precision to estimate ME, OM and DM intakes in

pre-weaned lambs consuming both milk and pellets, thus providing a means for farmers to develop feeding strategies for young lambs.

## 7.1 Introduction

Of the measurable factors affecting lamb growth, feed intake is the most variable and the most difficult to measure accurately (Coleman *et al.*, 1995). Under pastoral conditions, pre-weaned lambs obtain their nutrient intake from both milk and pasture making the prediction of milk and pasture intake difficult as neither of these dietary components can be measured directly. Therefore, feed intake values are typically predicted from values derived from those fed exclusively on milk or solid feed. The various techniques for measuring either milk or pasture intake often lack accuracy and precision and, therefore, do not give a true reflection of intake (Decruyenaere *et al.*, 2009a; Geenty, 2010). The inability to estimate milk and pasture intake with ease and accuracy seriously limits the management of feed intake and hence animal performance (Boval *et al.*, 2003). In order to determine nutrient balances and nutrient use efficiencies in young lambs, methods are needed to quantify the nutrient intake of lambs.

Faecal material consists primarily of the undigested residues of feed intake and may contain characteristics and nutritional information of the diet consumed (Coleman *et al.*, 1995). Therefore, predicting feed intake from faecal chemical composition may provide a more precise estimation of intake than other techniques. Faecal chemical components such as nitrogen (N), neutral detergent fibre (NDF), acid detergent fibre (ADF) and lignin have been used to predict intake in grazing sheep (Azevedo *et al.*, 2014; David *et al.*, 2014). However, there is no information on milk and solid feed intake prediction in young lambs from faecal chemical composition.

The aims of the present study were to investigate whether the dry matter (DM), organic matter (OM) and metabolisable energy (ME) intakes of lambs fed a combination of milk

and pellets could be predicted with sufficient accuracy from faecal and dietary chemical composition to be useful in making feed intake management decisions.

## **7.2 Materials and Methods**

The studies were conducted at Massey University 5 km South of Palmerston North, New Zealand during the months of August to December, 2014 and September to December, 2015. The study and animal handling procedures were approved by the Massey University Animal Ethics Committee. The study was divided into two phases:

- *Phase one* – generation of prediction equations to estimate milk and pellet intake from faecal chemical composition in pre-weaned lambs kept in metabolic crates which allowed for total faecal collection.
- *Phase two* – the equations developed from phase one were evaluated with lambs consuming a known amount of milk and pellets in individual pens and spot faecal samples (samples taken randomly at any given time) collected directly from the rectum.

### **7.2.1 Phase one: Experimental Design and Animals**

The designs of the two experiments were previously reported in Chapters Five and Six. Briefly, twenty-eight sets of Romney twin lambs were selected for the study at 24 hours post-partum in each experiment. One male lamb from each set was separated from the dam, moved indoors and hand-reared. The lambs were allocated to four treatment groups in each experiment based on various milk and pellet combinations (Table 7.1). Lambs from each treatment group in both experiments ( $n = 4$  and  $5$  per group in experiments one and two, respectively) were randomly selected and placed in metabolic

crates for four days at specific LW (17 kg for experiment one and 9 kg and 16 kg for experiment two) to allow for total faecal collections. The daily milk and pellet intake of the lambs were recorded. Total faeces excreted were collected and weighed daily. Faeces collected per lamb over the four-day period were pooled and sub-sampled for each period of the digestibility study to obtain a total of 54 faecal samples. All samples were freeze-dried, ground, and stored at -20°C for further analysis.

Table 7.1 Treatment and live weights of lambs at various stages of faecal collection in the two experiments

| Treatment <sup>1</sup>                   | n <sup>2</sup> |            |          | LW <sup>2</sup> at which measurement were made, kg |                                   |
|------------------------------------------|----------------|------------|----------|----------------------------------------------------|-----------------------------------|
|                                          |                | Initial LW | Final LW | Digestibility                                      | Spot <sup>3</sup> faecal sampling |
| <i>Experiment one (2014) – Chapter 5</i> |                |            |          |                                                    |                                   |
| MO                                       | 4              | 4.97       | 18.16    | 17                                                 | 8, 12, 16, 18                     |
| MP <sub>30</sub>                         | 4              | 5.11       | 18.74    |                                                    |                                   |
| MP <sub>60</sub>                         | 4              | 4.99       | 18.50    |                                                    |                                   |
| MP <sub>ad</sub>                         | 4              | 5.02       | 18.86    |                                                    |                                   |
| <i>Experiment two (2015) – Chapter 6</i> |                |            |          |                                                    |                                   |
| NMLP                                     | 5              | 5.34       | 18.98    | 9, 16                                              | 9, 12, 15, 18                     |
| NMHP                                     | 5              | 5.31       | 18.57    |                                                    |                                   |
| HMLP                                     | 5              | 5.57       | 19.01    |                                                    |                                   |
| HMHP                                     | 5              | 5.44       | 18.77    |                                                    |                                   |

<sup>1</sup>Treatment: MO = milk only; MP<sub>30</sub> = milk + 30% of *ad libitum* pellets intake; MP<sub>60</sub> = milk + 60% of *ad libitum* pellets intake; MP<sub>ad</sub> = pellets offered *ad libitum*; NMLP = normal-protein milk + low-protein pellet; NMHP = normal-protein milk + high-protein pellet; HMLP = high-protein milk + low-protein pellet; HMHP = high-protein milk + high-protein pellet

<sup>2</sup>n: number of lambs involved in the digestibility study

<sup>3</sup>Live weight

<sup>3</sup>Spot: samples taken randomly at any given time (A total of 40 samples balanced for all dietary treatment groups were taken)

### **7.2.2 Phase two: Evaluation of Prediction Equations**

A total of 148 spot faecal samples were collected directly from rectum of lambs during the hand-rearing periods. Samples from experiment one were collected at 8 kg, 12 kg, 16 kg and 18 kg whilst those from experiment two were collected at 9 kg, 12 kg, 15 kg and 18 kg LW (Table 7.1). Lambs involved in the total faecal collection study were excluded from the 18 kg LW spot sample collection for experiment one and the 9 kg and 18 kg spot sample collection for experiment two. All samples collected were stored at -20°C until further analysis. Forty of the 148 spot faecal samples over a range of LWs and feed intakes were selected, freeze-dried and analysed. The 40 were also balanced for all the dietary treatment groups.

### **7.2.3 Chemical Analysis**

All diet and faecal samples were analysed according to AOAC (1990) for nitrogen (N) by the Leco total combustion method (AOAC method 968.06) and dry matter (DM) using a convection oven at 105°C (AOAC methods 930.15 and 925.10). Ash was determined in a furnace at 550°C (AOAC method 942.05) and organic matter (OM) was calculated as DM minus ash content. Gross energy (GE) was determined by the bomb calorimeter (Leco, AC 350, Leco Corporation, St Joseph, MI, USA). Neutral detergent fibre (NDF) in both the pellet and faecal samples were determined using the Tecator Fibretec System (AOAC method 2002.04) following the method described by Robertson and Van Soest (1981). The determination of total DM, GE, N, OM and NDF excreted in the faeces were carried out by multiplying the measured content in the faecal sample by the quantity of faeces excreted. The composition of milk replacer and pellets used in experiments one and two are presented in Table 7.2.

### 7.2.4 Statistical Analysis

#### Phase one

The forward selection method of stepwise regression was used to select the best independent variable(s) (concentrations of NDF, N, ash and DM in diet and faeces and the LW of lambs) that best predicted intake of the dependent variables (dry matter intake [DMI], organic matter intake [OMI] and ME intake) in phase one using the PROC REG procedure (SAS, 2013). The significant ( $P < 0.05$ ) independent variable(s) selected in the stepwise regressions were used to develop the prediction of DMI, OMI and ME intake.

Table 7.2 Chemical analysis of milk replacer and pellets fed to lambs in experiments one and two.

| Item                       | Dietary composition <sup>1</sup> |                      | Experiment two <sup>2</sup> |      |                 |                 |                 |
|----------------------------|----------------------------------|----------------------|-----------------------------|------|-----------------|-----------------|-----------------|
|                            | Experiment one <sup>2</sup>      |                      |                             |      |                 |                 |                 |
|                            | MR                               | Pellets <sup>3</sup> | NM                          | MPC  | HM <sup>4</sup> | LP <sup>5</sup> | HP <sup>5</sup> |
| Dry matter, %              | 96.9                             | 88.2                 | 97.7                        | 96.4 | 97.4            | 87.3            | 87.5            |
| Ash, %                     | 5                                | 8.9                  | 5.4                         | 7.4  | 5.8             | 3.4             | 3.9             |
| Crude protein, %           | 24.4                             | 16.9                 | 24.0                        | 60.2 | 31.2            | 13.6            | 19.5            |
| Gross energy, kJ/g         | 22.7                             | 15.2                 | 22.8                        | 19.3 | 22.1            | 16.4            | 16.5            |
| Neutral detergent fibre, % | n/a <sup>6</sup>                 | 11.8                 | n/a                         | n/a  | n/a             | 21.6            | 20.1            |

<sup>1</sup>Diet: MR = Milk replacer; NM = Normal protein milk; MPC = Milk protein concentrate; HM = High-protein milk; LP = Low-protein pellets; HP = High-protein pellets

<sup>2</sup>Experiment one = Chapter Five; Experiment two = Chapter Six;

<sup>3</sup>Composed of barley, soya bean meal, canola, peas, wheat, maize, oats, molasses, vegetable oil, grass seed meal, minerals, vitamins, prebiotics, and essential oils

<sup>4</sup>Composed of 80% NM and 20% MPC

<sup>5</sup>Composed of wheat middlings, soya bean, barley, and molasses

<sup>6</sup>n/a = not applicable

The accuracy of the prediction equations were examined using relative prediction error (RPE) defined as the ratio between the root square of the MSPE and the mean of the actual intake values (Fuentes-Pila *et al.*, 1996; Fuentes-Pila *et al.*, 2003), the concordance correlation coefficient (CCC) (Lin, 1989; Nickerson, 1997) and the coefficient of determination ( $R^2$ ). According to Fuentes-Pila *et al.* (1996) a RPE value lower than 10% is an indication of satisfactory prediction, a RPE between 10% and 20 % indicates a relatively acceptable prediction, and a RPE greater than 20% indicates poor prediction. The values corresponding to the CCC and their interpretations are as follows: values from 0.21 to 0.40 indicate a fair prediction; 0.41 to 0.60 indicate a moderate prediction; 0.61 to 0.80 indicate a substantial prediction, and 0.81 to 1.00 indicate an almost perfect prediction (Fuentes-Pila *et al.*, 1996; Visser *et al.*, 2012).

### *Phase two*

The equations developed using the phase one data were validated according to their accuracy of predicting randomly selected records from phase two data. Linear regression analyses were conducted for the parameters (DMI, OMI and ME intake) using the measured values as the independent variables and the predicted values as the dependent variables to test whether the intercept was equal to zero and whether the slope was equal to unity. The  $R^2$  was used as an indicator of the precision, whereas the intercept and the slope indicated the accuracy of the model (Tobias *et al.*, 2006).

## 7.3 Results

### 7.3.1 Development of Prediction Equations (Phase one)

The daily milk DMI (DMI<sub>m</sub>) over the two experiments ranged from 189.9 g/d to 310.1 g/d and for pellets (DMI<sub>p</sub>) ranged from 0 g/d to 231.7 g / d. The ranges of daily organic matter intake (OMI) were from 178.6 g/d to 293.9 g/d and from 0 g / d to 221.3 g/d for milk (OMI<sub>m</sub>) and pellets (OMI<sub>p</sub>), respectively.

The best models to predict DMI<sub>m</sub> and OMI<sub>m</sub> included faecal N concentration of (fN) and the LW of lambs (Equation 7.1 and 7.2, respectively).

$$\text{DMI}_m, \text{ g/d} = 65.11 (\pm 10.57) + 2.98 (\pm 1.52) \text{ fN} + 12.91 (\pm 0.39) \text{ LW} \quad (7.1)$$

$$(R^2 = 0.96; \text{RPE} = 3.48\%; \text{CCC} = 0.98)$$

$$\text{OMI}_m, \text{ g/d} = 60.65 (\pm 9.73) + 2.63 (\pm 1.39) \text{ fN} + 11.84 (\pm 0.36) \text{ LW} \quad (7.2)$$

$$(R^2 = 0.96; \text{RPE} = 4.81\%; \text{CCC} = 0.97)$$

Neutral detergent fibre intake (NDF<sub>i</sub>) was predicted from faecal NDF (fNDF, %) on the basis that there was no NDF present in milk and therefore any fNDF must be a result of pellet intake. The simple regression between NDF<sub>i</sub> and fNDF and the multiple regression between NDF<sub>i</sub>, fNDF and LW are shown in Equations 7.3 and 7.4, respectively. The inclusion of LW in the estimated NDF<sub>i</sub> equation showed a significant effect ( $P < 0.001$ ). By including LW regression model, the RPE was reduced by 14.3% and  $R^2$  and CCC increased by 20% and 14%, respectively.

$$\text{NDF}_i = -0.87 (\pm 2.42) + 0.89 (\pm 0.10) \text{ fNDF} \quad (7.3)$$

$$(R^2 = 0.61; \text{RPE} = 45.4; \text{CCC} = 0.76)$$

$$\text{NDF}_i = -23.9 (\pm 3.55) + 0.91 (\pm 0.07)\text{fNDF} + 1.62 (\pm 0.22)\text{LW} \quad (7.4)$$

$$(R^2 = 0.81; \text{RPE} = 31.1; \text{CCC} = 0.90)$$

The DMIp and OMIp were estimated from the predicted  $\text{NDF}_i$ , and the concentration of NDF ( $\text{NDF}_p$ , %), and DM ( $\text{DM}_p$ , %) in the pellets using the equation

$$\text{DMIp, g/d (or OMIp)} = \left( \frac{\text{NDF}_i, \text{ g/d}}{\text{NDF}_p, \%} \right) \times \text{DM}_p \quad (7.5)$$

The measured pellet DM and OM intakes were plotted against the predicted DM and OM intake (Table 7.3).

Table 7.3 Relationship between measured and predicted pellet dry matter intake (DMIp) and organic matter intake (OMIp) of pre-weaned lambs offered milk and pellets concurrently

| Intake    | n  | Measured <sup>1</sup> | SD    | SE   | Predicted <sup>2</sup> | SD    | SE   | R <sup>2</sup> | RPE, % | CCC  |
|-----------|----|-----------------------|-------|------|------------------------|-------|------|----------------|--------|------|
| DMIp, g/d | 54 | 87.00                 | 61.34 | 8.35 | 84.99                  | 54.52 | 7.42 | 0.76           | 34.5   | 0.86 |
| OMIp, g/d | 54 | 82.08                 | 57.80 | 7.87 | 80.26                  | 51.70 | 7.04 | 0.77           | 33.9   | 0.87 |

<sup>1</sup>Measured: actual mean value obtained from experiments one and two

<sup>2</sup>Predicted: mean value obtained from prediction equations

SD = standard deviation

SE = standard error

RPE = relative predictive error

CCC = concordance correlation coefficient

Milk and pellet DMI, OMI and their metabolisable energy (ME) content were combined to predict the total DMI per day total OMI per and total ME intake per day (Table 7.4).

Table 7.4 Relationship between measured and predicted dry matter intake (DMI), organic matter intake (OMI) and metabolisable energy (ME) intake in pre-weaned lambs offered milk and pellets concurrently.

| Intake          | n  | Measured <sup>1</sup> | SD   | SE   | Predicted <sup>2</sup> | SD   | SE   | R <sup>2</sup> | RPE,% | CCC  |
|-----------------|----|-----------------------|------|------|------------------------|------|------|----------------|-------|------|
| DMI, g/d        | 54 | 344.3                 | 93.8 | 12.8 | 342.3                  | 88.8 | 12.1 | 0.89           | 8.83  | 0.94 |
| OMI, g/d        | 54 | 318.6                 | 87.1 | 11.9 | 316.8                  | 82.6 | 11.2 | 0.89           | 8.87  | 0.94 |
| ME intake, MJ/d | 54 | 1.71                  | 0.40 | 0.05 | 1.71                   | 0.39 | 0.05 | 0.93           | 6.04  | 0.96 |

<sup>1</sup>Measured: actual mean value obtained from experiments one and two

<sup>2</sup>Predicted: mean value obtained from prediction equations

SD = standard deviation

SE = standard error

RPE = relative predictive error

CCC = concordance correlation coefficient

### 7.3.2 Validation (Phase two)

The regression equations derived in phase one were validated against 40 independent spot faecal samples. The validated results showed that the equations developed to estimate daily milk OM (OM<sub>m</sub>; Eq.7.6) and DM (Eq.7.7) intakes in lambs were predicted with substantial accuracy as shown by their low RPE values (less than 10%) and high R<sup>2</sup> and CCC values (greater than 0.81).

$$\text{OMIm}_{\text{pre}} = 26.3 (\pm 6.64) + 0.92 (\pm 0.03) \text{OMIm}_{\text{mea}} \quad (7.6)$$

$$(R^2=0.97; \text{RPE} = 4.81\%; \text{CCC}= 0.97)$$

$$\text{DMIm}_{\text{pre}} = 27.9 (\pm 7.29) + 0.95 (\pm 0.03) \text{DMIm}_{\text{mea}} \quad (7.7)$$

$$(R^2=0.97; \text{RPE} = 7.16\%; \text{CCC}= 0.94)$$

For the pellets, the prediction accuracy of NDF<sub>i</sub>, DMIp and OMIp was not sufficient although the R<sup>2</sup> and CCC values indicated a substantial prediction. The RPE being greater than 20% indicated a non-satisfactory result (Table 7.5).

For total DMI (Fig 7.1), OMI (Fig. 7.2) and ME intake (Fig 7.3), a high accuracy of prediction was obtained, which was demonstrated by the acceptable RPE values of 15.1% and 9.48% for DMI, OMI and ME intake, respectively and high R<sup>2</sup> and CCC values (0.79 and 0.88 for DMI and 0.89 and 0.93 for ME intake, respectively). In all cases, the intercept was not different ( $P > 0.05$ ) from zero and the slope was different ( $P < 0.001$ ) from unity.

Table 7.5 Descriptive and equation statistics for validation data of pellet neutral detergent fibre (NDF<sub>i</sub>), dry matter intake (DMIp) and organic matter intake (OMIp).

|                           | Measured<br>(n= 40) | SD    | SE   | Predicted<br>(n=40) | SD    | SE   | R <sup>2</sup> | RPE,<br>% | CCC  |
|---------------------------|---------------------|-------|------|---------------------|-------|------|----------------|-----------|------|
| NDF <sub>i</sub> ,<br>g/d | 14.36               | 14.60 | 2.31 | 11.37               | 10.70 | 1.69 | 0.71           | 58.64     | 0.78 |
| DMIp,<br>g/d              | 91.6                | 88.9  | 14.1 | 72.0                | 67.5  | 10.7 | 0.64           | 61.42     | 0.75 |
| OMIp,<br>g/d              | 83.5                | 80.9  | 12.8 | 65.69               | 61.34 | 9.70 | 0.65           | 60.99     | 0.75 |

<sup>1</sup>Measured: actual mean value obtained from experiments one and two

<sup>2</sup>Predicted: mean value obtained from prediction equations

SD = standard deviation

SE = standard error

RPE = relative predictive error

CCC = concordance correlation coefficient

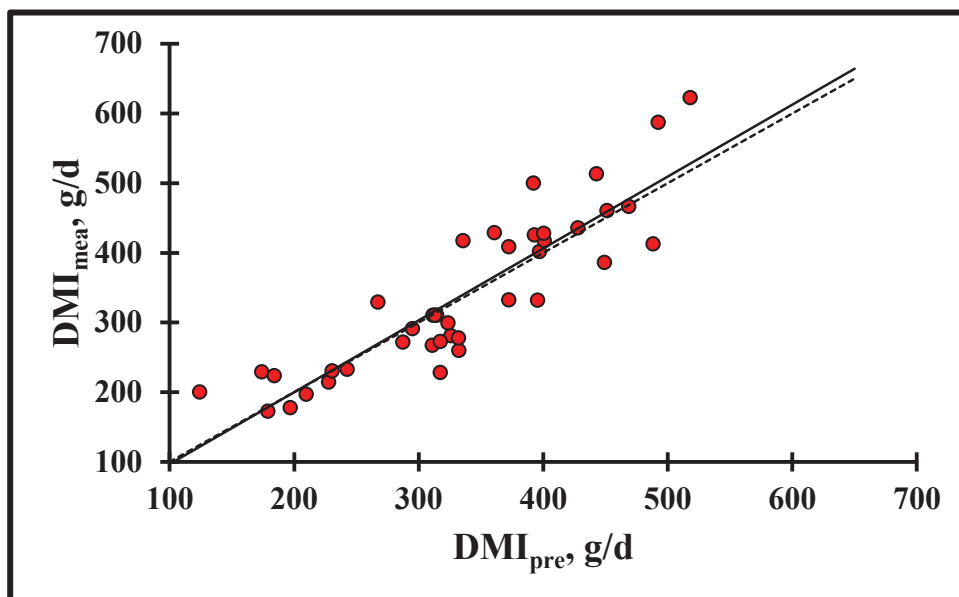


Figure 7.1 The relationship between measured dry matter intake (DMI<sub>mea</sub>) and predicted dry matter intake (DMI<sub>pre</sub>) of pre-weaned lambs consuming milk and pellets concurrently. The solid (–) line depicts the equation:  $DMI_{mea} = -6.18 (\pm 29.95) + 1.03 (\pm 0.09) DMI_{pre}$  ( $R^2 = 0.79$ ;  $RPE = 15.09\%$ ;  $CCC = 0.88$ ). The dashed (--) line depicts  $DMI_{mea} = DMI_{pre}$

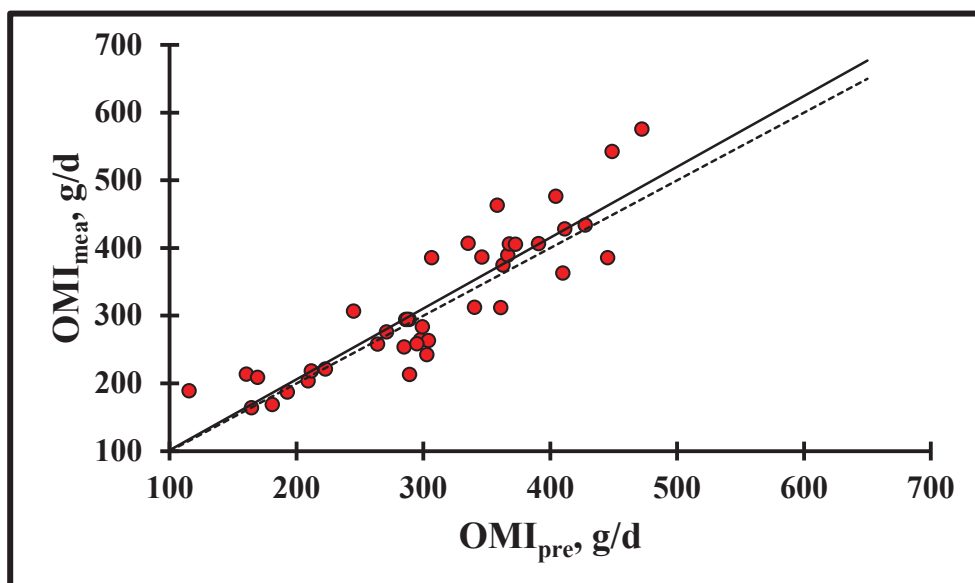


Figure 7.2 The relationship between measured organic matter intake (OMI<sub>mea</sub>) and predicted organic matter intake (OMI<sub>pre</sub>) of pre-weaned lambs consuming milk and pellets concurrently. The solid (–) line depicts the equation:  $OMI_{mea} = -3.44 (\pm 27.24) + 1.05 (\pm 0.09) OMI_{pre}$  ( $R^2 = 0.80$ ;  $RPE = 14.90\%$ ;  $CCC = 0.88$ ). The dashed (--) line depicts  $OMI_{mea} = OMI_{pre}$

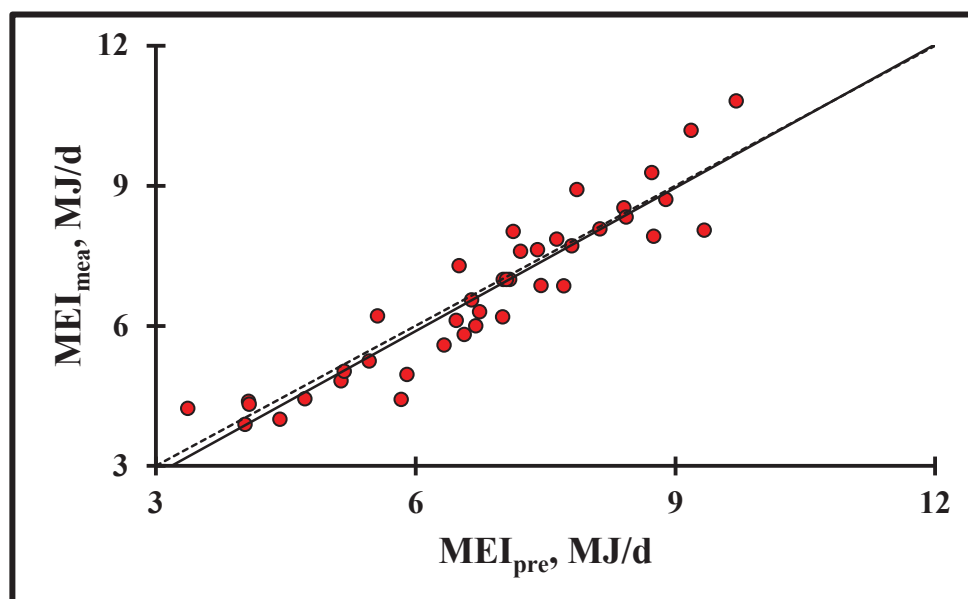


Figure 7.3 The relationship between measured metabolisable energy intake ( $MEI_{mea}$ ) and predicted metabolisable energy intake ( $MEI_{pre}$ ) of pre-weaned lambs consuming milk and pellets concurrently. The solid (—) line depicts the equation:  $MEI_{mea} = -0.26 (\pm 0.45) + 1.02 (\pm 0.06) MEI_{pre}$  ( $R^2 = 0.87$ ; RPE = 9.48%; CCC = 0.93). The dashed (--) line depicts  $MEI_{mea} = MEI_{pre}$

## 7.4 Discussion

The aim of the present study was to determine if DM, OM and ME intakes of lambs fed a combination of milk and pellets can be predicted with sufficient accuracy from dietary and faecal chemical composition. If possible, this would provide a means for farmers to develop more suitable feeding strategies for young lambs and also to optimise feed rations to meet the requirements of lambs under a variety of feeding conditions.

### 7.4.1 Prediction Equations (Phase one)

The significant linear regression between organic matter intake (OMI) and faecal nitrogen (fN) (Phase one) found in this study supported the concept of the relationship

between OMI and fN. This is based on the assumption that the amount of fN excreted per unit of organic matter (OM) ingested is constant (Lancaster, 1949; Strozinski and Chandler, 1972). However, this assumption was only confirmed for the prediction of milk OMI ( $OMI_m$ ), as the relationship between pellet OMI ( $OMI_p$ ) and fN was not significant. This was not in agreement with previous studies using older lambs and goats that reported a positive linear relationship between herbage OMI and fN (Boval *et al.*, 2003; Peripolli *et al.*, 2011; Azevedo *et al.*, 2014; David *et al.*, 2014). A review by Cordova *et al.* (1978) showed the inconsistent results from 25 studies with regards to the use of fN concentration as an indicator of pasture intake in grazing ruminants. Generally, the best association between fN concentration and N intake from solid feed occurred when the dietary nitrogen content was less than or equal to 2.4 g/kg DM (Wofford *et al.*, 1985), which may likely explain the no relationship between fN and  $OMI_p$  observed in the present study as the N concentration in the pellets were greater than 2.4 g/kg DM with the exception of the low-protein pellets. There are no published reports of OMI estimation from fN in young lambs consuming both milk and solid feed with which to make a direct comparison, but fN not selected by the stepwise regression model indicated a poor  $OMI_p$  prediction from fN (data not shown). Thus, results of the present study partially support the theoretical concept of the relationship between OMI and fN. Nevertheless, the prediction equations obtained for milk intake showed good precision and hence can be used to estimate milk OM and DM intakes in artificially reared lambs.

The most important variable for estimating pellet dry matter and organic matter intakes was the concentration of NDF. The relationship between dietary NDF and the concentration of NDF in faeces (fNDF) has been demonstrated previously by Fanchone

*et al.* (2007) in older sheep. In the present study, the concentrations of fNDF and pellet NDF ( $\text{NDF}_i$ ) provided the best estimate of pellet intake when stepwise procedures were used to obtain the best multiple regression equation. The significant linear relationship between fNDF and  $\text{NDF}_i$  found in the present study was consistent with the observations made by Wofford *et al.* (1985) in grazing cattle. However, we consider the model to have a low predictive value based on the very high RPE values obtained.

Faecal chemical compositions can have a low potential for predicting solid feed intake of ruminants (Holloway *et al.*, 1981). Therefore, it is important to include animal characteristics in intake prediction models. The potential feed intake of an animal is influenced by its demand for energy, its physical capacity for feed consumption such as live weight (or stomach capacity) and its physiological state (Allison, 1985; Mertens, 1987). The inclusion of live weight to the pellet and milk DMI prediction equations improved the  $R^2$  and CCC and decreased the RPE. A significant effect of LW on the  $\text{DMI}_{\text{pre}}$ ,  $\text{OMI}_{\text{pre}}$  and  $\text{MEI}_{\text{pre}}$  indicated that feed intake in young lambs was a linear function of their LW. The accuracy of the developed equations suggest they can be used to estimate intake of pre-weaned lambs at different live weights in an artificially rearing systems without access to grazing.

#### **7.4.2 Validation (Phase two)**

The validation results demonstrated that the prediction equations developed were sufficiently accurate for predicting ME intake from spot faecal samples. The validations were performed using a range of lamb treatments and live weights which should cover most situations in an artificial lamb rearing system.

The use of simple and multiple linear regression equations based on faecal composition to predict feed intake of lambs has been previously reported (David *et al.*, 2014). The authors predicted daily feed intake based on the total faecal collection, as the fN content used in the intake predictions were expressed in grams per day. This means that feed intake of lambs can only be predicted by conducting a parallel digestibility study. Results of the present study demonstrated it is possible to predict feed intake in pre-weaned lambs from the concentration of N and NDF in faecal material. A practical limitation for using the developed equations in the present study is the need for proximate laboratory analyses to determine the chemical composition of faecal and dietary samples and their associative costs. However, it eliminates the labour associated with total faecal collection and the discomfort of keeping lambs in crates. The possibility of using alternate laboratory analysis techniques such as the spectroscopic techniques, which have been successfully used to predict feed intake in sheep and cattle (Boval *et al.*, 2004; Fanchone *et al.*, 2007; Decruyenaere *et al.*, 2009b), should be considered as they may help reduce costs.

## **7.5 Conclusion**

The present study has shown that faecal N is a reliable index to predict the milk OM and DM intake of milk replacer in bottle-fed artificially-reared lambs but not pellet intake. The NDF concentration in both faeces and pellets was the most accurate in estimating pellet OM and DM intakes in young lambs. The robustness of the ME intake equation developed indicated that under similar conditions to that of the present study, the ME intake of pre-weaned lambs can be estimated with sufficient accuracy. Not only do developed ME intake equations provide information of theoretical interest to scientists,

but they have a significant practical application in farming situations by enabling farmers to develop more accurate feeding strategies for young lambs which will be more attuned to the lambs requirements. Unless validated with lambs grazing with their dams, these equations have been developed for indoor fed lambs.

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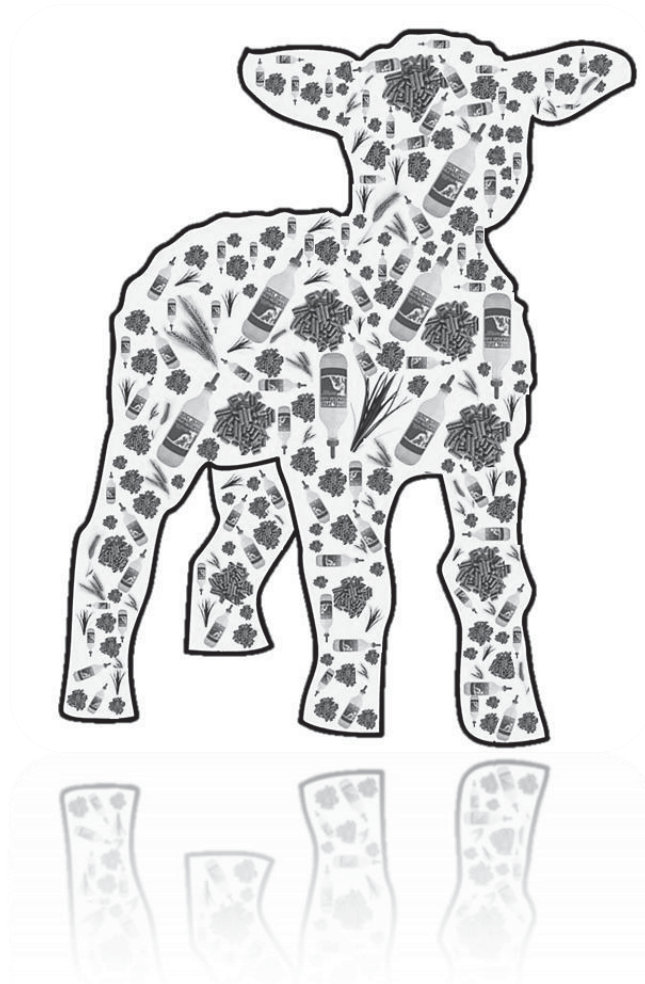
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## **Chapter 8      Modelling Lamb Growth: A Mechanistic Dynamic Model of Nutrient Utilisation, Growth and Body Composition in Pre-Weaned Lambs Reared Artificially**

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## ABSTRACT

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A mechanistic dynamic model was developed to simulate the growth and body composition of pre-weaned lambs reared artificially. The results of the simulated model were evaluated using the actual data from the three experiments involving 79 twin-born male lambs randomly allocated to different combinations of milk and pellets resulting in ten treatments groups. The accuracy of the traits simulated was evaluated by mean bias, concordance correlation coefficient (CCC), relative predictive error (RPE), and the coefficient of determination ( $r^2$ ). The model accurately simulated daily protein deposition (mean bias =  $0.07 \text{ g/d} \pm 1.5$ ;  $r^2 = 0.93$ ; RPE = 4.21 %; CCC = 0.96), water deposition (mean bias =  $-2.34 \pm 6.75 \text{ g/d} \pm 1.5$ ;  $r^2 = 0.97$ ; RPE = 6.23 %; CCC = 0.94) and ash deposition (mean bias =  $0.22 \pm 0.43 \text{ g/d} \pm 1.5$ ;  $r^2 = 0.83$ ; RPE = 9.00 %; CCC = 0.87) rates. Fat deposition in lambs was underestimated (paired t-test,  $P < 0.05$ ; mean bias =  $3.75 \pm 4.99 \text{ g/d}$ ) due to an over-estimation of maintenance energy requirement for lambs given restricted access to pellet intake and, therefore, resulting in an overall poor prediction of fat deposition rate ( $r^2 = 0.37$  RPE = 29.78%; CCC = 0.34). Empty body weight at slaughter of the lambs was accurately simulated ( $r^2 = 0.95$  RPE = 1.66%; CCC = 0.97). Although live weights (LW) and average daily gains (ADG) of the lambs were underestimated by the developed model (paired t-test,  $P < 0.05$ ), the accuracy of the overall simulated mean LW and ADG were substantial ( $r^2 = 0.90$  and  $0.99$ ; RPE = 3.73 % and 4.92 %; CCC = 0.89 and 0.97 for LW and ADG, respectively). The developed lamb growth model can be used to simulate, with reasonable accuracy, pre-weaned lamb performance under artificial rearing conditions at least. The simulation model needs to be further validated with other datasets.

## 8.1 Introduction

A better understanding of the growth process can be obtained when information and concepts are synthesised and integrated into growth simulation models (Black, 1995). Models put together concepts and observations in a context that can be used theoretically and also for computational purposes (Birkett and de Lange, 2001). Computer simulation models have been developed to simulate growth in various animal species e.g. sheep (Finlayson *et al.*, 1995), poultry (Gous *et al.*, 1999), pigs (De Lange *et al.*, 2003), beef cattle (Garcia *et al.*, 2008), and dairy calves (Souza *et al.*, 2016). Specifically, models to predict the growth and body composition of lambs consuming either liquid or solid feed only have been developed (Black, 1974; Sainz and Wolff, 1990; Osorio *et al.*, 2015). However, there are no growth models for pre-weaned lambs consuming both liquid and solid feed diets concurrently.

Pre-weaned lambs undergo two phases of development relative to their digestive function, accompanied by changing dietary intake (Searle *et al.*, 1972). Differences in the digestion process of milk and pellets and the associative effect on growth and body composition (Chapter 5 and 6) make it difficult to directly predict performance. Therefore, simulation models are an option for evaluating the impact of dietary changes on growth and body composition (Pettigrew, 2016), whilst evaluating the economic impacts of those decisions in order to optimise production systems. Pre-weaned lamb growth models should describe the underlying biological mechanism of growth with regards to the physical, biochemical, and metabolic pathways between variables and should also take into account between animal variations, thereby, requiring a mechanistic approach. Further, the changing dietary intake over time associated with the digestion process will require a dynamic approach.

Therefore, the aim of this chapter was to develop a mechanistic dynamic model to predict the growth and body composition of pre-weaned lambs under defined feeding conditions. These simulated results for lamb growth and body composition would then be compared with actual data from three experiments (Chapters 4, 5 and 6).

## **8.2 Model description**

The model equations were derived from data from the three experiments in this thesis (Chapters 4, 5 and 6), data from other studies conducted at Massey University and information available in the literature. For an effective application of growth models, the performance variables of the lamb must be accurately characterised. The main variables required are the initial live weight, body protein deposition potential, protein and energy maintenance requirements, partitioning of energy and protein intakes above maintenance for protein and fat deposition and the daily feed intake (Schinckel and de Lange, 1996).

The present model simulates the partitioning of actual individual lamb feed intake (metabolisable energy [ME] intake and crude protein [CP] intake) from Chapters 4, 5 and 6 into retained protein, fat, ash and water contents in the empty body of pre-weaned lambs. The average daily gain (ADG), empty body weight (EBW) and live weights (LW) of the lambs at slaughter were also simulated by the model. A diagrammatic representation of the nutrient partitioning model is presented in Figure 8.1. The nutrients inputs used to simulate growth and body composition are protein, energy, and neutral detergent fibre (NDF) intakes. The model calculates on a day-to-day basis the body deposition rates in response to protein and energy intake and the total body composition. The concepts and representations used will be discussed hereafter.

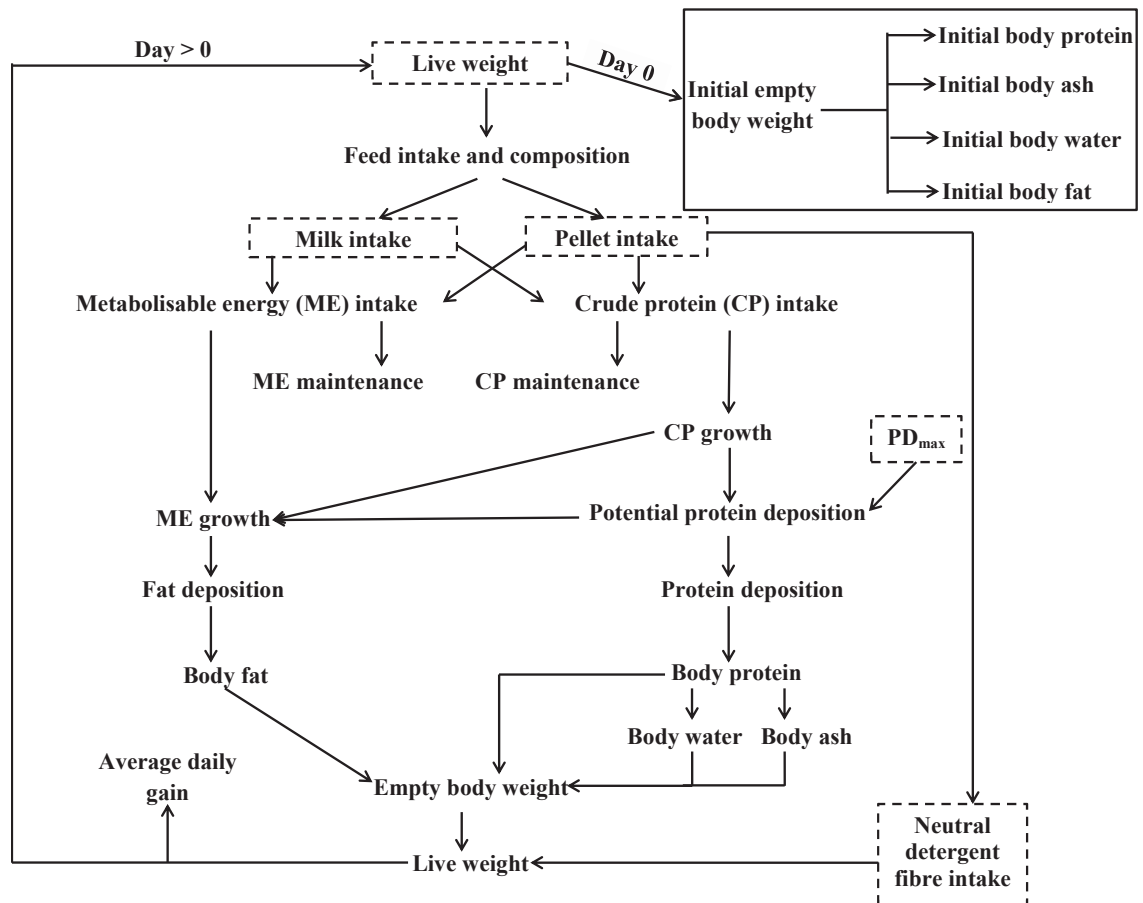


Figure 8.1 A schematic representation of energy and protein partitioning in a simple pre-weaned lamb growth model during artificial rearing. Upper solid box indicates the estimation of the initial body composition of lambs based on the baseline slaughter data from Chapters 5 and 6. Dashed boxes contain the model inputs. Arrows indicate the directional flow of parameters.  $PD_{max}$  = maximum protein deposition.

### 8.2.1 Feed intake estimation

The actual milk ( $MR_i$ ) and pellet ( $PEL_i$ ) intakes of the lambs were considered as input parameters in the model. A continuous input of feed intake is assumed in the present model. The actual crude protein intake (CPI) and the neutral detergent fibre intake ( $NDF_i$ ) were calculated as milk and pellet intake multiplied by the nutrient content of each feed (Eq. 8.1 and 8.2, respectively).

$$\text{CPI} = (\text{MR}_i \times \text{CP}_{\text{MR}}, \text{ g/kg DM}) + (\text{PEL}_i \times \text{CP}_{\text{PEL}}, \text{ g/kg DM}) \quad (8.1)$$

$$\text{NDF}_i = \text{PEL}_i \times \text{NDF}_{\text{PEL}}, \text{ g/kg DM} \quad (8.2)$$

The ME values of milk and pellet were calculated from the digestibility study conducted in Chapters 5 and 6. Metabolisable energy content for milk was considered to be 0.96 of the gross energy (GE) content and that of pellets was 0.71\*GE content. The ME intake (MEI) was calculated as milk and pellet intake multiplied by the ME content of each feed (Eq. 8.3).

$$\text{MEI} = (\text{MR}_i \times \text{ME}_{\text{MR}}, \text{ MJ/kg DM}) + (\text{PEL}_i \times \text{ME}_{\text{PEL}}, \text{ MJ/kg DM}) \quad (8.3)$$

### **8.2.2 Estimation of initial body composition**

The empty body weight (EBW) in the present model was defined as LW minus the contents of the gastrointestinal tract and drained blood at slaughter. The initial live weight ( $\text{LW}_0$ ) was an input parameter used to calculate the initial empty body weight ( $\text{EBW}_0$ ). The equation for calculating  $\text{EBW}_0$  was derived from the baseline slaughter groups in Chapters 5 and 6 (Eq 8.4).

$$\text{EBW}_0 = 0.88 \times \text{LW}_0 \quad (8.4)$$

The initial body protein ( $\text{P}_0$ ), initial body fat ( $\text{F}_0$ ), initial body water ( $\text{W}_0$ ), initial body ash ( $\text{A}_0$ ) were calculated as a function of  $\text{EBW}_0$ , based on the baseline slaughter data recorded in Chapters 5 and 6 according to the following Equations (8.5 to 8.8):

$$\text{P}_0 = 0.176 \times \text{EBW}_0 \quad (8.5)$$

$$\text{F}_0 = 0.022 \times \text{EBW}_0 \quad (8.6)$$

$$W_0 = 0.758 \times EBW_0 \quad (8.7)$$

$$A_0 = 0.044 \times EBW_0 \quad (8.8)$$

### 8.2.3 *Modelling growth and body composition*

The following calculations were done on a daily basis. The protein and energy for maintenance is generally a power function of live weight. The ME and CP requirements for maintenance requirement ( $ME_m$ , Eq. 8.9 and  $CP_m$ , Eq. 8.10 respectively) were calculated from metabolic LW ( $LW^{0.75}$ ) based on the equations obtained in Chapters 4 and 5, respectively.

$$ME_m = 0.45 \times LW^{0.75} \quad (8.9)$$

$$CP_m = 2.74 \times LW^{0.75} \quad (8.10)$$

The ME and CP available for growth ( $ME_g$  and  $CP_g$ , respectively) were calculated as the difference between the total MEI (and CPI) and maintenance requirements (Eqs 8.11 and 8.12).

$$ME_g = MEI - ME_m \quad (8.11)$$

$$CP_g = CPI - CP_m \quad (8.12)$$

Daily protein deposition rate is determined as a minimum value allowed by the available  $CP_g$  or  $ME_g$ , or the maximum protein deposition rate ( $PD_{max}$ ) (Bastianelli and Sauvant, 1997). The potential protein deposition rate  $PD_{pot}$  was calculated from either the  $CP_g$  remaining after an inevitable catabolism of 17% (Chapter 5) or the  $PD_{max}$  of 45 g/d (Chapter 6), whichever is smallest (Eq. 8.13).

$$PD_{\text{pot}}, \text{ g/d} = \text{minimum} [0.83 \times CP_g, PD_{\text{max}}] \quad (8.13)$$

The ME required for protein deposition ( $ME_{PD}$ ) was calculated from the energetic cost of protein deposition of 0.044 MJ/kg (ARC, 1981) and the  $PD_{\text{pot}}$  value obtained (Eq. 8.14).

$$ME_{PD} = 0.044 \times PD_{\text{pot}} \quad (8.14)$$

Fat deposition in lambs depends on the amount of energy available after supplying the needs for maintenance and lean growth (Sainz and Wolff, 1990). The ME available for fat deposition ( $ME_{FD}$ ) was calculated from the difference between  $ME_g$  and  $ME_{PD}$  (Eq. 8.15).

$$ME_{FD} = ME_g - ME_{PD} \quad (8.15)$$

The model assumed that any additional protein that was not utilised for protein deposition was catabolised and the energy gained was deposited as fat ( $adME_{FD}$ ). The calculated energy yield from deaminated protein was taken to be 0.0115 MJ ME / g CP (Whittemore and Fawcett, 1976) (Eq. 8.16).

$$adME_{FD} = 0.0115 \times (CP_g - PD_{\text{pot}}) \quad (8.16)$$

The fat deposition (FD) was calculated by dividing the sum of  $ME_{FD}$  and  $adME_{FD}$  by the energetic cost of fat deposition in lambs of 0.076 MJ ME / g fat (0.039 / 0.51; where 0.039 MJ/kg is the energy content of fat (SCA, 1990) and 0.51 is the efficiency of ME utilisation for FD (Chapter 4 and 5) as shown in Equation 8.17.

$$FD = (ME_{FD} + adME_{FD}) / 0.076 \quad (8.17)$$

The simulated body protein (P) and fat (F) in lambs on day one ( $P_1$ ,  $F_1$ ) were calculated based on Eqs. 8.18 and 8.19, respectively.

$$P_1 = P_0 + PD_{\text{pot}} \quad (8.18)$$

$$F_1 = F_0 + FD \quad (8.19)$$

Body composition data from Chapters 4, 5 and 6 and Morel *et al.* (2016) were used to derive prediction equations to predict body water (W) and ash (A) in the lambs body on day one ( $W_1$ ,  $A_1$ ) from body protein on day one ( $P_1$ ) (Eqs 8.20 and 8.21).

$$W_1 = P_1^{0.801} \times 4.366 \quad (r^2 = 0.95) \quad (8.20)$$

$$A_1 = (P_1 \times 0.147) + 0.072 \cdot (r^2 = 0.82) \quad (8.21)$$

The simulated EBW on day one ( $EBW_1$ ) was calculated as the sum of the chemical body components on day one according to Equation 8.22

$$EBW_1 = P_1 + F_1 + W_1 + A_1 \quad (8.22)$$

The simulated LW of the lambs on day one ( $LW_1$ ) was calculated as a function of EBW and of dietary  $NDF_i$  using a prediction equation derived from Chapters 4, 5 and 6 (Eq.8.23).

$$LW_1 = (1.14 \times EBW_1) + (0.0047 \times NDF_{i1}) \quad (r^2 = 0.89) \quad (8.23)$$

Equations 8.9 to 8.23 were used to simulate the daily growth and body composition up to the day of slaughter (n). The protein deposited per day (PD/d), fat deposited per day (FD/d), water deposited per day (WD/d), and ash deposited per day (AD/d) were

calculated as the difference between the body composition of the lambs on the day of slaughter ( $P_n$ ,  $F_n$ ,  $W_n$ ,  $A_n$ ; where  $n$  is the day of slaughter) and the initial body composition ( $P_0$ ,  $F_0$ ,  $W_0$ ,  $A_0$ ) divided by the number of experimental days ( $x$ ) according to Equations 8.24 to 8.29.

$$PD/d = (P_n - P_0)/x \quad (8.24)$$

$$FD/d = (F_n - F_0)/x \quad (8.25)$$

$$WD/d = (W_n - W_0)/x \quad (8.26)$$

$$AD/d = (A_n - A_0)/x \quad (8.27)$$

The EBW at slaughter was calculated as the sum of the chemical body components on the day of slaughter ( $n$ ) according to Equation 8.28.

$$EBW_n = P_n + F_n + W_n + A_n \quad (8.28)$$

The ADG was calculated as the difference between the LW of the lambs at slaughter ( $LW_n$ ) and the  $LW_0$  divided by the number of experimental days ( $x$ ) (Eq. 8.29)

$$ADG = (LW_n - LW_0)/x \quad (8.29)$$

#### **8.2.4 Model evaluation**

The simulated individual lamb daily protein (PD/d), fat (FD/d), water (WD/d) and ash (AD/d) deposition rates and ADG were compared to the actual data from experiments in Chapters 4, 5 and 6. Briefly, the experiments were conducted with 79 pre-weaned lambs. Eight of the lambs were used as the baseline slaughter group and the remaining

71 lambs were allocated to different nutritional treatment groups in each experiment fed different milk and pellet combinations as described in Chapters 4, 5 and 6, resulting in 10 treatment groups (Table 8.1).

#### **8.2.5 Statistical analysis**

The paired T-TEST ( $\alpha = 0.05$ ) procedure of SAS (2013) was used to determine if the simulated means differed from the actual means within treatment groups. Evaluation of the model was conducted by regressing the actual group values (independent variables) against the simulated values (dependent variables) (Piñeiro *et al.*, 2008). To measure precision of the models predictions, the coefficient of determination ( $r^2$ ) was used to determine the total variance explained by the regression model (and also how much of the linear variation in the actual values is explained by the variation in the simulated values) (Smith and Rose, 1995; Tobias *et al.*, 2006). The model bias (mean bias) was computed subtracting the simulated mean value from the actual mean value. The model was evaluated using relative predictive error (RPE) and the concordance correlation coefficient (CCC) (Lin, 1989). An RPE value lower than 10% is an indication of satisfactory prediction, and a RPE greater than 20% indicates poor prediction (Fuentes-Pila *et al.*, 1996). A CCC value of 0.81 to 1.00 indicates an almost perfect prediction, 0.61 to 0.80 indicates a substantial prediction, 0.41 to 0.60 indicates a moderate prediction and values from 0.21 to 0.40 indicate a fair prediction (Fuentes-Pila *et al.*, 1996; Visser *et al.*, 2012).

Table 8.1 Summary of the data used in the development and evaluation of the growth and body composition simulation model

| Breed                                | Treatment | n | Feed intake        |             | Nutrient intake <sup>2</sup> per kg dry matter |           |           | Mean lamb variables <sup>3</sup> |          |                  |       |
|--------------------------------------|-----------|---|--------------------|-------------|------------------------------------------------|-----------|-----------|----------------------------------|----------|------------------|-------|
|                                      |           |   | Milk replacer, g/d | Pellet, g/d | CP, g/kg                                       | ME, MJ/kg | NDF, g/kg | Initial LW                       | Final LW | Age <sub>s</sub> | ADG   |
| Experiment one – Chapter Four (2013) |           |   |                    |             |                                                |           |           |                                  |          |                  |       |
| Suffolk                              | MO        | 8 | 188.4              | 0           | 253.4                                          | 22.5      | 0         | 6.12                             | 13.67    | 59.75            | 128.4 |
| Suffolk                              | MP        | 7 | 185.1              | 144.4       | 231.3                                          | 18.51     | 63.13     | 6.11                             | 18.68    | 59.57            | 214.6 |
| Experiment two– Chapter Five (2014)  |           |   |                    |             |                                                |           |           |                                  |          |                  |       |
| Romney                               | MO        | 7 | 202.4              | 0           | 251.6                                          | 22.6      | 0         | 4.97                             | 18.16    | 93.00            | 143.6 |
| Romney                               | MP30      | 7 | 204.1              | 34.93       | 243.5                                          | 21.18     | 17.92     | 5.11                             | 18.74    | 80.84            | 171.7 |
| Romney                               | MP60      | 7 | 202.5              | 57.80       | 239.4                                          | 20.47     | 27.16     | 4.99                             | 18.50    | 76.43            | 179.5 |
| Romney                               | MPad      | 7 | 199.3              | 101.8       | 232.8                                          | 19.34     | 41.76     | 5.02                             | 18.86    | 66.71            | 211.3 |
| Experiment three– Chapter Six (2015) |           |   |                    |             |                                                |           |           |                                  |          |                  |       |
| Romney                               | NMLP      | 7 | 230.1              | 81.65       | 224.2                                          | 20.34     | 58.95     | 5.34                             | 18.98    | 62.00            | 224.0 |
| Romney                               | NMHP      | 7 | 226.7              | 88.40       | 239.8                                          | 20.27     | 58.61     | 5.31                             | 18.57    | 58.14            | 233.5 |
| Romney                               | HMLP      | 7 | 232.4              | 100.8       | 274.6                                          | 19.56     | 68.75     | 5.57                             | 19.01    | 54.00            | 255.0 |
| Romney                               | HMHP      | 7 | 230.6              | 102.1       | 292.7                                          | 19.54     | 65.10     | 5.44                             | 18.77    | 52.00            | 265.4 |
| Descriptive statistics               |           |   |                    |             |                                                |           |           |                                  |          |                  |       |
| Mean                                 |           |   | 209.8              | 70.19       | 248.4                                          | 20.46     | 39.57     | 5.41                             | 18.13    | 66.11            | 201.7 |
| SE                                   |           |   | 2.27               | 6.42        | 2.42                                           | 0.17      | 3.33      | 0.10                             | 0.23     | 1.58             | 5.89  |
| SD                                   |           |   | 19.12              | 54.10       | 20.39                                          | 1.41      | 28.09     | 0.81                             | 1.92     | 13.30            | 49.64 |

<sup>1</sup>Treatment: MO = milk only; MP<sub>30</sub> = milk + 30% of *ad libitum* pellets intake; MP<sub>60</sub> = milk + 60% of *ad libitum* pellets intake; MP<sub>ad</sub> = pellets offered *ad libitum*; NMLP = normal-protein milk + low-protein pellet; NMHP = normal-protein milk + high-protein pellet; HMLP = high-protein milk + low-protein pellet; HMHP = high-protein milk + high-protein pellet.

<sup>2</sup>Nutrient intake: refers to the combined intake of milk replacer and pellet (ME = metabolisable energy; CP: crude protein; NDF = neutral detergent fibre)

<sup>3</sup>Variables: LW = live weight, kg; Age<sub>s</sub> = Age at slaughter, d; ADG = average daily gain, g/d

### 8.3 Results

#### *Daily protein deposition rate*

The model accurately (paired t-test,  $P > 0.05$ ) simulated the daily protein deposition rates in all the treatment groups (Fig. 8.2) with the exception of MPad'14 which was underestimated by 3.03 g /d (paired t-test = 3.00,  $P < 0.05$ ) and HMLP'15 group which was overestimated by 2.63 g /d (paired t-test = -3.17,  $P < 0.05$ ) compared to the actual means.

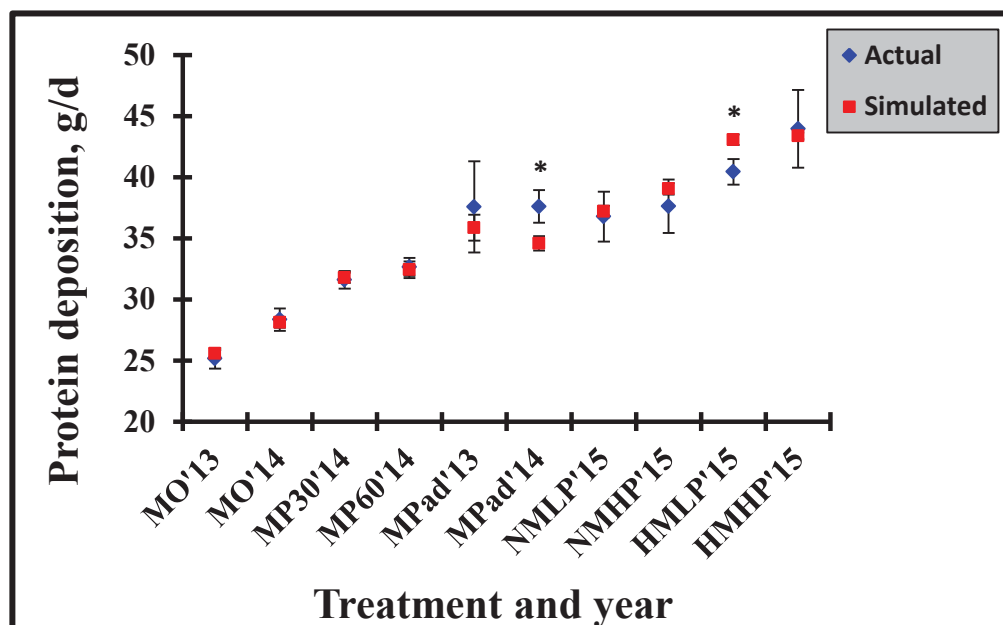


Figure 8.2 Comparison of the actual and simulated daily protein deposition rates of pre-weaned lambs consuming different milk and pellets combinations based on the proposed model. MO'13 = milk only (2013); MO'14 = milk only (2014); MP30'14 = milk + 30% of *ad libitum* pellets intake (2014); MP60'14 = milk + 60% of *ad libitum* pellets intake (2014); MPad'13 = pellets offered *ad libitum* (2013); MPad'14 = pellets offered *ad libitum* (2014); NMLP = normal-protein milk + low-protein pellet (2015); NMHP = normal-protein milk + high-protein pellet (2015); HMLP = high-protein milk + low-protein pellet (2015); HMHP = high-protein milk + high-protein pellet (2015). Error bars = standard error of means. \*\*, \*Simulated mean differs from actual mean at  $P < 0.05$ .

*Daily fat deposition rate*

The daily fat deposition rates (FD/d) for five of the treatment groups were poorly simulated by the developed model (Fig. 8.3). Simulated Fd for the milk-only fed groups (MO'13 and MO'14), and the MP30'14, MP60'14 and MPad'14 treatment groups were underestimated and differed from actual Fd (t test,  $P < 0.01$ ).

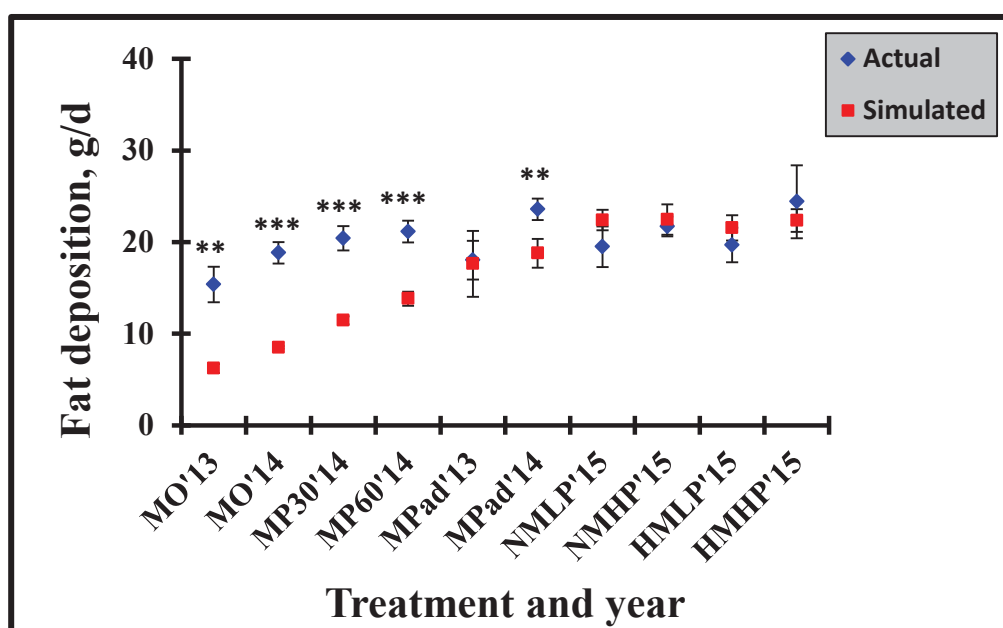


Figure 8.3 Comparison of the actual and simulated daily fat deposition rates of pre-weaned lambs consuming different milk and pellets combinations based on the proposed model. MO'13 = milk only (2013); MO'14 = milk only (2014); MP30'14 = milk + 30% of *ad libitum* pellets intake (2014); MP60'14 = milk + 60% of *ad libitum* pellets intake (2014); MPad'13 = pellets offered *ad libitum* (2013); MPad'14 = pellets offered *ad libitum* (2014); NMLP = normal-protein milk + low-protein pellet (2015); NMHP = normal-protein milk + high-protein pellet (2015); HMLP = high-protein milk + low-protein pellet (2015); HMHP = high-protein milk + high-protein pellet (2015). Error bars = standard error of means. \*\*, \*\*\* Simulated mean differs from actual mean at  $P < 0.01$ ,  $P < 0.001$ .

### Daily water deposition rate

The daily water deposition rates (WD/d) for the treatment groups were accurately simulated by the model (Fig. 8.4) with the exception of MO'13 (mean bias = -9.59;  $t$  test = -2.91;  $P < 0.05$ ), MO'14 (mean bias = -12.13 g,  $t$  test -7.18 =  $P < 0.001$ ), MP30'14; (mean bias = -6.82 g;  $t$  test = -3.87;  $P < 0.01$ ), and MP60'14; (mean bias = -7.91;  $t$  test = -4.66;  $P < 0.01$ ) which were overestimated and differed from actual WD/d.

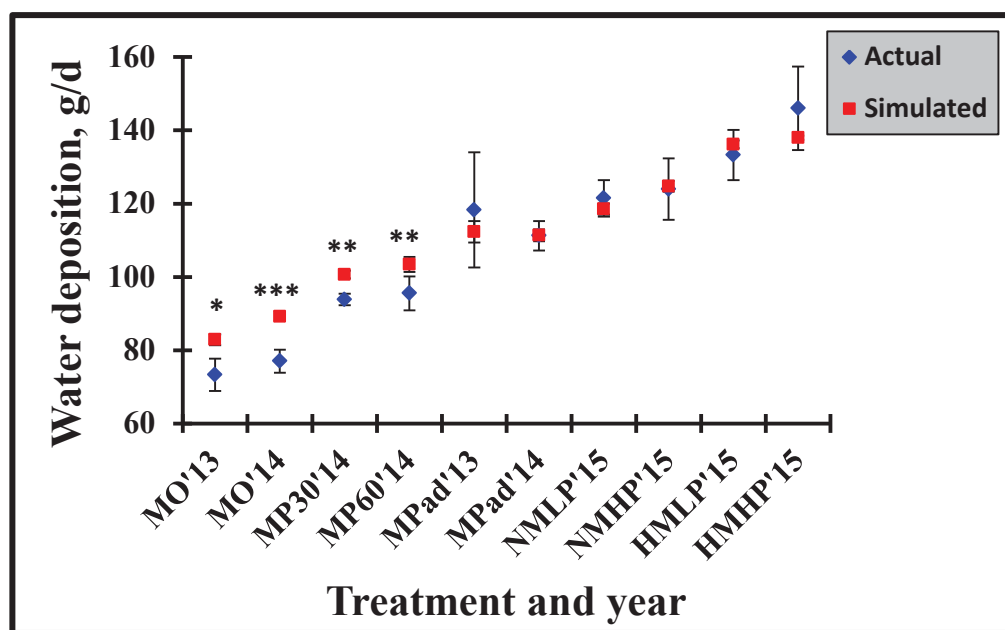


Figure 8.4 Comparison of the actual and simulated daily water deposition rates of pre-weaned lambs consuming different milk and pellets combinations based on the proposed model. MO'13 = milk only (2013); MO'14 = milk only (2014); MP30'14 = milk + 30% of *ad libitum* pellets intake (2014); MP60'14 = milk + 60% of *ad libitum* pellets intake (2014); MPad'13 = pellets offered *ad libitum* (2013); MPad'14 = pellets offered *ad libitum* (2014); NMLP = normal-protein milk + low-protein pellet (2015); NMHP = normal-protein milk + high-protein pellet (2015); HMLP = high-protein milk + low-protein pellet (2015); HMHP = high-protein milk + high-protein pellet (2015). Error bars = standard error of means. \*, \*\*, \*\*\* Simulated mean differs from actual mean at  $P < 0.05$ ,  $P < 0.01$ ,  $P < 0.001$ .

### Daily ash deposition rate

The pattern of ash deposition produced by the model (Fig. 8.5) was accurate for all the treatment groups (t test,  $P > 0.05$ ).

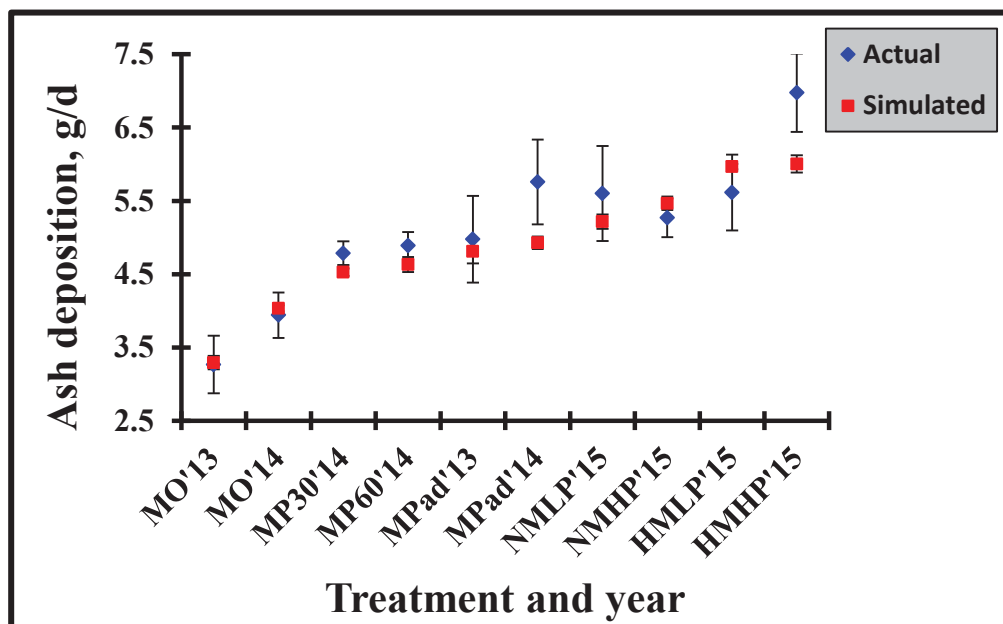


Figure 8.5 Comparison of the actual and simulated daily ash deposition rates of pre-weaned lambs consuming different milk and pellets combinations based on the proposed model. MO'13 = milk only (2013); MO'14 = milk only (2014); MP30'14 = milk + 30% of *ad libitum* pellets intake (2014); MP60'14 = milk + 60% of *ad libitum* pellets intake (2014); MPad'13 = pellets offered *ad libitum* (2013); MPad'14 = pellets offered *ad libitum* (2014); NMLP = normal-protein milk + low-protein pellet (2015); NMHP = normal-protein milk + high-protein pellet (2015); HMLP = high-protein milk + low-protein pellet (2015); HMHP = high-protein milk + high-protein pellet (2015). Error bars = standard error of means.

### Average daily gain

The simulated average daily gain (ADG) of MPad'14, NMLP'15 and HMLP'15 were overestimated compared to actual ADG (paired t-test,  $P < 0.05$ ), by 15.84 g/d, 11.05 g/d and 15.14 g/d, respectively (Fig 8.6). There was a tendency for the simulated ADG of HMHP'15 to be underestimated by 22.72 g/d (t test = 2.21,  $P = 0.06$ ).

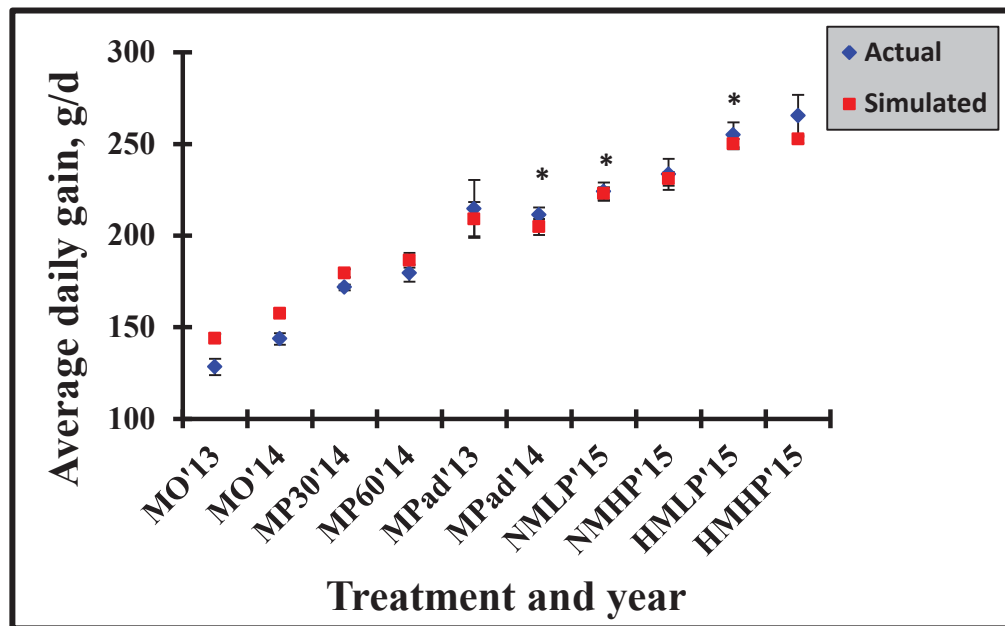


Figure 8.6 Comparison of the actual and simulated average daily gain of pre-weaned lambs consuming different milk and pellets combinations based on the proposed model. MO'13 = milk only (2013); MO'14 = milk only (2014); MP30'14 = milk + 30% of *ad libitum* pellets intake (2014); MP60'14 = milk + 60% of *ad libitum* pellets intake (2014); MPad'13 = pellets offered *ad libitum* (2013); MPad'14 = pellets offered *ad libitum* (2014); NMLP = normal-protein milk + low-protein pellet (2015); NMHP = normal-protein milk + high-protein pellet (2015); HMLP = high-protein milk + low-protein pellet (2015); HMHP = high-protein milk + high-protein pellet (2015). Error bars = standard error of means. \*Simulated mean differs from actual mean at  $P < 0.05$ .

#### Overall model performance

The paired t-test indicated that the model underestimated ( $P < 0.05$ ) the final LW of the lambs by 2.42%, ADG by 4.24% and FD by 18.50 % (Table 8.2). With the exception of FD which was poorly simulated, the developed model accurately simulated the growth and body composition of the artificially reared lambs (Table 8.2) as indicated by  $r^2$  values greater than 0.80, rand CCC values greater than 0.81 and RPE values lower than 10%.

Daily fat deposition rate was re-run by reducing the maintenance energy for lambs fed milk only diets and those with restricted access to pellets to  $0.34 \text{ MJ ME} / \text{kg LW}^{0.75}$  (Walker and Norton, 1971; Thomson *et al.*, 1979), while maintaining the  $0.45 \text{ MJ ME} / \text{kg LW}^{0.75}$  for lambs with *ad libitum* access to pellets to test if the simulated FD would improve (Fig 8.7). The FD for the treatment groups were accurately simulated by the model with the exception of NMLP'15 and NMHP'15, which were overestimated ( $P < 0.05$ ) and differed from actual FD. However, the overall actual and simulated means were not different (t test,  $P > 0.05$ ) and the  $r^2$  and CCC values increased from 0.37 to 0.40 and from 0.34 to 0.56 respectively while the RPE decreased from 29.78% to 14.93%.

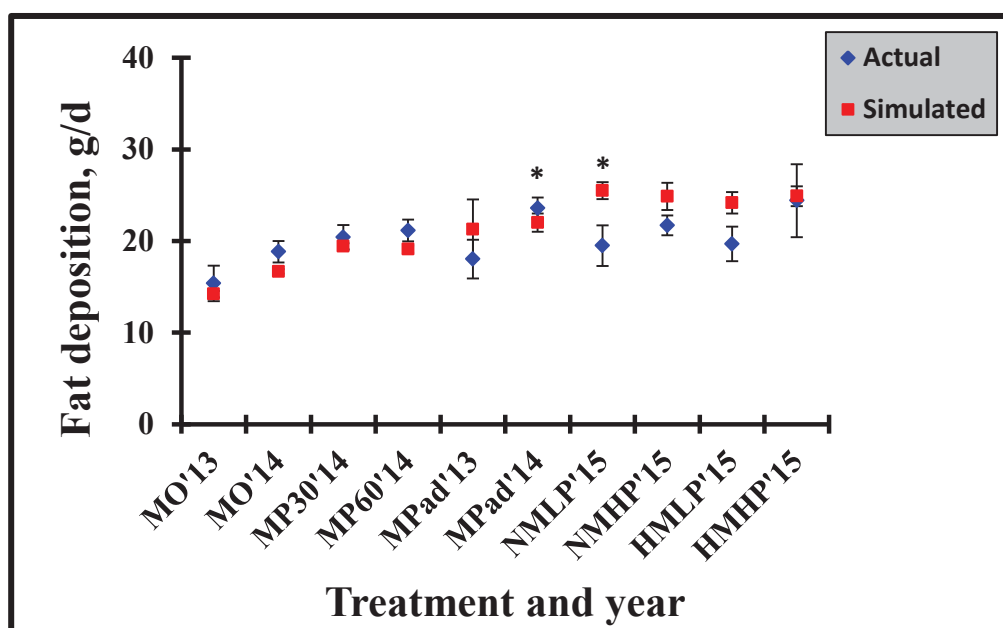


Figure 8.7 Comparison of the actual and simulated daily fat deposition rates of pre-weaned lambs consuming different milk and pellets using maintenance energy requirement values of  $0.34 \text{ MJ ME} / \text{kg LW}^{0.75}$  for lambs with restricted access to pellets and  $0.45 \text{ MJ ME} / \text{kg LW}^{0.75}$  for lambs with *ad libitum* access to pellets. MO'13 = milk only (2013); MO'14 = milk only (2014); MP30'14 = milk + 30% of *ad libitum* pellets intake (2014); MP60'14 = milk + 60% of *ad libitum* pellets intake (2014); MPad'13 = pellets offered *ad libitum* (2013); MPad'14 = pellets offered *ad libitum* (2014); NMLP = normal-protein milk + low-protein pellet (2015); NMHP = normal-protein milk + high-protein pellet (2015); HMLP = high-protein milk + low-protein pellet (2015); HMHP = high-protein milk + high-protein pellet (2015). Error bars = standard error of means. \*Simulated mean differs from actual mean at  $P < 0.05$ .

Table 8.2 Statistical indicators of model performance: Mean bias, co-efficient of determination ( $r^2$ ), relative predictive error (RPE) and concordance correlation co-efficient (CCC) for the growth and body composition of pre-weaned lambs consuming various combinations<sup>1</sup> of milk and pellets.

| Trait                   | n  | Mean <sup>2</sup> ± standard deviation |                | Statistical indicators       |                                |       |        |      |
|-------------------------|----|----------------------------------------|----------------|------------------------------|--------------------------------|-------|--------|------|
|                         |    | Actual, g/d                            | Simulated, g/d | Mean bias<br>SD <sup>3</sup> | Paired t-<br>test <sup>4</sup> | $r^2$ | RPE, % | CCC  |
| Live weight, kg         | 10 | 18.19 ± 1.61                           | 17.75 ± 1.34   | 0.16 ± 0.54                  | 2.61*                          | 0.90  | 3.73   | 0.89 |
| Empty body weight, kg   | 10 | 15.46 ± 1.18                           | 15.47 ± 1.15   | -0.01 ± 0.27                 | -0.11 <sup>ns</sup>            | 0.95  | 1.66   | 0.97 |
| Average daily gain, g/d | 10 | 202.7 ± 45.7                           | 194.1 ± 36.6   | 8.58 ± 9.73                  | 2.79*                          | 0.99  | 4.92   | 0.97 |
| Protein deposited, g/d  | 10 | 35.18 ± 5.69                           | 35.12 ± 5.88   | 0.07 ± 1.56                  | 0.14 <sup>ns</sup>             | 0.93  | 4.21   | 0.96 |
| Fat deposited, g/d      | 10 | 20.27 ± 2.64                           | 16.52 ± 6.14   | 3.75 ± 4.99                  | 2.38*                          | 0.37  | 29.78  | 0.34 |
| Water deposited, g/d    | 10 | 109.4 ± 23.89                          | 111.7 ± 18.37  | -2.34 ± 6.75                 | -1.10 <sup>ns</sup>            | 0.97  | 6.23   | 0.94 |
| Ash deposited, g/d      | 10 | 5.11 ± 1.02                            | 4.89 ± 0.84    | 0.22 ± 0.43                  | 1.63 <sup>ns</sup>             | 0.83  | 9.00   | 0.87 |

<sup>1</sup>Combinations: MO'13 = milk only (2013); MO'14 = milk only (2014); MP30'14 = milk + 30% of *ad libitum* pellets intake (2014); MP60'14 = milk + 60% of *ad libitum* pellets intake (2014); MPad'13 = pellets offered *ad libitum* (2013); MPad'14 = pellets offered *ad libitum* (2014); NMLP = normal-protein milk + low-protein pellet (2015); NMHP = normal-protein milk + high-protein pellet (2015); HMLP = high-protein milk + low-protein pellet (2015); HMHP = high-protein milk + high-protein pellet (2015).

<sup>2</sup>Mean = calculated from the average of each treatment group

<sup>3</sup>SD = standard deviation

<sup>4</sup>Paired t-test: ns = not significant ( $P > 0.05$ ), \*  $P < 0.05$ .

## 8.4 Discussion

The principal objective in developing this model was to simulate the effects of varying nutrient input on pre-weaned lamb growth and body composition using actual information of feed intake and diet characteristics under artificial rearing conditions. Published lamb performance models have tended to focus on predicting ADG and body composition in older lambs (ARC, 1980; Cannas *et al.*, 2006; NRC, 2007; Osorio *et al.*, 2015) thus limited information is available on simulated body composition in pre-weaned lambs. Hence, there is a need to develop models to predict the changes in lamb growth and body composition with dietary changes. This model needs to be useable at an applied level.

### 8.4.1 Model Assumptions

#### *Feed intake*

Due to the significant variation in the initiation and consumption of solid feed in young lambs (Chapter 4), developing a general equation to predict feed intake based on age or live weight for lambs may not accurately reflect the actual intake of lambs. Therefore, the present model included the actual milk and pellet intake of lambs as an input parameter with the assumption that any poorly simulated outcome would be due to factors other than feed intake.

#### *PD<sub>max</sub>*

The present model assumed a maximum value for daily protein deposition (PD<sub>max</sub>). The PD<sub>max</sub> value of 45 g / d (Chapter 6) was considered a constant throughout the simulation period. The accurate prediction of protein deposition in the present model indicates that a PD<sub>max</sub> of 45 g/d in pre-weaned lambs was justifiable.

*Fat deposition*

The present model assumed that any extra energy available in the lamb's body after maintenance requirements and protein deposition rates have been satisfied was deposited as fat. Therefore, inaccurate estimation of maintenance energy requirements could have implications on fat deposition which may explain the poorly simulated daily fat deposition rate. Factors that increase energy expenditure per unit live weight such as increased protein (lean tissue) deposition rate (Sainz and Wolff, 1990; Schinckel and de Lange, 1996; Hoch and Agabriel, 2004) and greater liver and gastrointestinal tract (GIT) weights (Ferrell *et al.*, 1976; Canas *et al.*, 1982; Ferrell, 1988) reduce the amount of energy available for fat deposition. Therefore, diets that increase protein deposition rates or the weights of liver and GIT tissues may increase maintenance energy requirements. However, these notions were not taken into consideration when estimating fat deposition in the present model as a constant maintenance energy requirement value of  $0.45 \text{ MJ ME} / \text{kg LW}^{0.75}$  was used.

As there were no variations in the amount of milk intake consumed per day by lambs within each of the three experiments (Chapters 4, 5 and 6) due to controlled intake, maintenance requirement could not be calculated separately for milk and pellets. It appears that the maintenance requirement value used in the present model was influenced by the variation in pellet intake and hence the model overestimated the requirements of the milk only fed lambs and lambs with restricted access to pellets intake (Fig 8.3). Rerunning the model by reducing the maintenance energy requirement for lambs with restricted access to pellets to  $0.34 \text{ MJ ME} / \text{kg LW}^{0.75}$  (Walker and Norton, 1971; Thomson *et al.*, 1979), whilst maintaining the  $0.45 \text{ MJ ME} / \text{kg LW}^{0.75}$  for lambs with *ad libitum* access to pellets improved the simulated daily fat deposition in lambs. These findings demonstrate that overestimating maintenance energy

requirements of lambs underestimates their daily fat deposition rates during the pre-weaning period.

#### **8.4.2 Overall model performance**

The ADG of lambs, although underestimated was effectively simulated as indicated by the high  $r^2$  and CCC values. The protein deposition of an animal is considered the most important determinant of weight gain, due to the high water content of protein-rich tissues (Bastianelli and Sauvant, 1997). Therefore, the rate of protein deposition has a greater impact on the growth rates of lambs than fat deposition (Ferrell *et al.*, 1979; Hoch and Agabriel, 2004). This finding was demonstrated in the present result by the substantial ADG prediction even when fat deposition was poorly simulated, confirming previous studies.

The mean bias value obtained indicated that the ADG calculated by the present model was underestimated by 4.24%. Comparing ADG models based on the mean bias, previous models predicting ADG in older lambs using the Cornell Net Carbohydrate and Protein System for Sheep (CNCPS-S) (Cannas *et al.*, 2006) or the California Net Energy System (Osorio *et al.*, 2015) have similarly underestimated ADG with a difference of 0.45%, and 1.85% respectively with the exception of the Small Ruminant Nutrition System (SRNS) which over simulated ADG by 0.84% (Linsky, 2013). However, higher  $r^2$  and/or CCC values were obtained in the present study ( $r^2 = 0.99$ ; CCC = 0.97) than those reported in the studies of Cannas *et al.* (2006) ( $r^2 = 0.82$ ; CCC = 0.90) and Osorio *et al.* (2015) ( $r^2 = 0.74$ ; CCC = 0.86), suggesting the current model was more accurate. A standard deviation of 37 g obtained in the present ADG model was higher than the 23 g reported by Osorio *et al.* (2015). A larger standard deviation reflects the variability in gain due to the dietary, environmental and breed difference

(Whetsell *et al.*, 2006). Additionally, the amount of blood in gain was not accounted for in the present model which may have contributed also to the underestimation of ADG.

The use of body protein mass to simulate body water and body ash has been previously reported in pig growth simulations models (De Lange, 1995; De Lange *et al.*, 2003). In the present model the sum of these body tissues including body fat was used to estimate the EBW of the lambs, whilst LW was calculated as a function of EBW and NDF intake (Williams *et al.*, 1992). Overall, the accuracy of predicting EBW was better than that of LW, indicating that the method of describing gut fill in the present model was not completely accurate. Nevertheless, the model was not designed to account for the amount of blood in the body. Accounting for blood in the body would have improved the simulated LW simulation as it was underestimated.

## **8.5 Conclusion**

The present lamb growth model was reasonably accurate and can be used to simulate pre-weaned lamb performance. However, fat deposition was poorly simulated but could be improved with altered estimations of the maintenance energy requirements of lambs during the pre-ruminant and transitional stage of growth. Overall, these results offer evidence that generalised equations could be used to predict lamb performance during the pre-weaning period. The simulated model needs to be validated with other datasets to further evaluate its performance.

## **8.6 Implications**

A reasonably accurate lamb growth model has been designed with the potential of evaluating pre-weaning lamb performance and optimising production systems for the

sheep industry. The model is simple to use. The main constraint of the present model is that it only applies to milk and pelleted diets for which the digestibility and nutrient composition are known. Thus, in future, efforts should be devoted to developing feed intake prediction equations in order to build independent models. The present model focused on the growth and chemical composition of the whole empty body of the lambs. However, simulating the growth and composition separately for carcass and non-carcass tissues needs to be considered in future models. This would allow the industry to determine the economics of various options. The developed model focused entirely on pre-weaned lambs. Thus if we want to model lamb growth post-weaning or even maturity, simulating protein requirements in terms of crude protein may not be ideal for lambs post weaning due to the changes in diet and the digestive physiology of lambs which needs to be taken into consideration. Therefore, the use of metabolisable protein rather than crude protein would be ideal post weaning.

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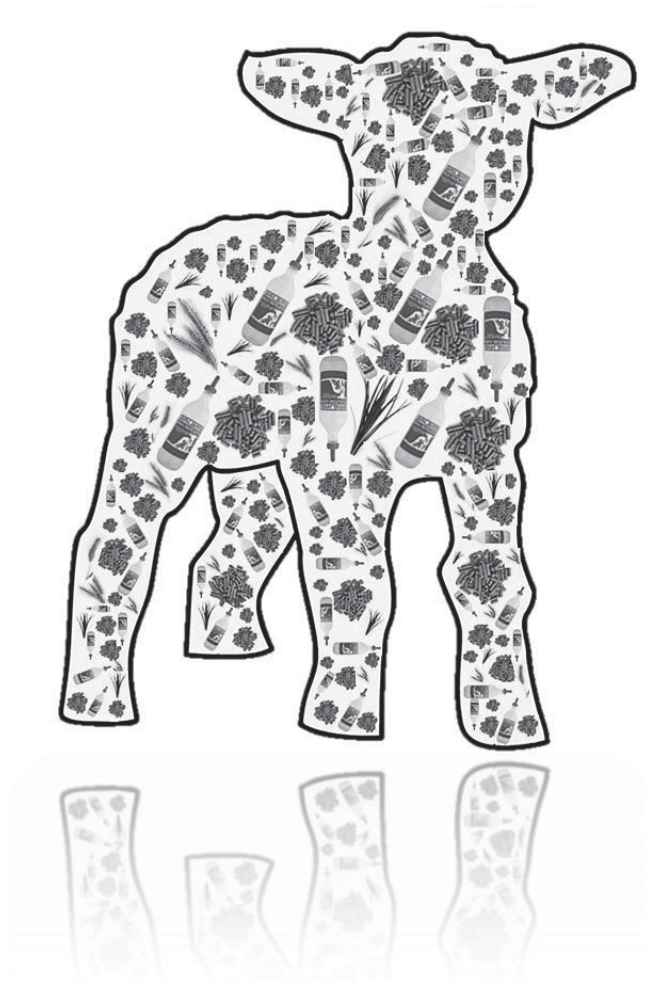
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## Chapter 9      General Discussion

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## 9.1 Introduction

Lamb meat sales are worth approximately NZ\$ 2.6 billion annually to the New Zealand economy (Beef & Lamb New Zealand, 2016). Increasing the efficiency and profitability of lamb production is a key goal for the New Zealand sheep industry. Lamb growth varies in response to nutrient supply; therefore understanding why there is variation in response could be important for improving the efficiency of nutrient utilisation. Nutrient requirements and utilisation by animals vary with diet type and management conditions (Galvani *et al.*, 2008). Pasture intake in young lambs (especially twin lambs) make up about 34% of their total dry matter intake at three weeks of age (Geenty *et al.*, 1985). However, estimation of metabolisable energy requirement for maintenance and growth for lambs pre-weaning have been limited to milk-only fed lambs (Jagusch and Mitchell, 1971; Chiou and Jordan, 1973). Thus, the nutrient requirements of pre-weaned lambs consuming both milk and solid feed need investigation. Currently there are no tools to measure feed intake in pre-weaned lambs while at pasture. Moreover, under extensive grazing systems, it is difficult to accurately measure both milk and pasture intake. It was therefore proposed that raising lambs artificially would serve as a model to represent pre-weaned lamb growth at pasture with their dams and may enable a better understanding of the relationship between lamb nutrient intake and growth.

The aims of this thesis were to gain a clearer understanding of nutrient intake and utilisation for growth and body composition in pre-weaned lambs consuming both milk and solid feed, to derive equations for predicting feed intake and to develop a growth simulation model which can be used as a tool for developing feeding strategies for lambs.

Briefly, Chapter 3 investigated the relationship between prenatal ewe characteristics and twin-lamb pre-weaning growth, Chapter 4 investigated the effects of two feeding regimens (milk replacer only vs milk replacer and pellets) on lamb growth, organ development and how much of the variation in lamb growth could be explained by metabolisable energy (ME) intake under controlled feed intake conditions. Chapter 5 examined the effects of incremental levels of pellets in addition to milk replacer on pre-weaned lamb growth, body composition, milk replacer and pellet utilisation and the use of ME for maintenance and growth. In Chapter 6, the effects of different protein to energy ratios achieved via milk replacer and pellets on lamb growth body composition and, nitrogen and energy balances were determined. Chapter 7 developed prediction equations to estimate the feed intake of pre-weaned lambs consuming milk and pellets concurrently. In Chapter 8, data from the three lamb growth trials (Chapter 4, 5 and 6) were used to develop a growth simulation model to predict pre-weaned lamb growth and body composition.

In this section (Chapter 9), the general outcome of the experiments, their results, conclusions and implications for lamb rearing are discussed. In addition, limitations and weaknesses of the research are identified and discussed. This section ends with an overview of main conclusions of the research and recommendations for future research.

## **9.2 Summary of main findings and conclusions drawn**

### **9.2.1 *Lamb growth and organ development***

Lamb growth in the first six weeks of life is largely determined by the amount of milk obtained from the dam (Chapter 3). Although the a significant proportion of variation in twin lamb growth to 6 weeks was explained by the milk production of the dams,

weaning weight at about 12 weeks was not related to 42-day accumulated milk yield, due to lamb growth after about 6 weeks being heavily dependent on pasture as the nutrient source. Therefore, it was concluded that lamb growth from 6 to 12 weeks of life may be significantly influenced by factors other than milk production (Chapter 3). Chapters 4, 5 and 6 were, thus, designed to understand how milk and solid feed intake affected lamb growth and how these diets could be altered to improve lamb growth.

Chapters 4 and 5 showed that addition of pellets to the milk diet of lambs was one way of improving pre-weaning growth rates in the first eight weeks of life. Nevertheless, modelling the protein to energy requirements of lambs (Chapter 5) indicated that protein intake relative to energy intake was limiting lamb growth during this period. The pre-weaning period is where the greatest growth rate can be achieved (Muir *et al.*, 2003). Therefore, improving lamb growth rates during this period offers more options to farmers as lambs can go directly to slaughter at weaning or have a higher store value (Schreurs *et al.*, 2010) and this can partly be achieved by altering dietary composition which was the focus of Chapter 6.

Over the three studies, the average daily gains of lambs were greatest for lambs in the 2015 study (Chapter 6) with those lambs reaching the 18 kg target live weight (LW) before age 60 days (Fig.9.1). Lambs in Chapter 6 maintained growth rates comparable to lambs in an accelerated lamb production system (deNicolo *et al.*, 2008). This outcome highlighted the benefit of feeding lambs the right CP:ME ratio to optimise growth rate. With this knowledge and a better understanding of how lamb pre-weaning growth rate can be manipulated through dietary means, it may be possible to significantly increase the production efficiency of lambs which would subsequently improve financial returns to farmers.

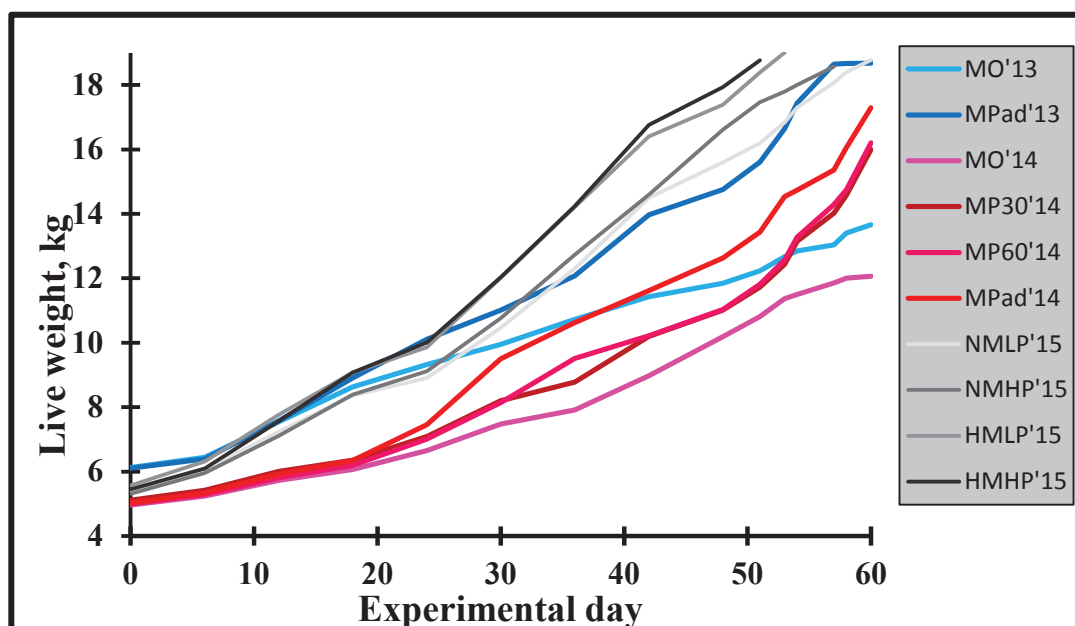


Figure 9.1 Live weights of lambs in the first 60 days of life from the three artificial rearing studies conducted in this thesis. MO'13 = milk only; MPad'13 = pellets offered *ad libitum*, MO'14 = milk only; MP30'14 = milk + 30% of *ad libitum* pellets intake; MP60'14 = milk + 60% of *ad libitum* pellets intake; MPad'14 = pellets offered *ad libitum*, NMLP = normal-protein milk + low-protein pellet; NMHP = normal-protein milk + high-protein pellet; HMLP = high-protein milk + low-protein pellet; HMHP = high-protein milk + high-protein pellet ('number = year of study).

Lambs in the experiments reported in Chapters 5 and 6 were fed milk replacer as a proportion of their LW and it was expected that the lambs with *ad libitum* access to pellets in Chapter 5 would have growth rates comparable to the lambs fed normal milk in Chapter 6. However, this was not the case. Lambs in Chapter 6 were fed five times daily in the first two weeks of life in contrast to four times daily in Chapter 5. This increase in feeding frequency increased the total milk intake of lambs in Chapter 6 and may have contributed to the improved growth rates.

Feeding lambs to a similar slaughter weight regardless of the diet type, composition or time taken to reach slaughter weight had no effect on carcass weight (Chapter 5 and 6).

However, the weight of specific organs such as stomach, liver and intestines differed depending on the diet type (milk vs. pellets) or diet quality (low-protein vs. high-protein). For example, Baldwin (2000) showed that increases in the weight of the gastrointestinal organs and liver in lambs fed pellets in addition to milk were likely in response to the absorptive and metabolic requirements associated with solid feed intake. Whereas lambs fed high-protein milk had increased liver and kidney weights in comparison to their counterparts fed low-protein milk due to increased metabolic activity of these organs (Chapter 6). Overall, results of this thesis showed that weights of different visceral organs are influenced by diet type and quality.

Solid feed intake measurements not only explained a greater proportion of variation in lamb growth, it also revealed just how early lambs began to eat solid feed (Chapter 4). Previous research recommended introducing lambs to creep feed between one and two weeks of age (Hamada *et al.*, 1976). However, the results of Chapter 4 suggest introducing lambs immediately post-birth may be a management option to increase lamb growth and could allow for early weaning at six to eight weeks of age. Growth rate of early weaned lambs depends on their ability to rapidly increase pasture intake following weaning (Hill *et al.*, 2010). However, digestion will be limited if rumen development is inadequate. Early pellet intake may encourage:

- rumen development, thus allowing adult capacity to be reached earlier (Geenty and Sykes, 1983),
- more rapid development of rumen micro flora (usually reaching adult status by 3 weeks of age) (Joyce and Rattray, 1970)

- increased production of volatile fatty acids in the rumen (Liu *et al.*, 2016) which would in turn stimulate the development of rumen papillae, which is where the end products of digestion are absorbed (van Houtert, 1993).

Indeed, Chapter 5 showed that earlier rumen development in lambs improved the efficiency of ME utilisation for growth.

### **9.2.2 Body composition**

The importance of improving pre-weaning growth rates needs to be balanced against the possibility of any adverse effects on carcass composition (with markets mostly requiring leaner carcasses) and the increased cost of artificial lamb rearing. In Chapters 4 and 5, it was demonstrated that diet type (milk only or milk + pellets) had little influence on the chemical body composition when lambs were fed to a fixed live weight during the pre-weaning growth period. Generally, the proportion of protein in the LWs of lambs (g/kg LW) up to 18 kg was greater than that of fat. However, the incremental proportion of fat was greater than that of protein, indicating that the lambs started to deposit more fat below 20 kg LW.

Body composition of lambs varied greatly in response to the quality of the diet offered (Chapter 6). Little attention has been paid in the literature to the distribution of protein and fat between the various body components in lambs of similar age and weight as those in this thesis. Chapter 6 showed that the deposition of protein and fat in carcasses were affected by the CP:ME intake ratio of lambs with the leanest carcasses being produced when a ratio of approximately 14 g/MJ was offered to lambs. The effect of CP:ME ratio from the milk replacer on body composition showed up the strongest. In practical terms, this suggests that in addition to selecting for ewes with high milk yield

(Chapter 3) farmers should also select ewes with high CP:ME content in their milk. Additionally, changing the composition of commercial milk replacers to a CP:ME ratio of approximately 13 (Chapter 6) will help maximise lamb growth to weaning.

#### *Protein and energy interrelationships*

The protein and energy dependent phases of growth indicate a preferential use of protein for protein deposition provided that the fat:protein ratio in the gain exceeds a certain minimum and there is enough protein per unit energy that is required for protein deposition (Bastianelli and Sauvant, 1997). Before the present study, there have been inconclusive results in the literature as to whether these protein and energy dependent phases of growth exist in growing lambs (Titgemeyer, 2003; Schroeder and Titgemeyer, 2008). In general, results from this thesis showed that increased lamb ME intake was associated with increased protein deposition (“A” in Fig 9.2). However, as ME intake increased further, there was proportionally less protein intake per unit ME (“B” in Fig 9.2). Consequently, there was no further response in protein deposition. Nevertheless, when protein intake was increased at a similar level of ME intake (“C” in Fig. 9.2), lambs responded by increasing protein deposition.

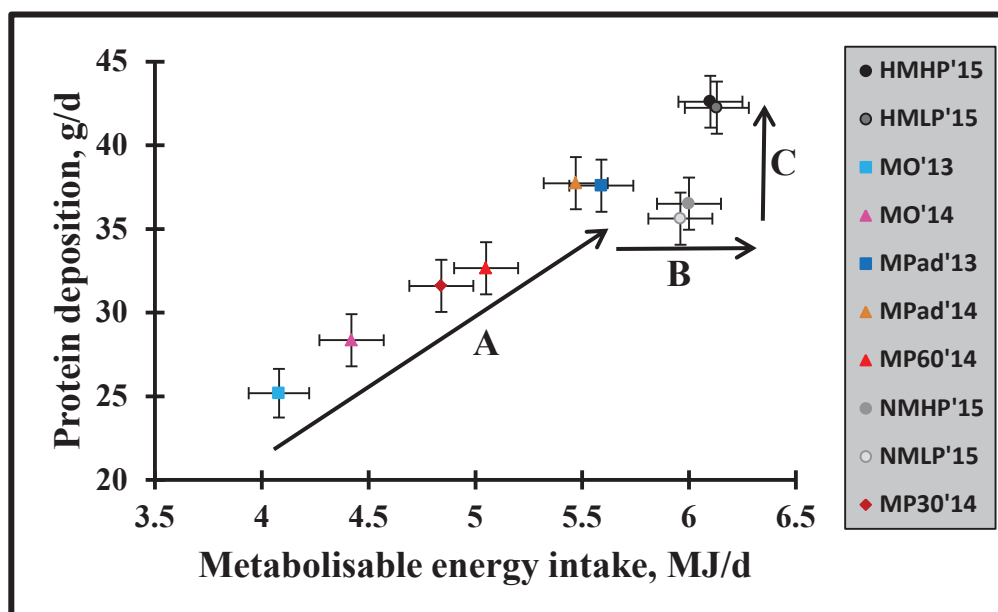


Figure 9.2. The effect of different metabolisable energy intake levels on protein deposition in lambs during the pre-weaning phase of growth. “A” represents protein deposition response in lambs to increasing ME intake, “B” and “C” = represents protein deposition response in lambs at similar ME intake but higher protein to energy intake ratio. MO’13 = milk only; MPad’13 = pellets offered *ad libitum*, MO’14 = milk only; MP30’14 = milk + 30% of *ad libitum* pellets intake; MP60’14 = milk + 60% of *ad libitum* pellets intake; MPad’14 = pellets offered *ad libitum*, NMLP = normal-protein milk + low-protein pellet; NMHP = normal-protein milk + high-protein pellet; HMLP = high-protein milk + low-protein pellet; HMHP = high-protein milk + high-protein pellet (‘number = year of study). Error bars = standard error of means.

The response of protein deposition to ME intake indicates that the efficiency of protein deposition in pre-weaned lambs is affected not only by ME intake but by the ratio of protein to energy in the diet. Maximum protein deposition was thus reached at a CP:ME ratio of about 14 (Fig.9.3) confirming that protein intake was limiting lamb growth as indicated in Chapter 5.

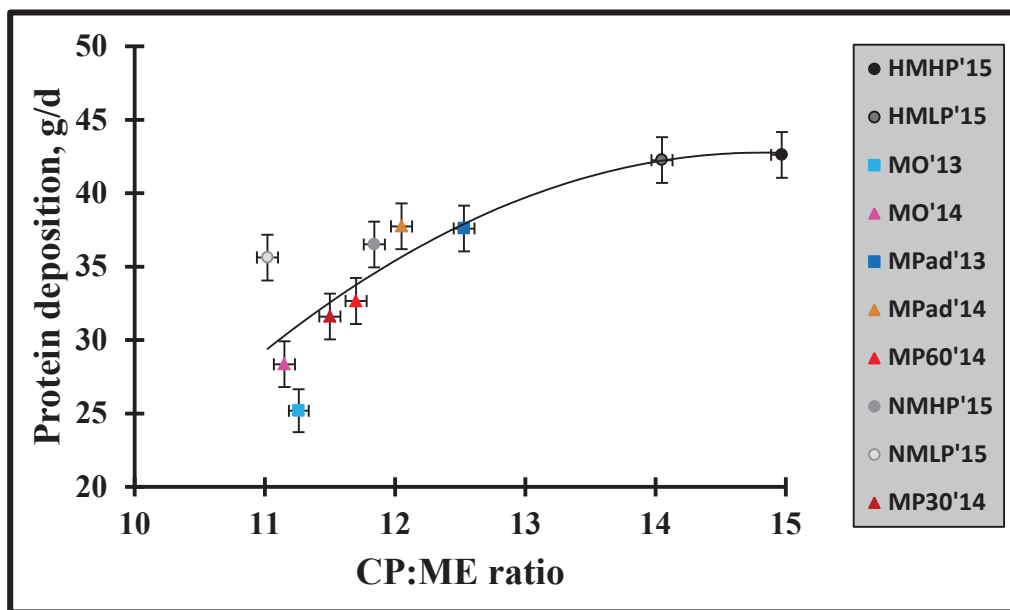


Figure 9.3 The relationship between CP:ME ratio and protein deposition in lambs during the pre-weaning phase of growth. MO'13 = milk only; MPad'13 = pellets offered *ad libitum*, MO'14 = milk only; MP30'14 = milk + 30% of *ad libitum* pellets intake; MP60'14 = milk + 60% of *ad libitum* pellets intake; MPad'14 = pellets offered *ad libitum*, NMLP = normal-protein milk + low-protein pellet; NMHP = normal-protein milk + high-protein pellet; HMLP = high-protein milk + low-protein pellet; HMHP = high-protein milk + high-protein pellet ('number = year of study). Error bars = standard error of means.

Fat deposition responded to increases in both ME intake (Fig 9.4) and CP:ME ratio (Fig 9.5). In both Figures, increasing either ME intake or the CP:ME ratio at proportionately low levels of protein intake, increased fat deposition ("A" and "B" in Fig. 9.4 and 9.5). These observations indicated that at any level of energy intake, when protein intake per unit energy is insufficient, any extra ME intake will be diverted into fat deposition. However, when protein intake per unit ME was adequate, lambs deposited more protein in gain, thus fat deposition decreased ("C" in Fig 9.4 and 9.5).

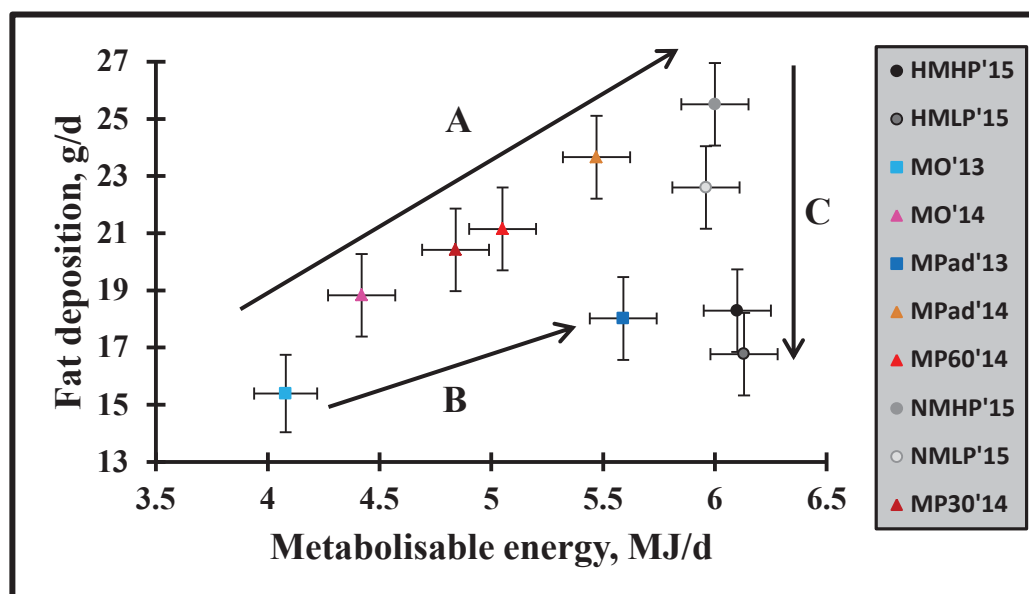


Figure 9.4. The effect of different metabolisable energy intake levels on fat deposition in lambs during the pre-weaning phase of growth. “A” and “B” represents fat deposition response in lambs to increasing ME intake in Romney and Suffolk lambs, respectively “C” represents fat deposition response in lambs at similar ME intake but different protein to ME ratios in Romney lambs. MO’13 = milk only; MPad’13 = pellets offered *ad libitum*, MO’14 = milk only; MP30’14 = milk + 30% of *ad libitum* pellets intake; MP60’14 = milk + 60% of *ad libitum* pellets intake; MPad’14 = pellets offered *ad libitum*, NMLP = normal-protein milk + low-protein pellet; NMHP = normal-protein milk + high-protein pellet; HMLP = high-protein milk + low-protein pellet; HMHP = high-protein milk + high-protein pellet (number = year of study). Error bars = standard error of means.

Another observation from the thesis experiments was the between-breed differences in terms of the relative rates of protein and fat deposition. Generally, in Figures 9.2 and 9.3, the Suffolk lambs (MO’13 and MPad’13) responded similarly to increases to either CP:ME ratio or ME intake as the Romney lambs (all treatment except MO’13 and MPad’13) with regards to protein deposition. However, the Suffolk lambs deposited less fat in comparison to the Romney lambs, even at limiting CP:ME intake ratio (“B” in Fig 9.4). These differences in fat to protein ratio reflect differences in genotype (Kirton *et al.*, 1995). Thus, at similar levels of ME intake (B in Fig 9.4) the less protein consumed by the Suffolk lambs, the less fat deposited per unit protein (Fig 9.5),

suggesting that any additional energy consumed was preferentially used for protein deposition.

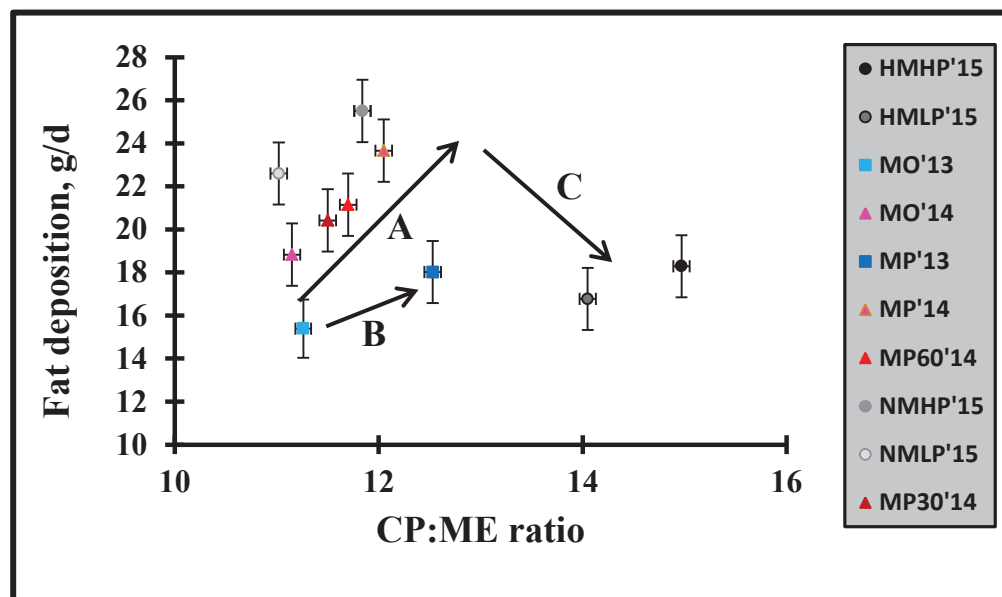


Figure 9.5 The effect of varying the crude protein to metabolisable energy (CP:ME) ratio on fat deposition in lambs during the pre-weaning phase of growth. “A” and “C” represents fat deposition response in lambs to increasing CP:ME intake ratios in Romney lambs. “B” represents fat deposition response in lambs to increasing CP:ME intake ratios in Suffolk lambs. MO'13 = milk only; MPad'13 = pellets offered *ad libitum*, MO'14 = milk only; MP30'14 = milk + 30% of *ad libitum* pellets intake; MP60'14 = milk + 60% of *ad libitum* pellets intake; MPad'14 = pellets offered *ad libitum*, NMLP = normal-protein milk + low-protein pellet; NMHP = normal-protein milk + high-protein pellet; HMLP = high-protein milk + low-protein pellet; HMHP = high-protein milk + high-protein pellet ('number = year of study). Error bars = standard error of means.

Overall, fat deposition was inversely related to protein deposition (Fig 9.6) suggesting that when CP:ME intake is not limiting, lambs utilised protein intake for protein deposition after a minimum fat:protein ratio of about 0.4 had been deposited. However, when CP:ME intake was limiting, lambs preferentially deposited more fat at the

expense of protein in gain. Nevertheless, some breed differences may occur with regards to the level of fat deposited per unit protein.

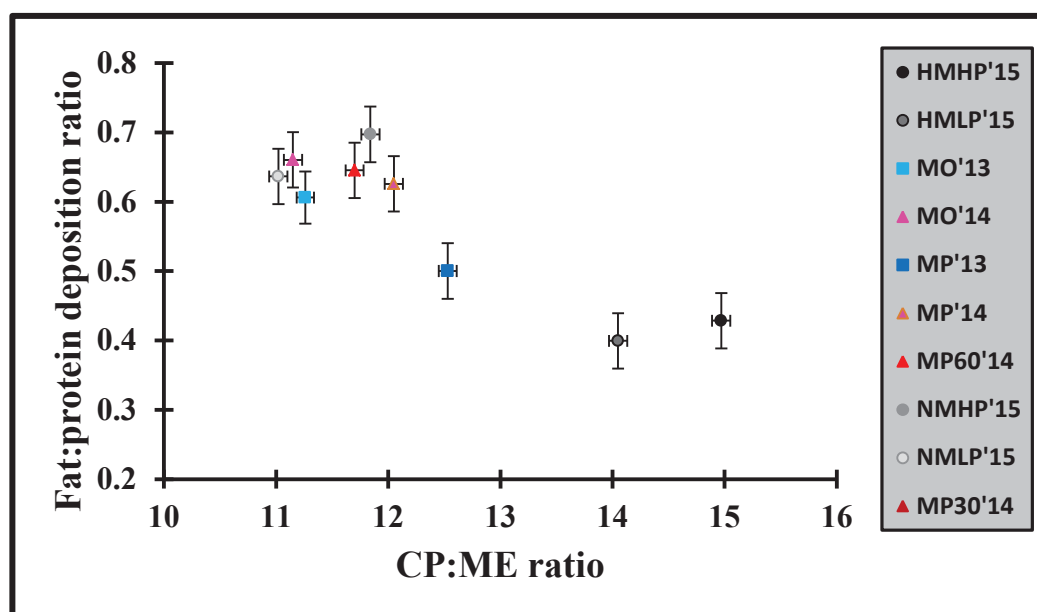


Figure 9.6 The relationship between CP:ME ratio and fat:protein deposition rate in lambs during the pre-weaning phase of growth. MO'13 = milk only; MPad'13 = pellets offered *ad libitum*, MO'14 = milk only; MP30'14 = milk + 30% of *ad libitum* pellets intake; MP60'14 = milk + 60% of *ad libitum* pellets intake; MPad'14 = pellets offered *ad libitum*, NMLP = normal-protein milk + low-protein pellet; NMHP = normal-protein milk + high-protein pellet; HMLP = high-protein milk + low-protein pellet; HMHP = high-protein milk + high-protein pellet ('number = year of study). Error bars = standard error of means.

### 9.2.3 Maintenance and growth energy requirements

One of the objectives of this thesis was to evaluate the maintenance ( $ME_m$ ) and growth ( $ME_g$ ) energy requirements of young lambs when consuming milk and pellets. Results from Chapters 4 and 5 showed  $ME_m$  of 0.40 and 0.45 MJ ME/kg  $LW^{0.75}$  were similar to previously reported values. However, modelling the body composition of lambs (Chapter 8) demonstrated that overestimating the  $ME_m$  (i.e., using the abovementioned

ME<sub>m</sub> of 0.45 MJ ME/kg LW<sup>0.75</sup>) of milk-only fed lambs underestimated their daily fat deposition rates (Chapter 8). While, underestimating the ME<sub>m</sub> of lambs offered milk replacer and *ad libitum* access to pellets (i.e., using the ME<sub>m</sub> of 0.34 MJ ME/kg LW<sup>0.75</sup> (Walker and Norton, 1971; Thomson *et al.*, 1979)) overestimated their fat deposition (Fig 9.7).

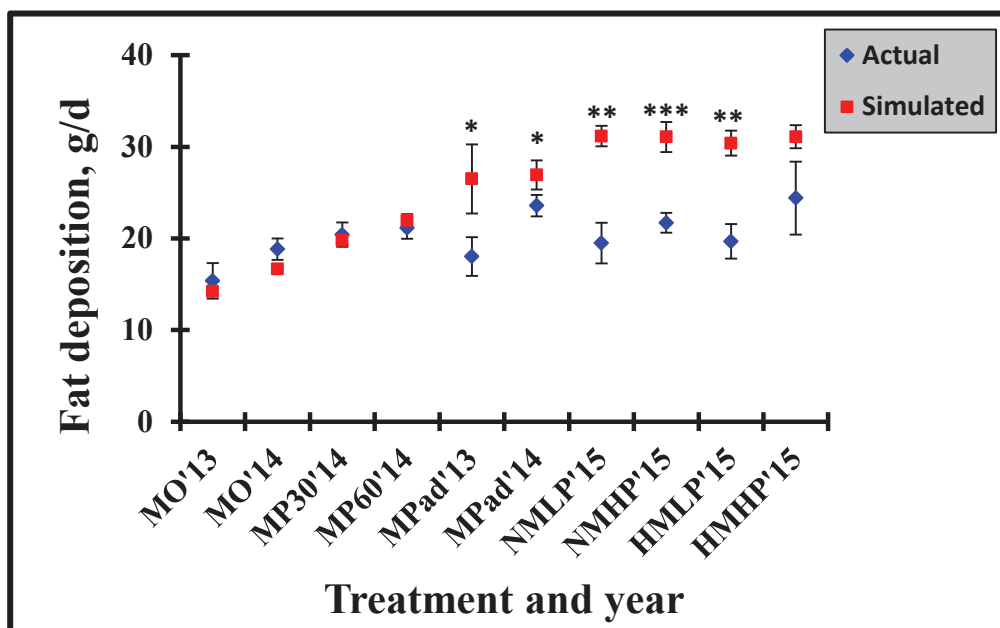


Figure 9.7 Comparison of the observed and predicted average daily gain of pre-weaned lambs consuming different milk and pellets using a maintenance energy requirement of 0.34 MJ ME/kg LW<sup>0.75</sup> across treatment groups MO'13 = milk only; MPad'13 = pellets offered *ad libitum*, MO'14 = milk only; MP30'14 = milk + 30% of *ad libitum* pellets intake; MP60'14 = milk + 60% of *ad libitum* pellets intake; MPad'14 = pellets offered *ad libitum*, NMLP = normal-protein milk + low-protein pellet; NMHP = normal-protein milk + high-protein pellet; HMLP = high-protein milk + low-protein pellet; HMHP = high-protein milk + high-protein pellet ('number = year of study). Error bars = standard error of means \*. \*\*, \*\*\*Simulated mean differs from actual mean at P < 0.05, P < 0.01, P < 0.001.

Daily protein, water and ash deposition rate were unaffected the changing ME<sub>m</sub> estimations (data not shown). A greater amount of energy is required to maintain

protein than fat (Emmans, 1994) and therefore at the same live weight, the  $ME_m$  of pre-weaned lambs with greater body fat content may decrease. These results confirmed that maintenance energy requirement of pre-weaned lambs is an important factor affecting body fat content in pre-weaned lambs. Therefore, inaccurate estimation of maintenance energy requirements would have implications for fat deposition rates

The  $ME_g$  values of 12.9 MJ/kg live weight gain (LWG) (Chapter 4) and 13.8 MJ/kg LWG (Chapter 5) reported in the present thesis were greater than the ARC (1980) recommendations (6.4 – 7.8 MJ/kg LWG). It is generally accepted that the ME requirement for LWG is higher in growing animals that gain more fat than those that gain more protein (Luo *et al.*, 2004; Nicol and Brookes, 2007). Pre-weaned lambs generally have a greater proportion of protein than fat in their empty bodies (Walker and Norton, 1971; Searle *et al.*, 1972). However, results in Chapter 5 showed that fat deposition in lambs increased by 8% while there was only a 4% increase in protein deposition in LWG. Although the total protein content in empty body gain of lambs was greater than the fat content, the greater percentage increase in fat may have resulted in the abovementioned higher  $ME_g$  values. These results imply that previously reported  $ME_g$  values may no longer be valid for modern genotypes of fast-growing lambs suggesting further research is necessary

### **9.3 Methodological Considerations**

The various experiments in this thesis focused on the use of milk only and milk and pellet combinations to examine lamb growth and composition. However, milk was fed as a proportion of the lambs' live weight and, therefore, there were no variations in the total milk intake of lambs which limited the estimation of maintenance and growth

energy requirements separately for the milk-only fed lambs. The design of the experimental Chapters (4, 5 and 6) limited the understanding of the separate effects of milk and solid feed on variation occurring in intakes, growth, digestion and nutrient retention. If these experiments were to be undertaken again, it is recommended that different controlled levels of milk and solid feed are included. These would enable the effects of each diet type as well as their interactions on measured lamb variables to be better understood.

It is important to have a feeding plan pre-weaning to aid rumen development and avoid growth checks at weaning time (Bimczok *et al.*, 2005; Todorov, 2012). The addition of roughage to the diet of lambs has been shown to increase the development of the reticulo-rumen tissue (Poe *et al.*, 1969). However, use of roughage in addition to pellets in the studies in this thesis would have made accurate measurement of intake more difficult. These studies had a clear defined LW end point of 18 kg. However, in a commercial scenario where lambs would be slaughtered at a much heavier weight and greater age, roughage would be advised. Therefore, future studies could consider examining the addition of roughage in the form of hay or cut grass to familiarise lambs to pasture intake.

#### **9.4 Practical implications and recommendations**

New Zealand lamb production is based on a seasonal pasture-based system to ensure ewes are lactating and lambs growing in spring when pasture growth and quality are high (Bensemann and Shadbolt, 2015). The results of this thesis indicated the possibility of supplementing the liquid diets of artificially reared lambs during the pre-weaning period with pelleted feed to improve their average daily gains. Improving growth rates

of lambs during the pre-weaning stage have been shown to subsequently improve post-weaning growth rates (Bhatt *et al.*, 2009; Galvani *et al.*, 2014) and thereby offering more options to farmers. It is also possible that pellets could be made available to lambs grazing with their dams and this can serve as a means of improving lamb growth rates especially for multiple-born lambs. The use of pellet as a supplement is used in creep feeding systems in parts of the world (Galvani *et al.*, 2014; Martínez *et al.*, 2015). However, the cost effect of this under New Zealand's pasture based system would need further evaluation.

### *Financial performance*

If farmers were to adopt the high protein to energy feeding strategy for artificial reared lambs (e.g. in the sheep milking industry), its benefit must be balanced against the financial effect. Therefore, the cost of feeding the different milk replacers and pellets per kg LWG and per kg hot carcass weight gain (HCG) were calculated based on the 2015 experiment only (Chapter 6).

### Cost of milk replacer and pellets

|                                                                             |                 |
|-----------------------------------------------------------------------------|-----------------|
| Normal-protein milk (NM)<br>(Milligans Feed Ltd, Oamaru, NZ)                | NZ\$ 4.95 / kg  |
| Milk protein concentrates (MPC)<br>(Fonterra, Palmerston North, NZ)         | NZ\$ 9.98 / kg  |
| High-protein milk (HM) @ 80% NM + 20% MPC                                   | NZ\$ 5.96 / kg. |
| Pellets (low and high protein)<br>(Massey University, Palmerston North, NZ) | NZ\$ 0.75 / kg  |

The unit cost of each feed was multiplied by the total milk and pellets consumed by lambs over the experimental period to obtain the total feed cost. The total feed cost was divided by the LWG or HCG of lambs to obtain their feed cost per kilogram gain. The total feed cost per kilogram LWG and feed cost per kg HCG were greater ( $P < 0.05$ ) for the HM fed lamb than NM milk replacer (i.e. a milk protein level effect) but was unaffected ( $P > 0.05$ ) by pellet protein intake level (Table 9.1).

Table 9.1 The cost of artificially rearing lambs fed two protein levels in milk replacer and two protein levels in pellets to 18 kg live weight

| Item (NZ\$)                   | Treatment <sup>1</sup> (Chapter 6). |       |        |       | SE <sup>2</sup> | P-value |        |                  |
|-------------------------------|-------------------------------------|-------|--------|-------|-----------------|---------|--------|------------------|
|                               | Milk                                |       | Pellet |       |                 | Milk    | Pellet | M×P <sup>3</sup> |
| n                             | NM                                  | HM    | LP     | HP    |                 |         |        |                  |
|                               | 14                                  | 14    | 14     | 14    |                 |         |        |                  |
| Milk cost / lamb              | 67.34                               | 72.01 | 71.91  | 67.45 | 1.83            | 0.08    | 0.10   | 0.69             |
| Pellet cost / lamb            | 3.76                                | 3.93  | 3.87   | 3.81  | 0.26            | 0.65    | 0.87   | 0.83             |
| Feed cost / lamb              | 71.10                               | 75.94 | 75.78  | 71.26 | 1.89            | 0.08    | 0.10   | 0.72             |
| Feed cost/kg LWG <sup>4</sup> | 5.30                                | 5.67  | 5.60   | 5.36  | 0.11            | 0.02    | 0.17   | 0.91             |
| Feed cost/kg HCG <sup>5</sup> | 10.12                               | 11.10 | 10.86  | 10.36 | 0.23            | 0.01    | 0.14   | 0.8              |

<sup>1</sup>Treatment: NM = Normal protein milk (24.0% CP); HM = High-protein milk (31.2% CP); LP = Low-protein pellet (13.6% CP); HP = High-protein pellet (19.5% CP)

<sup>2</sup>SE: standard error.

<sup>3</sup>M×P: milk×pellet interaction

<sup>4</sup>Live weight gain

<sup>5</sup>Hot carcass gain: calculated as the difference between the final hot carcass weight and the average hot carcass weight of the baseline group.

It was clear that the use of 20 % MPC to increase the CP:ME ratio of milk replacer was expensive and thus increased cost of raising lambs artificially. However, based on the results obtained in Chapters 5 and 6, it appeared the CP:ME intake of HM fed lambs was in excess of the lambs' requirements. Therefore, reducing proportion of MPC in the HM replacer from 20% to 13% will reduce the CP % content of HM replacer to 28.7% which will in turn reduce the total feed cost, feed cost /kg LWG and feed cost /kg

HCG per lamb by NZ\$ 4.75, NZ\$ 0.35 and NZ\$ 0.69 respectively. Considering the benefits of increased CP:ME ratio in milk replacer, such as greater daily gains and increased lean deposition, it will be worthwhile to find cheaper milk protein substitutes that can increase the protein content in milk replacers.

### **9.5 Recommendations for future research**

The experiments in this thesis focused on the pre-weaning lamb growth and composition. It would be of interest to know the effects of the different CP:ME ratios during the pre-weaning periods, if any, on post weaning growth rates, body composition of lamb carcass at slaughter and potential long term effects on replacement animals. This will help farmers decide if it is economic to feed lambs higher CP:ME diets early in life.

The results of this thesis indicate that both diet type and quality are important determining factors regulating liver and rumen development during the transitional period in pre-weaned lambs. However, it is unclear if these developmental processes affect digestive and absorptive capacities in the pre-weaned lamb. Further research is needed to elucidate the factors and mechanisms which control these important processes.

The greater proportion of lamb growth explained under controlled intake conditions and the model developed in this thesis highlighted the importance of finding cheaper methods to estimate lamb feed intake in pre-weaned lambs. The major drawback of the faecal and dietary index technique is the need for laboratory analyses to determine the chemical composition of faeces and diet samples. Consideration should be given to the spectroscopic techniques such as the near infrared and Fourier transform infrared

spectroscopies which have been used to predict diet quality and feed intake in pigs and older ruminants (Boval *et al.*, 2004; Fanchone *et al.*, 2007; Decruyenaere *et al.*, 2009; Schiborra *et al.*, 2015). These spectroscopic techniques are rapid, relatively simple, reproducible, require only small amounts of material with minimum sample preparation.

The developed model focused on the growth and chemical composition of the whole empty body of the lambs. However, simulating the growth and composition separately for carcass and non-carcass tissues needs to be considered in future models to allow the simulation of carcass growth and composition which is of economic and practical interest to farmers. Further, consideration should be given to simulating the protein to energy aspect of the model post-weaning. This thesis highlighted that the CP:ME requirements of lambs limits growth in early life but may be in excess in later life. Thus, it will be worthwhile to develop growth models from birth to slaughter or even further to maturity using different protein to energy ratios to help understand their implications on growth.

## **9.6 Overall summary and conclusions**

A series of studies has been undertaken to determine the effects of various combinations of milk replacer and pellets on the performance of pre-weaned lambs. Briefly, the studies have led to the following conclusions:

- Addition of solid feed to diet of pre-weaned lambs improves their growth rates, efficiency of gain and enhances rumen development. Additionally, this early rumen development improves the efficiency of ME utilisation for growth.
- Increasing daily energy metabolisable intake does not alter the total chemical body composition of the lambs fed to a fixed live weight.

- Increasing the crude protein content of milk replacer and therefore, the corresponding CP:ME ratio of intake increases the average daily gain and gain to feed ratio in pre-weaned lambs. Further, the protein content in their empty bodies increases whilst the fat content decreases.
- Feeding pre-weaned lambs in excess of their CP:ME requirements enables maximum potential protein deposition rates to be reached pre-weaning.
- The maintenance energy requirement of pre-weaned lambs is an important factor affecting body fat content. Therefore, under or over estimation of maintenance energy requirements will have implication on the prediction of fat deposition rates.

This study has contributed to the knowledge on rearing of lambs artificially with various combinations of milk and pellets. These are important findings which will provide a useful platform for future studies aiming to manipulate feeding strategies to improve lambs growth pre-weaning.

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## Appendix 1

DRC 16



**MASSEY UNIVERSITY**  
GRADUATE RESEARCH SCHOOL

**STATEMENT OF CONTRIBUTION  
TO DOCTORAL THESIS CONTAINING PUBLICATIONS**

(To appear at the end of each thesis chapter/section/appendix submitted as an article/paper or collected as an appendix at the end of the thesis)

We, the candidate and the candidate's Principal Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated below in the *Statement of Originality*.

**Name of Candidate:** Antoinette Simpah Danso

**Name/Title of Principal Supervisor:** Professor Patrick C.H. Morel

**Name of Published Research Output and full reference:**

Danso, A. S., Morel, P. C. H., Kenyon P. R., and Blair, H. T. 2016. Relationships between prenatal ewe traits, milk production, and preweaning performance of twin lambs. *Journal of Animal Science*, 94. Doi:10.2527/jas.2016-0337.

**In which Chapter is the Published Work:** Chapter 3

Please indicate either:

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and / or
- Describe the contribution that the candidate has made to the Published Work:

The candidate analysed all data pertaining to the published work with assistance from Professor Morel in developing an appropriate statistical model. The candidate wrote the first draft of the manuscript, with changes and corrections completed with input from supervisors (co-authors).

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**Name/Title of Principal Supervisor:** Professor Patrick C.H. Morel

**Name of Published Research Output and full reference:**

Danso, A. S., Morel, P. C. H., Kenyon, P. R., and Blair, H. T. 2014. BRIEF COMMUNICATION: Effect of early life diet on lamb growth and organ development. Proceedings of the New Zealand Society of Animal Production, 74: 205-208.

**In which Chapter is the Published Work:** Chapter 4

Please indicate either:

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- Describe the contribution that the candidate has made to the Published Work:

The candidate designed and conducted the experiment, analysed all the experimental data with assistance from Professor Morel in developing an appropriate statistical model. The candidate wrote the first draft of the manuscript, with changes and corrections completed with input from supervisors (co-authors).

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**STATEMENT OF CONTRIBUTION  
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**Name of Candidate:** Antoinette Simpah Danso

**Name/Title of Principal Supervisor:** Professor Patrick C. H. Morel

**Name of Published Research Output and full reference:**

Danso, A. S., Morel, P. C. H., Kenyon, P. R., and Blair, H. T. Effect of different feeding regimens on energy and protein utilization and partitioning for maintenance and growth in pre-weaned lambs reared artificially. *Journal of Animal Science*, 94.  
doi:10.2527/jas.2016-0871

**In which Chapter is the Published Work:** Chapter 5

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