Artificial rearing strategies to optimise new-born lamb growth and development

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Abstract

A series of artificial lamb rearing experiments was conducted to investigate the effects of milk replacer protein to energy ratio (CP:ME), pellet fibre level and age at weaning on lamb growth and body composition, rumen development and rumen bacterial population. A mechanistic, dynamic, pre-weaned lamb growth and body composition simulation model was also validated using data generated from these experiments.

The feeding of milk replacers with a high CP:ME ratio (15.89 g/MJ compared to a traditional industry value of 10.96 g/MJ) and adjustment of replacer CP:ME ratio (16.46 g/MJ to 10.96 g/MJ) to meet the lamb's changing nutritional requirement as it aged resulted in higher pre-weaning lamb growth rates. Feeding a high CP:ME milk replacer also reduced carcass fat levels. When fed pellets *ad libitum*, an incrementally adjusted CP:ME milk replacer resulted in similar pre-and post-weaning growth rates as when fed milk replacer with a consistently high CP:ME ratio (12.28 g/MJ).

Early milk weaning of lambs at 42 days of age did not impair growth rate to 57 days age but, reduced carcass fat, regardless of pellet fibre level. Early weaning of lambs also improved rumen n-butyric content and feeding low fibre pellets (NDF 116.76 g/kg) increased rumen n-valeric content. Early weaned lambs had increased rumen dorsal wall thickness.

Nutrient intake from solid feed positively influenced rumen volatile fatty acid content, while both nutrient intake and rumen volatile fatty acid content positively impacted rumen physical development. The relative abundance of rumen bacteria phyla and genera were altered by weaning age and pellet fibre level, with Firmicutes being more abundant in milk-fed lambs at 57 days of age, while Bacteroidetes were the prominent phylum in

early-weaned lambs. *Prevotella* was the prominent bacteria genus in early-weaned lambs. The most abundant bacteria genus in milk-fed lambs at 57 days of age was *Succinovibrio.*

The existing pre-weaned lamb growth model was found to predict overall lamb growth accurately when utilising data collected in the present studies. However, it was not as accurate for predicting body composition components. In summary, results from thesis will contribute to improving new-born lamb growth and development in artificial rearing systems.

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Abbreviations

ADF = Acid detergent fibre

ADG = Average daily gain

CCC = Concordance correlation coefficient

CMR = Commercial milk replacer

CP = Crude protein

CPI = Crude protein intake

CP_{g} = Crude protein requirements for growth

CP_{m} = Crude protein requirements for maintenance

DM = Dry matter

DMI = Dry matter intake

EBW = Empty body weight

GIT = Gastrointestinal tract

HFP42 = Commercial milk replacer, high fibre concentrate pellets and early weaning at 42 days of age

HFP57 = Commercial milk replacer, high fibre concentrate pellets and milk feeding to 57 days of age

HPLF42’19 = High protein milk replacer for two weeks followed by commercial milk replacer, low fibre pellet and early weaning, 2019 experiment

HPM = High protein milk replacer

HPMNP'17 = High protein milk and normal pellets, 2017 experiment

LFP42 = High protein milk replacer to 16 days followed by commercial milk replacer, low fibre concentrate pellets and early weaning at 42 days of age

LSD = Least significant difference

LW = Live weight

MB = Milk blend

MBNP'17 = Milk blend and normal pellets, 2017 experiment

MBNP'18 = Milk blend and normal pellets, 2018 experiment

ME = Metabolisable energy

MEI = Metabolisable energy intake

$ME_{g\,=}$ Metabolisable energy requirements for growth

ME_m = Metabolisable energy requirement for maintenance

MLT = Thickness of muscle layer

MP_m = Metabolisable protein requirements for maintenance

NDF = Neutral detergent fibre

NDFI = Neutral detergent fibre intake

NMHF42'19 = Commercial milk replacer, high fibre pellet and early weaning, 2019 experiment

NMHF57’19 = Commercial milk replacer and high fibre pellet feeding to 57 days of age, 2019 experiment

NMNP’17 = Commercial milk replacer and normal pellets, 2017 experiment

NMNP’18 = Commercial milk replacer and normal pellets, 2018 experiment

$PC_{(md)}$ = Principal components related to rumen metabolic development

$PC_{(pd)}$ Principal components related to rumen physical development

PSL:STL = Ratio between papillae outer boundary of the stratum corneum and respective straight length of rumen tissue

R^2 = Coefficient of determination

RPE = Relative predictive error

SD = Standard deviation

SE = Standard error

VFA = Volatile fatty acid

Chapter 1 General Introduction

General Introduction

The dairy sheep industry is expanding in New Zealand, with a projected goal of earning over $200 million in exported sheep dairy products by 2030 (McMillan et al., 2014b). McKusick et al. (1999) reported that the suckling of lambs in the first month of lactation reduced the harvestable milk yield by 35 - 40 % to 170 - 180 days of lactation in the USA sheep milking industry. In New Zealand, losses of harvestable milk yield of ewes were 20 to 25 % and 45 to 50 %, respectively for the first 30 or 60 days due to lamb suckling (McMillan et al., 2014a). Therefore, to achieve the goal of high milk production, and to reduce the loss of harvestable milk, cost-effective, artificial lamb rearing or early milk weaning systems are required and need to be evaluated.

Lamb production is also a major economic contributor to the New Zealand red meat market. Higher lambing percentages, approximately 130 % currently (Beef + Lamb New Zealand, 2020c), mean an increase in the number of multiple-born lambs which have slower growth and lower survival to weaning. Improving artificial lamb rearing systems or early weaning strategies would benefit both the dairy sheep industry, by reducing milk consumed by lambs, and the lamb meat industry, potentially allowing rearing of orphaned/mis-mothered and small multiple born lambs. However, artificial lamb rearing has been given little attention in New Zealand until the recent growth of the dairy sheep industry and little practical information is available (Nieper, 2017).

Faster lamb growth and higher survival pre-weaning contribute significantly to farm profitability. Greater lamb growth rate reduces the time required to reach the target live weight or allows the production of heavier lambs (Beef + Lamb New Zealand, 2014, Muir et al., 2003). A series of artificial lamb rearing experiments conducted in New Zealand sought to optimise the rearing regimen, thereby achieving greater lamb growth rates (Jensen et al., 2017, Nieper, 2017, Danso et al., 2014 , Danso et al., 2016, Danso et al.,

2018). Feeding of milk replacer and pellets increased the growth performance of artificially reared lambs compared to lambs fed only milk replacer. Further, lambs were shown to require a higher protein to energy (CP:ME) ratio at lower weights (13.1 g/MJ at 5 kg live weight), but requirement decreased as lambs grew (10.9 g/MJ at 18 kg live weight (Danso et al., 2016)). However, this CP:ME requirement of young lambs was not being met as low CP:ME ratios of approximately 10-11 g/MJ were reported for ewe's milk and typically available commercial milk replacers (van der Linden, 2010, Paten et al., 2013, Paten et al., 2017). While at older ages, lambs were being provided with a diet that contained a high CP:ME ratio of approximately 15.3 -16.2 g/MJ for ryegrass and white clover based diet or grain-based pellets (Danso et al., 2016, Golding et al., 2011, Somasiri et al., 2016). Hence, Danso et al. (2016) reported that in most of the current feeding and rearing systems, the lamb growth rate was limited due to a mismatch between the lamb's CP:ME requirement and the CP:ME intake. Hence, balancing the CP:ME ratio of milk replacer and/or pellets to meet the lambs changing requirement would improve lamb growth pre-weaning. The subsequent impact of adjusting the pre-weaning diet CP:ME ratio on post-weaning lamb growth is unknown and warrants further study.

While early milk weaning of young ruminants reduces the cost of milk feeding (Jones and Heinrichs, 2007), it is important that early weaning is accompanied by a smooth transition to solid feed with a focus on early rumen development to minimise post-weaning lamb growth check. There is limited information available on rearing regimens to stimulate rumen physical development and fermentative capacity in artificially reared lambs.

In-silico simulation of lamb growth and body composition would enable farmers to assess and adapt appropriate lamb feeding regimens that suit individual farming conditions

without the need for *in-vivo* experimentation. Dynamic and mechanistic models exist which simulate the growth, milk and/or feed intake and body composition of lambs from birth to a stipulated endpoint, i.e. post-weaning, slaughter or maturity (Sainz and Wolff, 1990, Finlayson et al., 1995, Johnson et al., 2012, Graham et al., 1976, Osorio et al., 2015). However, there appears to be only one stimulation model for pre-weaned, artificially reared lamb which predicts both growth and body composition (Anim-Jnr et al., 2020). This model has not been yet validated with an independent dataset.

This thesis aimed to investigate alternative artificial rearing strategies to optimise new-born lamb growth and development. Firstly, a literature review on artificial lamb rearing, pre-and post-weaning lamb nutritional requirements and rumen development during early life was carried out to identify knowledge gaps (Chapter 2). Based on the identified knowledge gaps on the optimum protein to energy ratio of milk replacer to improve the pre-weaning lamb growth, Chapter 3 was designed to investigate the effect of milk replacer protein to energy ratio (CP:ME) on growth and body composition of pre-weaned lambs reared artificially to 22 kg live weight. Chapter 4 examined the effect of dietary CP:ME ratio on the growth performance of pre-and post-weaned lambs to 22 kg live weight. In Chapter 5, the influence of pellet fibre level, in addition to milk replacer composition, and age at weaning (42 vs 57 days of age) on growth and body composition of lambs reared artificially was evaluated. Chapter 6 investigated the impact of varying pellet fibre levels and milk replacer composition on rumen development of artificially-reared, early-weaned lambs. The effect of pellet fibre levels and age at weaning on the composition of established rumen bacterial communities of artificially-reared lambs was investigated in Chapter 7. Using the data generated in the aforementioned artificial lamb rearing experiments, the mechanistic, dynamic pre-weaned lamb growth and body

composition simulation model of Anim-Jnr et al. (2020) was validated in Chapter 8. Finally, a summary of the findings of this thesis, limitations, implications and areas of future research were discussed in Chapter 9.

Chapter 2 Literature Review

2.1 New Zealand sheep industry

The New Zealand sheep industry is a well-established sector with a current population of approximately 27.4 million sheep (Beef + Lamb New Zealand, 2020b). Extensive sheep farming is observed mainly in hill or high country areas whilst intensive sheep farms are typically in the highly productive lowlands (Morris, 2013). Romney, a dual-purpose breed, is the prominent sheep breed farmed in New Zealand, and it contributes approximately 50% of the sheep population (Beef + Lamb New Zealand, 2020b).

Sheep numbers have declined from 1990/91 to 2018/19 by 53% while lamb production reduced only by 9% (Beef + Lamb New Zealand, 2020b) (Figure 2.1). Lamb production was maintained at an approximately similar level via an increase in lambing percentage, lamb growth rate and carcass weight (Morris, 2013). Lambing percentages are approximately 92% in extensive sheep farming operations in the high country and approximately 130% in intensive farming systems, based on the number of lambs tailed per 100 ewes mated (Morris and Kenyon, 2014).

The dairy sheep flocks in New Zealand vary from small flocks to large scale farms with over 20,000 ewes (Peterson and Prichard, 2015). The number of milking ewes in New Zealand is approximately 25,000 and the largest dairy sheep flock, Blue River Dairies has 15,000 milking ewes (Hutching, 2016). There are five main dairy sheep producers in New Zealand: Blue River Dairies, Kingsmeade, Waituhi Kuratau Maori trust, Andy and Kat Gunson and Neudorf Dairy. These producers primarily contribute to sheep cheese production (Peterson and Prichard, 2015), but some are involved in yoghurt production (Peterson and Prichard, 2015). Blue River Dairies produce human infant milk formula based on sheep milk, targeting the export market, mainly China (Peterson and Prichard, 2015, Nicoll, 2018). The growing global demand for sheep dairy products has led New

Zealand to set a goal of earning over $200 million through the export of sheep dairy products by 2030 (McMillan et al., 2014b).

Figure 2.1 Sheep numbers and lamb production in New Zealand (Beef + Lamb New Zealand, 2020b)

The suckling of lambs in the first month of lactation reduces harvestable milk yield by 35 - 40% during a 170 -180 day ewe lactation (McKusick et al., 1999). Hence, producers focus on low cost early weaning methods for lambs (McMillan et al., 2014a) or rearing of lambs artificially (Caroprese et al., 2016).

McKusick et al. (2001) reported that the commercial milk yield of ewes was higher when lambs were artificially lamb reared (remove from their dams 24 h after birth) compared to a commercial milk production system which allows lambs to suckle for 9 h/d and machine milked ewes once a day after lambs had been separated for a 15 h period. However, in the system which machine milked ewes once a day, but allowed lambs 9 h/d of suckling and weaned lambs at 28 days had the greatest financial return from milk and

lamb sold (at 4 months) compared to both artificial and conventional rearing systems (McKusick et al., 2001). New Zealand dairy lamb rearing system typically removes lambs from ewes at 2 days of age (Peterson and Prichard, 2015, Nieper, 2017). Therefore, the purpose of lamb rearing (be it for raising lambs to slaughter or as dairy replacement stock) and the type of rearing regimen used are major factors to be considered by farmers to potentially maximize their profit.

Ram lambs from the dairy industry and female lambs that are not used as replacement or expansion of the dairy sheep herd can be valued as input for meat production, similar to New Zealand dairy-beef industry. New Zealand exports lamb worldwide and lamb exports are composed of 2% carcasses and 98 % cuts (Beef + Lamb New Zealand, 2020a). The major market for lamb is North Asia which is 50% of New Zealand total lamb exports. China is the largest single country market for New Zealand lamb exports (Beef + Lamb New Zealand Economic Service, 2020).

The increasing development of the sheep dairy industry (Peterson and Prichard, 2015) will contribute to the economy through dairy production and indirectly through meat production. These facts emphasise the importance of investigating strategies for cost-effective and sustainable artificial rearing strategies for lambs.

2.1.1 Importance of heavy young ruminants at weaning

Meat producers tend to slaughter young animals and manage lambs to target higher weight gain through intensive feeding (Urbano et al., 2017). Lamb muscle and bone growth and their overall growth are high compared to mature sheep (Reis et al., 2001). The ovine species is characterized by efficient weight gain and high carcass quality in the first six months of life (Reis et al., 2001). Hence, proper nutrition during this period is essential.

Weaning weight is a key factor that determines the time required to grow lambs to market weight (Urbano et al., 2017). A faster growth rate of lambs from birth to weaning results in heavier weights or they can be slaughtered at a younger age (Beef + Lamb New Zealand, 2014, Muir et al., 2003). Greater growth rates are associated with improved feed efficiency (Rattray, 1981). Hence, it increases the pasture availability for other sheep stock classes managed together in a conventional farming system (Beef + Lamb New Zealand, 2014). The ewe lambs will reach their target breeding weight earlier if they are growing faster and that will ensure replacement within the ewe flock (Beef + Lamb New Zealand, 2014).

Feeding young animals to meet their nutrient requirements is vital to maximizing growth. Most of the artificial lamb rearing systems in New Zealand use milk replacers and solid feed meals to meet feed demands (Jensen et al., 2017). However, milk replacer feeding is expensive, less viable for meat production systems and there is a lack of specialized products for lambs (Urbano et al., 2017).

The improvement of lamb growth rate during the pre-weaning period is one of the target goals of the New Zealand sheep industry to increase the total weight of lambs weaned per ewe bred (Morris and Kenyon, 2014). Considering the benefits of heavy young lambs at weaning and the expected targets of industry, more research focused on improving the pre-weaning growth performances of lambs is needed.

2.2 Artificial rearing

Artificial rearing is the separation of lambs from ewes and hand-rearing them. Artificial rearing should ensure efficient and healthy growth of lambs through the use of milk replacers and solid feed and it needs to be economically viable for commercial-scale (Owen and Davies, 1970). The rearing of 3-4 lambs by a nurse ewe is associated with

lower lamb growth and survival (Notter et al., 2018). An increase in the number of lambs born per ewe has encouraged artificial rearing of lambs (Umberger, 2009, David et al., 2014) to reduce lamb mortality (Umberger, 2009). This ensures a high number of these multiple born lambs reach the market as the prolificacy of ewes increases (Owen and Davies, 1970). By increasing lamb survival, artificial lamb rearing can have a positive effect on the reproductive efficiency of ewes (Napolitano et al., 2008). Additionally, artificial rearing can be used for situations where ewes reject their offspring (Urbano et al., 2017). It is also reported that a higher profit can be obtained from highly productive dairy sheep breeds during the first month of lactation lambs are reared on commercial milk replacers increasing harvestable ewe milk yields (Margetín et al., 2014).

The harvesting of ewe milk for processing in the dairy sheep industry requires the early separation of lambs and artificial rearing (Caroprese et al., 2016), similar to the dairy cow industry. However, colostrum feeding during the first 18-24 hours of life is essential to ensure appropriate nutrition and immunity status of lambs (Umberger, 2009). Hence, colostrum feeding from the dam should be practised within the first 18 to 24 hours to ensure healthy lambs for artificial rearing (Umberger, 2009). If there is a shortage of ewe colostrum or rejection of lambs by the ewe, cow colostrum or colostrum replacers can be fed for lambs in artificial rearing systems (Umberger, 2009).

In the natural rearing of lambs, the frequency of suckling is high (7-8 suckling events during a six-hour) during the first two weeks post-partum (Fletcher, 1971). It then decreases abruptly during the second and third weeks and thereafter declines more slowly before becoming relatively uniform through to the twelfth week of lactation (Fletcher, 1971). Therefore, it would be expected that artificial rearing may need to align with natural rearing behaviours for efficient lamb live weight gain, however, feeding

frequency in artificial rearing systems has been reported to have no effect on live weight gain or protein deposition in lambs (Walker et al., 1966). A recent study showed that the use of a colostrum alternative, followed by milk replacer in an artificial lamb rearing system is a successful as natural rearing (Belanche et al., 2018).

2.2.1 Artificial rearing systems of lambs

Artificial rearing of lambs on a large scale was not common in New Zealand until the recent growth of the dairy sheep industry (Nieper, 2017). Generally, dairy sheep production systems focus on artificially rearing lambs on milk replacers (Emsen et al., 2004). However, little practical validated information is available on the artificial rearing of lambs in New Zealand (Nieper, 2017). It has been reported that artificial lamb rearing can be performed with milk replacers (birth to three weeks of age, indoor) and solid feed meal, with lambs gradually transitioned to pasture with continued access to milk replacers and meal (4-5 weeks of age), then milk weaning at 6-8 weeks of age and weaning off meal (10-12 weeks of age) to a complete pasture diet (Jensen et al., 2017).

The milk replacers for lambs can be produced from cow's colostrum, cow's milk, goat milk or sheep milk (Emsen et al., 2004, Kintzel, 2014, Thompson et al., 1993). However, the calf milk replacer usage for lamb rearing is limited due to lower fat content (19 %) and higher lactose content (40 %) (Berger and Schalapper, 1993). Further, the excess lactose content of cow's milk can result in severe scouring and may lead to lamb deaths (Umberger, 2009).

The nutritional composition of high-quality lamb milk replacer generally consists of 22-24 % crude protein, 25-35 % crude fat, 0.5-1 % crude fibre, 22-25 % lactose, 5-8 % ash and 20000, 5000 IU per pound of vitamin A, and D, respectively and 50-100 IU per pound of vitamin E (Umberger, 2009). These nutrient composition values closely resemble the

composition of ewe's milk (Paten et al., 2013, van der Linden, 2010). The use of sheep milk replacer is limited by its relatively high cost (Umberger, 2009). Thus, Urbano et al. (2017) reported that the production of milk replacers using low-cost ingredients while still matching the biological values of ewe's milk is a challenge (Urbano et al., 2017).

The mortality of lambs in artificial rearing systems is another limitation and with death rates of lambs of 10 to 30 % reported (Bull and Binnie, 2006). One of the reasons for lamb deaths is abomasal bloat, which occurs in hand-reared lambs at 2-4 weeks of age in once a day or *ad libitum* warm milk feeding systems (Bull and Binnie, 2006). There are a number of reported issues associated with milk replacer feeding, such as digestive disorders, abomasal bloat and poor digestibility (Owen and Davies, 1970). Additionally, the increase in labour requirement due to more frequent feedings, high lamb mortality and the high cost of milk replacers are factors that limit the practice of artificial rearing of lambs (Umberger, 2009).

Different feeding procedures are used in artificial rearing systems depending on the number of lambs to be reared, the farmer preference and the farming situation. However, appropriate sanitation is necessary regardless of the system (Martin et al., 2010). Milk replacers can be fed by bottle feeding, milk/teat bars, multi-nipped container, bucket feeding, or automated feeders and each system has its benefit and limitations (Table 2.1).

Rumen development needs to have adequately occurred before weaning of artificially reared lambs (Belanche et al., 2018) and the introduction of solid feed at an early stage in artificial rearing is encouraged to improve rumen development (Baldwin et al., 2004a). This helps to prepare lambs for post-weaning diets and increase their post-weaning body weight gain (Bimczok et al., 2005). A starter solid feed with high palatability and 18-20 % crude protein in the dry matter can be used for young lambs (Umberger, 2009). The

appropriate age of solid feed or creep feed introduction differ in many studies (i.e. 2 days (Danso et al., 2014)), 7 days (Liu et al., 2016), 7-10 days (Sun et al., 2018). However, early starter feeding improves rumen development in lambs compared to later provision (Liu et al., 2016). The early weaning of lambs to a solid feed is important to reduce the cost of milk replacer (Umberger, 2009).

Table 2.1 Advantages and limitations of milk feeding systems

Feeding system	Advantages	Limitations	Citation
Bottle feeding	Easy to monitor milk intake Inexpensive Reduce the risk of disease spread Easy to clean	Time-consuming Require regular feeding patterns Risk of digestive problems	Nakielny (2013), Martin et al. (2010)
Milk/teat bars	Easy to manage Less time consumption Home-made	Wastage of excess milk	Nakielny (2013), Heaney and Leger (1990), Martin et al. (2010)
Multi-nipped container	Can be used for a small group of lambs	Difficulty to train the lambs Wastage of milk	Heaney and Leger (1990)
Automated feeders	Reduce labour requirement Constant milk supply Can use on the commercial scale	Capital outlay is high	Nakielny (2013), Martin et al. (2010)

2.2.2 Impact of artificial rearing

2.2.2.1 Growth

Many studies have reported that artificially reared lambs result in the same live weight at weaning as natural rearing, although others have reported poor growth rate and body weight compared to those reared naturally (Table 2.2). Higher carcass yields in artificially reared lambs compared to ewe reared lambs have been reported (Belanche et al., 2018, Napolitano et al., 2002). Further, restricted ewe milk feeding in pre-weaning has been shown to have little impact on slaughter weight, empty body weight, hot and cold carcass weights (Table 2.2).

The higher pre-weaning lamb growth rate seen in artificial rearing systems was achieved by altering the composition of the milk replacer (Table 2.2). The fatty acid profile of lamb carcasses can also be altered by manipulating the fatty acid composition of milk replacers. A study has shown that the polyunsaturated/saturated fatty acid ratio in the lamb carcass is improved, towards the requirements of a human diet, in artificially reared lambs fed milk replacer containing less saturated fatty acids and more mono-and poly-unsaturated fatty acids compared to ewe's milk (Napolitano et al., 2002).

2.2.2.2 Welfare

Lambs are unable to distinguish their mother immediately after birth (Napolitano et al., 2008), with the bond between lamb and ewe developing within 18 hours after birth (Nowak et al., 1989). The development of this bond is feeding mediated (Napolitano et al., 2008). In artificial rearing, lambs are separated from their mothers suddenly and hand-reared, thus, lambs may experience emotional stress (Napolitano et al., 2008). It has been found that gradual separation of lambs from ewes has beneficial effects compared to a sudden separation (Sevi et al., 2003) but both methods are reported to reduce lamb weight and cause behavioural, endocrine and immune disturbances (Sevi et al., 2003).

Lamb adaptation to the artificial teat is more rapid during the first three days after birth (Sevi et al., 1999). Feeding of a 50:50 mixture of ewes' milk and commercial milk replacer increases lamb milk intake compared to milk replacer only feeding during the first three days post-separation (Sevi et al., 1999). Gradual separation of lambs improves milk intake and growth, though these were still lower compared to ewe-reared lambs (Sevi et al., 1999). Artificially reared lambs show increased social interactions with their pen mates than lambs reared on their dams (Napolitanoa et al., 2003) and they may gain benefit from pen mates through establishing new social relationships. Gentle handling by

their human carers will lessen the stress experienced by lambs due to maternal separation (Caroprese et al., 2016). In calf rearing, this has been shown to make them easier to handle and less susceptible to stress associated with subsequent human involved management practices (Lensink et al., 2000).

Table 2.2 Growth performances of lambs reared artificially compared to those reared by their dams

Diet	Rearing period*	Performances of lambs to weaning*	Citation
Ewe colostrum and ewe milk vs ewe colostrum and milk replacer vs 50g alternative colostrum supplementation and milk replacer	45 d	Lambs in all treatments reached a similar weaning weight Higher growth rate, heavier final weight and lower dressing percentage in ewe reared lambs compared to artificially reared lambs	Belanche et al. (2018)
Ewe milk vs calf milk replacers + lamb starter	2 d - 6 w	Higher body weight at 2 and 4 weeks in ewe milk-fed lambs At 6 weeks body weight is not different for both groups Higher lamb survival in artificially reared lambs then ewe reared	Emsen et al. (2004)
Ewe milk vs milk replacer	2- 40 d	Higher growth rate and carcass weight in ewe milk-fed lambs	Lanza et al. (2006)
Ewe milk vs commercial milk replacer vs whole powdered cow milk	2 to 28-33 d	Higher body weight at 3-5 d in ewe reared lambs. Lambs in all treatments reached a similar weaning weight	Hernández-Castellano et al. (2015a)
Ewe's milk vs milk replacer	5 w	The same daily weight gain Final live weight and carcass weights were not significantly affected Higher carcass yield in artificially reared lambs Improved polyunsaturated/saturated fatty acid ratio in carcass toward the required in the human diet (0.45) by milk replacer feeding	Napolitano et al. (2002)
Ewe's milk vs milk replacer vs a mix of milk replacer and ewe's milk (50:50)	5 w	Ewe reared lambs gained more than artificially reared lambs Milk mix fed lambs gained more than milk replacers fed lambs	Sevi et al. (1999)
Ewe's milk vs milk replacer	7 w	Higher weight gain ewe milk and milk replacer fed lambs from 0 to 14 d and from 15 to 30 d, respectively Feeding does not affect gain in the overall period Higher warm and cold carcass yields in ewe reared lambs Higher dressing percentage was in ewe reared, gently handled lambs	Napolitano et al. (2006)
Ewe milk for the whole period vs ewe milk till 15 d, rest concentrate and Lucerne hay *ad libitum* vs ewe milk till 15 d, concentrate and Lucerne hay *ad libitum* till 30 d, and rest concentrate and Lucerne hay *ad libitum*	9 w	Restricted ewe milk feeding was not affected on slaughter weight, empty body weight, hot and cold carcass weights The same growth rate for all lambs until 15 d Higher growth of lambs fed ewe's milk only from 16th to 30th From 30-63 d milk till 15 d, rest concentrate and Lucerne hay *ad libitum* fed lambs had higher growth	Maiorano et al. (2009)
Ewe milk vs milk replacer vs cow-milk vs buffalo-milk at 10% of body weight, starter ration for all at 5th week	1w-12w	Average daily gain and total weight gain are similar for ewe milk-fed and buffalo-milk fed lambs and it is higher than those offered cow-milk or milk replacer	Anjum et al. (2014)
Ewe milk vs gelatinized milk replacer (MRg-Linseed) vs non-gelatinized milk replacer (MRng-Ca-soap)	2 w - 3 m	Higher average daily gain and feed conversion ratio in MRg-Linseed and MRng-Ca-soap fed lambs than ewe milk-fed lambs	Bhatt et al. (2018)
Ewe milk or milk replacers	12 w	High daily weight gain and better morphometric indices showed in ewe reared lambs until weaning	Ward et al. (2017)

*, d = days and w = weeks

2.3 Rumen development

2.3.1 In the neonatal ruminant

The stomach of ruminants contains four compartments: the rumen, reticulum, omasum and abomasum. The rumen of new-born ruminants is incompletely developed both anatomically and physiologically (Warner et al., 1956) and disproportionate compared to adult ruminants (Chiba, 2014).

The neonatal rumen accounts for 30 % of the stomach mass (Church, 1975) with an increase in capacity to 70 % of the stomach mass during the weaning process (Warner et al., 1956). At maturity, the proportion of the entire stomach system accounted for by the rumen, reticulum, omasum and abomasum of small ruminants is approximately 75 %, 8 %, 4 % and 13 %, respectively (Membrive, 2016). The complex stomach of mature ruminant animals contributes 55 % of the digestive tract (Kumar and Pitta, 2015).

The development of the pre-stomach in a young ruminant goes through three phases; the non-ruminant phase (birth to 3 weeks), transition phase (3-8 weeks) and ruminant phase (post 8 weeks) (Membrive, 2016). The transition from non-ruminant to ruminant state occurs with the shift from milk to solid feed intake, leading to the establishment of anaerobic microbes and fermentation, differentiation and growth of papillae, development of absorption and metabolic pathways, maturation of salivary apparatus and development of rumination behaviour (Khan et al., 2016).

2.3.1.1 Anatomy

The rumen occupies the left half of the abdominal cavity in the adult ruminant. It is the largest of the four compartments and serves as a storage site, fermentation vat and houses a diverse microbial population in mature ruminants (NRC, 2007). Milk bypasses the rumen during the pre-mature stage of young ruminants due to the presence of the oesophagal groove (Gupta et

al., 2016). The oesophageal groove is between the cardia and reticulo-omasal orifice in young ruminants and consists of two sections, a reticular portion and a short omasal portion (NRC, 2007). The lips of the groove close, diverting digesta to the abomasum (NRC 2007). Cunningham and Klein (2008) reported that approximately 90 % of milk reaching the cardia flowed into the omasum while 10 % reach the rumen. Therefore, the non-functional and undeveloped rumen does not play a significant role in digestion (Govil et al., 2017). This temporary tube responds to suckling or swallowing of milk by young ruminants (Ørskov et al., 1970). After two months of age, the groove starts working less efficiently in calves (Membrive, 2016), but there is limited information in lambs.

The rumen starts to grow at two to three weeks of age and will continue until six months of age in calves (Govil et al., 2017). Rumen physical development involves an increase in mass, volume and the growth of finger-like projections on the inner epithelial layer called papillae (Baldwin, 2000, Baldwin and Connor, 2017). The neonatal undeveloped rumen remains if dietary requirements for rumen development are not provided (Govil et al., 2017). Solid feed intake which begins at approximately three weeks of age triggers the transition from the non-ruminant state to a true ruminant (Dias et al., 2017). Delaying weaning can result in inadequate rumen development during the early life of ruminants (Lawrence and Pierce, 1983), which may ultimately lead to economical losses, due to reduced post-weaning growth and poor digestive efficiency.

The reticular-rumen of the young ruminant is smaller, with thinner, slightly transparent walls (Warner et al., 1956), has lower volume capacity and lacks the presence and colouration of the papillae (Church, 1975). The rumen wall is comprised of two layers, an epithelial and a muscular layer. The exterior muscular layer supports the interior epithelial layer (Gupta et al., 2016). Papillae create a large surface area over which to absorb digestion end-products from

rumen fermentation (Gupta et al., 2016). Rumen epithelial cells in neonatal animals are not as keratinized as these in mature animals (Gilliland et al., 1962).

2.3.1.2 Rumen Physiology

The establishment of conditions suitable for rumen micro-flora, availability of substrates, a water-based environment, the outflow of materials from the rumen by muscular activity and absorption of nutrients by the rumen epithelium, are all required the development of the rumen functionality (Govil et al., 2017). Dramatic physical and metabolic changes of ruminal epithelium need to occur prior to weaning in lambs (Baldwin and Jesse, 1992). The papillae are non-functional at birth, hence, there is little or no absorption or metabolism of VFA in the rumen (Govil et al., 2017) in early life. Young ruminants must develop sufficient rumen capacity and functionality in order to thrive post-weaning.

Ruminal epithelial metabolic adaptations are associated with the physical development of tissues and shifts in the fate of glucose and butyrate carbon (Baldwin and Jesse, 1992). The oxidation of glucose and butyrate in the rumen of neonates occurs at varying levels (Baldwin and Jesse, 1992). The oxidation rate of glucose is low at birth by ruminal cells and increases gradually by two weeks of age and continues until six weeks of age. Then the rate is reduced to below the level observed at birth post six weeks of age. Butyrate oxidation increases gradually from birth, peaks at four days old, and declines during the pre-weaning period. Oxygen consumption by the rumen epithelium of a pre-mature ruminant is higher than an adult due to the use of glucose as the primary substrate for oxidation (Annison et al., 1957). Hence, glucose becomes the preferred substrate for neonatal rumen epithelial cells (Giesecke et al., 1979), where it acts as an energy substrate or produces reducing equivalents for biosynthetic reactions (Baldwin and Jesse, 1992). The rumens' ability to oxidise glucose reduces immediately before weaning and thereafter VFAs are used as the primary oxidative substrate

for energy (Baldwin and Jesse, 1992). Solid feed consumption is required for rumen metabolic maturation which is indicated by a change of oxidative substrate from glucose to butyrate (Lane et al., 2000).

Intake of solid feed stimulates microbial colonization and the production of VFA. Calves fed good quality grass are able to digest 75 % of the dry matter and 84 % of the cellulose at one month of age (Membrive, 2016). The concentrations of VFA in the rumen were elevated in lambs fed solid feed compared to milk replacer at 12 weeks of age (Lane et al., 2000). Although VFA's play an important role in rumen development (Baldwin and McLeod, 2000), the stimulatory effect of different VFAs are not all equal (Heinrichs and Lesmeister, 2005). For example, the stimulatory effect of butyrate on papillae growth is greater than that of propionate (Heinrichs and Lesmeister, 2005), followed by acetate. The greater the VFA concentration and the earlier or longer the period of exposure to VFA, the greater the rumen metabolic development (Lane et al., 2000).

The neonatal ruminant's rumen has minimal ketogenic capacity compared to the mature animals (Giesecke et al., 1979). Rumen ketogenesis, as indicated by β-hydroxybutyrate (βHBA) production has been found to occur independently of solid feed intake and VFA concentration (Lane et al., 2000), and should begin to increase between four to ten weeks of age (Giesecke et al., 1979). However, βHBA production is not evident in lambs fed only milk replacer at six weeks of age (Lane et al., 2000). The βHBA production, by rumen epithelial cells, was increased ten-fold at weaning (56 days of age) compared to βHBA levels from birth to 42 days of age (Baldwin and Jesse, 1992).

Little reticulo-rumen muscular activity is observed at the birth in ruminants, and regurgitation and rumination are absent (Julien et al., 2015). Quigley (1998) reported that rumen contractions in calves occur at three weeks of age and cud-chewing at seven days of age. Earlier

supplementation of dry feed promotes rumen activities (Julien et al., 2015). Rumen motility and muscularization in calves can be enhanced by forage bulk and large particle sizes (Khan et al., 2016).

Rumination is an inherent behaviour of ruminants. Irregular chewing movements are observed in milk only fed lambs at three to five days of age, even in the absence of feed in the mouth (Membrive, 2016). It has been observed that rumination effectively occurs in the majority of calves by two weeks of age (Swanson and Harris, 1958). Time spent ruminating and solid feed intake are highly correlated in young calves (Swanson and Harris, 1958). Rumination time increases with the increase in the solid feed intake after weaning (Hepola et al., 2008).

2.3.1.3 Rumen Bacteriology

The ruminant gastrointestinal tract, including the pre-mature rumen, is sterile at birth (Abecia et al., 2017). Ruminal microflora is required to convert plant materials into products that can be utilized by ruminant animal (Jami et al., 2013). Microbial colonization of the pre-mature rumen does not depend on the feed composition (Jayne-Willlams, 1979). During this period the pre-mature rumen contains only saliva, mucus and desquamated epithelial cells (Jayne-Willlams, 1979).

During the pre-mature stage of a lamb's life, although the vast majority of milk ingested does not pass through the rumen, due to the presence of oesophageal groove, some does spill into the rumen. This may provide a trace of nutrients for microbial population (Fonty et al., 1987). Further, Fonty et al. (1983) reported that the rumen of one-week old lambs contained few or no energy substrate for cellulolytic bacteria (i.e. cellulose or its hydrolysis products). Bryant et al. (1958) also stated that cellulolytic bacteria which colonized the rumen during the first week of a lamb's life do not rely on plant cell wall material for nutrients. Hence, these cellulolytic bacteria depend on other non-cellulolytic species to fulfil their nutritional

requirements. Aerobic bacteria have been found in the rumen's of one-day old calves (Govil et al., 2017) and by one week of age in lambs, the rumen is populated by anaerobic species including cellulolytic and methanogenic bacteria and anaerobic fungi (Fonty et al., 1987). The composition of microflora inhabiting the pre-mature rumen of lambs differs from that of an adult sheep (Skillman et al., 2004). For example, the bacteria genera *Propwni bacterium, Clostridium, Peptostrepto coccus* and *Bifidobacterium* are found in high quantities in the pre-mature rumen content of lambs' whilst they are not predominant in the adult ruminant. A large number of *Clostridium* is found in pre-mature lamb microflora, while adult animals do not contain non-sporulating species (Fonty et al., 1987). As there is an increase in the dry feed intake as the ruminant animal matures, there is a change in the number and type of bacteria, from aerobic to anaerobic and facultative aerobic (Davis and Drackley, 1998). Skillman *et al*., (2004) reported that the dam plays an important role in the establishment of micro-flora in the offspring, with bacteria species introduced from different sources including, colostrum, skin, saliva, vaginal canal and faecal materials in the absence of a dam. The type of feed offered, housing and handling can affect the acquisition and establishment of the anaerobic rumen ecosystem in artificially reared calves (Beharka et al., 1998), but there is limited information about about microflora establishment in lambs.

The populations of milk-associated microbes in the rumen reduce with maturity, and there is increasing evidence that the microbes present in an adult ruminant are strongly influenced by the microflora that establishes during the first week of a young ruminants' life (Dias et al., 2017). A mature ruminants' rumen contains a bacterial population of 10^8-10^{11} per gram of digesta which is dominated by obligatory anaerobic bacteria. The anaerobic bacterial count is 1000-fold greater than the facultative bacterial count (Membrive, 2016). Generally, 15 to 20 bacterial genera can be observed in the rumen in high population. The most prominent bacterial

species in the adult rumen are *Fibrobacter succinogenes*, *Ruminococcus albus*, and *Ruminococcus flavefaciens* (Nagaraja, 2016). Rumen bacteria are capable of degrading cellulose, hemicellulose and starch, utilize lipid, protein and urea and detoxifying tannin and saponin in feeds (Agarwal et al., 2015). *Ruminobacter amylophilus, Selenomonas ruminantium, Streptococcusn bovis* and species of *Lactobacillus* and *Bifidobacterium* are the prominent amylolytic bacteria and *Prevotella* sp., *Ruminobacter amylophilus, Butyrivibrio fibrisolvens, Streptococcus bovis, Selenomonas ruminantium*, and *Megasphaera elsdenii* are the most active proteolytic bacteria found in the mature rumen (Nagaraja, 2016). Major lipid hydrolysing bacteria in the rumen include *Anaerovibrio lipolytica* and *Butyrivibrio fibrisolvens* (Nagaraja, 2016).

2.3.2 Dietary factors affecting rumen development

Colostrum, milk or milk replacers and solid feed intake (pasture and pellets) all differently contribute to rumen development. The growth of rumen epithelial cells enhances papillae length, width and thickness of the interior ruminal wall (Baldwin et al., 2004a). Solid feed intake of young ruminants causes the production of VFA by microbial digestion in the rumen, which promote the morpho-physiological shifts (rumen papillae development and increase in volume) required to achieve a fully functional rumen by weaning (Warner et al., 1956, Baldwin and Connor, 2017). The low rumen pH resulting from VFA production stimulates VFA absorption and plays a catalytic role in rumen papillary differentiation (Sutton et al., 1963). Total rumen papillae development is generally achieved by approximately two to three months of age (Membrive, 2016). The mechanical/physical stimulation of solid feed in the rumen can increase the rumen weight and musculature but does not influence papillary development (Baldwin and Connor, 2017). It has been reported that rumen papillae surface area, height and circumference were increased by concentrate supplementation (Danso et al., 2014), however,

it does not appear to change rumen muscle development in lambs (Rickard and Ternouth, 1965).

The majority of studies examining rumen development have been done with calves, there are a limited number of studies that examine the effect of ewe milk or milk replacers and solid feed intake on rumen development of lambs. The outcomes of these studies have been summarised in Table 2.3. Based on the studies on calves, in addition to grain-based concentrated diet feeding, the inclusion of adequate fibre level is important for the rumen muscular (Beiranvand et al., 2014) and volume development and avoid the clumping of papillae (Greenwood et al., 1997b). It appears to be only one study examining the effect of starter fibre level on the rumen development of lambs post-weaning (Xie et al., 2020). They found that increasing the fibre level of starter concentrate (from 14 % to 22 and 26 % of NDF) improves rumen fermentation environment and reduces hyperkeratosis and plaque formation and finally, result in heavier lambs. Limited research on fibre level of diet on rumen development suggests the need for future studies to better understand and improve performances of lambs in artificially rearing systems.

Table 2.3 Effect of pre- and post-weaning diets on rumen development of lambs

Pre-weaning diet	Feeding period (days)	Effect on rumen development at weaning	Post-weaning diet	Effect on rumen development at post-weaning	Citation
Ewe's milk only	7-84	Little influence on rumen weight, rumeno-reticulum volume and papillae development (length, circumference, surface area)	N/A	N/A	Abou Ward (2008)
Ewe's milk only	10-56	Little influence on rumen papillae growth (length, width, density) and the concentration of acetate, propionate, butyrate and total VFA in the rumen	N/A		Sun et al. (2018)
Ewe's milk + concentrates	10-56	Reduced the rumen pH, increased concentration of acetate, propionate, butyrate and total VFA, increased BHBA concentration, increased length, width and surface of rumen papillae, increase cell layers in the stratum corneum, stratum granulosum and total epithelia.	N/A	N/A	Sun et al. (2018)
Ewe's milk + concentrate	7-84	Increased weight of rumen and volume of rumeno-reticulum, papillary circumference and total papillary surface area	N/A	N/A	Abou Ward (2008)
Milk replacer + concentrate	59	Greater papillae length, circumference, and surface area	N/A	N/A	Danso (2016)
Milk replacer + concentrate	18-56	Increased reticulum-rumen weight	N/A	N/A	Cavini et al. (2015)
Milk replacer + concentrate	10-38	Influenced the VFA production, rumen papillary growth (length and width)	N/A	N/A	Yang et al. (2015)
Milk replacer + concentrate + alfalfa	10-38	Increase the rumen papillae length, ratio of duodenal villus height to the crypt depth	N/A	N/A	Yang et al. (2015)
Milk replacer + concentrate + alfalfa	10-38	Stimulated proliferation of fibrolytic bacteria, promote the presence of some saccharolytic bacteria and short-chain fatty acid producers	N/A	N/A	Yang et al. (2018)
Milk replacer + VFA infusion	7-56	Longer rumen papillae development, oxidize less glucose and produce more acetoacetate	N/A	N/A	Lane and Jesse (1997)
Milk replacer + concentrate + Sodium butyrate (3.6 g/DM kg)	18-56	Longer rumen papillae development	N/A	N/A	Cavini et al. (2015)
Solid feed + Acetic acid (4% w/w)	21-56	Long and slender papillae development	N/A	N/A	Rickard and Ternouth (1965)
Solid feed + Propionic acid (4% w/w)	21-56	Shorter and thicker papillae development, no papillae pigmentation, developed papillary bodies and thicker stratum corneum	N/A	N/A	Rickard and Ternouth (1965)
Solid feed + Butyrate acid (4% w/w)	21-56	Shorter and thicker papillae development, develop papillary bodies and thicker stratum corneum	N/A	N/A	Rickard and Ternouth (1965)
Concentrate + 15% coarse alfalfa (3-4 cm long)	21-63	Developed thinner stratum corneum and thicker muscularity of rumen wall, darker brown epithelium colour	N/A	N/A	Norouzian and Valizadeh (2014)
Concentrate diet without alfalfa hay	21-63	Developedthick keratinized layer /stratum corneum, thin muscular layer and lighter colour in epithelium	N/A	N/A	Norouzian and Valizadeh (2014)
Milk replacer	1-48	N/A	Milk replacer (49-84 days)	Increase in BHBA production (Ketogenesis)	Lane et al. (2000)
Milk replacer	1-48	N/A	Milk replacer + pelleted lamb starter (49- 77 days), Pellets only (77 - 84 days)	Development of fewer and larger papillae/cm^2 Elevated intra- ruminal VFA concentration	Lane et al. (2000)
Milk replacer + starter with 14 % NDF[1]	21-60	N/A	Starter with 14 % NDF to 90 days	Greater papillae width and keratin layer and epithelium thicknesses, lower muscular layer thickness No effect on papillae length	Xie et al. (2020)
Milk replacer + starter with 18, 22 and 26 % NDF[1]	21-60	N/A	Starter with 18, 22 and 26 % NDF to 90 days	Similar muscle layer thickness, papillae width. Thinner epithelial in 26 % NDF fed lambs compared to 18 and 22 %, Thinner muscle layer in 18% compared to 22 % No effect on papillae length	Xie et al. (2020)

N/A; Not applicable for the study
[1] NDF= Neutral detergent fibre

2.4 Pre-weaned lamb energy requirements

Lambs require nutrients for both maintenance and growth during the pre-weaning period. Ewe milk or milk replacers is the major source of nutrients during the pre-weaning period (Krishnamoorthy and Moran, 2012), with solid feed (Jensen et al., 2017) becoming more important as the lamb ages. Appropriate energy and protein intake pre-weaning is vital to achieving optimal live weight gains (Danso et al., 2018), while the solid feed intake also helps with rumen development and the establishment of rumen microflora (Hoover and Stokes, 1991). However, all the energy (Gross energy: GE) contained in the ingested feed is not utilized by the animal, as energy is lost through faeces (FE), urine (UE), methane production (CH_4E) and heat production (Figure 2.2).

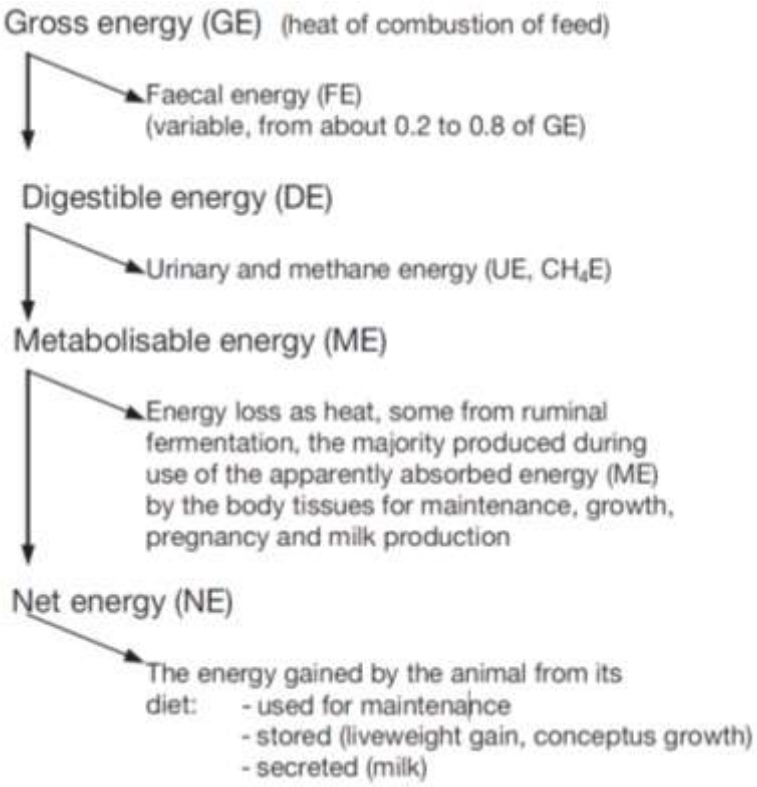

Figure 2.2 The partitioning of feed gross energy in animals (Gross energy, GE; faecal energy, FE; digestible energy, DE; urine energy, UE; methane energy, CH_4E; metabolisable energy, ME; net energy, NE), Adapted from CSIRO (2007).

Metabolisable energy (ME) is used to express the energy value of a feed or ration that is available to the animal (Figure 2.2, CSIRO, 2007). Metabolisable energy is calculated by subtracting FE, UE and CH_4E losses from GE, or by subtracting UE and CH_4E losses from digestible energy (DE) as demonstrated in the following formulas (CSIRO, 2007):

$ME = GE – (FE + UE + CH_4E)$ or

$ME = DE – (UE + CH_4E)$

The daily ME requirement of a lamb depends on its requirement for maintenance, growth, activity, and thermoregulation (ARC, 1980, CSIRO, 2007).

2.4.1 Metabolisable energy requirements for maintenance (ME_m)

The energy intake per day at zero energy balance of animals is referred to as metabolisable energy for maintenance (ME_m) (Dawson and Steen, 1998). The ME_m can be calculated as a function of body weight (kJ/kg $W^{0.75}$; where LW=live weight). Alternatively, ME_m requirement can be determined by plotting energy retention against ME intake and when the linear function is extrapolated to meet the inflexion, the point at zero energy retention gives the ME_m requirement (Dawson and Steen, 1998).

The ME_m of an animal depends on the genotype, age, gender, physiological state, type of ration and level of feeding (CSIRO, 2007). Ferrell (1988) also reported that variation in mass and energy expenditure of the liver and gastrointestinal tract affect (by 20-25%) the total animal energy expenditure or requirement. Table 2.4 demonstrates that limited research has been conducted to determine the ME_m of lambs fed milk replacers and/or pellets or solid feed simultaneously during the pre-weaning period when reared either indoors or outdoors irrespective of gender.

Table 2.4 The metabolisable energy requirement for maintenance (ME_m) of lambs

Reference	Breed	Sex of Lamb	Indoor or Outdoor	Feed type	ME_m MJ/kg $LW^{0.75}d^{-1}$
ARC (1980) (recommendation)	-	Male	-	Milk/milk replacer (Liquid diet)	0.40
ARC (1980) (recommendation)	-	Female	-	Milk/milk replacer (Liquid diet)	0.35
ARC (1980) (recommendation)	-	Male	Outdoor	Milk	0.50
ARC (1980) (recommendation)	-	Male	Outdoor	Solid feed	0.43
ARC (1980) (recommendation)	-	Female	Outdoor	Milk	0.43
ARC (1980) (recommendation)	-	Female	Outdoor	Solid feed	0.38
Jagusch and Mitchell (1971)	Crossbred	Male	Indoor	Milk	0.61
Sanz Sampelayo et al. (1995)	Segurena	Male	Indoor	Milk replacer	0.39
Jassim et al. (1996)	Awassi	-	Indoor	Pellet	0.48
Danso et al. (2016)	Romney-cross	Male	Indoor	Milk replacer with pellet	0.40

2.4.2 Metabolisable energy requirements for growth (ME_g)

The metabolisable energy requirement for growth (ME_g) depends on the animal's growth rate, which includes the increase in the size of the animal and both structural and functional development. The ME_g varies with age, breed, sex, type of ration ingested, activity level, feeding level, feed quality and physiological stage of the animal (CSIRO, 2007, SCA, 1990). The carcass of an animal is composed of water, fat, protein, minerals and small amounts of carbohydrates (Heinz and Hautzinger, 2010). The quantity of each component in the body varies with the animals' growth (Heinz and Hautzinger, 2010). The major determinant of body growth is the deposition of protein (Oddy and Sainz, 2002, Bastianelli and Sauvant, 1997) compared to fat in the early life stage.

The energy requirement for protein deposition is the summation of energy deposited as protein and energy cost for protein deposition. Protein deposition is accomplished parallel with minimum lipid retention (Bastianelli and Sauvant, 1997). The rate of fat deposition is low in early postnatal lambs (Black, 1983) and progressively increases until they reach

approximately 70% of their mature weight (Oddy and Sainz, 2002). The live weight gain per unit of energy available is five to six times greater (CSIRO, 2007) in early life stage lambs, as more protein deposition is occurring compared to fat deposition (Oddy and Sainz, 2002). This is because there is more water accumulation in protein-rich tissues (Bastianelli and Sauvant, 1997). The energy cost of fat and protein deposition in milk-fed lambs was reported to be 63 kJ ME/g and 30kJ ME/g, respectively (Kielanowski, 1965), while the energy cost of fat and protein deposition in lambs fed milk replacer during the first three weeks of age was 46.4 kJ ME/g and 35.5 kJ ME/ g, respectively (Walker and Norton, 1971). Table 2.5 summarises the reported pre-weaning lamb MEg requirements from previous studies.

Table 2.5 The metabolisable energy requirement (ME_g) for the growth of lambs

Reference	Breed	Sex of lamb	Indoor or Outdoor	Feed type	MEg (MJ/day/kg gain)
Danso et al. (2016)	Romney	Male	Indoor	Milk	13.8
Filho et al. (2011)	Santa Ines	Male	Indoor	Concentrate and hay	23.34
Silva et al. (2010)	Santa Ines	Male	Indoor	Concentrate	18.15

2.4.3 Metabolisable energy utilization efficiency

Metabolisable energy utilization efficiency (k) is defined as the ratio between net energy (NE) and ME content (k=NE/ME) (Ma et al., 2016, Filho et al., 2011). In an animal fed at maintenance level or fasted, the efficiency of ME utilization for maintenance is denoted as k_m, whilst the efficiency of ME utilization for growth is referred to as k_g (ARC, 1980). Reported k_m and k_g values for lambs are summarized in Table 2.6. The k_g depends on fat and protein deposition (CSIRO, 2007) and the theoretical values for the efficiency of fat and protein synthesis are in young lambs, 0.72 and 0.84, respectively (Baldwin, 1968). Highly variable k_g values have been reported in previous studies.

Table 2.6 The efficiency of metabolisable energy utilization for maintenance (k_m) and growth (k_g) of lambs

Reference	Breed	Sex of lamb	k_m	k_g
Silva et al. (2010)	Santa Ines	Male	0.66	0.36
Filho et al. (2011)	Santa Ines	Male	0.70	0.43
Danso et al. (2016)	Romney	Male	-	0.53
Pereira et al. (2017)	Santa Ines	Male	0.60	0.28
Pereira et al. (2018)	Morada Nova	Male	0.58	0.36
Pereira et al. (2018)	Morada Nova	Female	0.58	0.28
Oliveira et al. (2018)	Hair sheep	-	0.63	0.36

2.5 Pre-weaned lamb protein requirements

Protein plays a major role in both maintenance and weight gain. The protein requirement of an animal varies with genetic potential for lean deposition, the growth stage of the animal and body composition (Silva et al., 2007). Protein is an expensive feedstuff in livestock systems (Galvani et al., 2009). Thus, improved information and understanding on the protein requirements of young lambs would be of benefit to farming operations.

Protein requirements of ruminants can be expressed as metabolisable protein, which includes microbial protein and rumen undegraded dietary protein (NRC, 2007). This mainly depends on microbial protein synthesis and ruminal outflow of intact feed proteins (NRC, 2007). As demonstrated by Stern et al. (1983) an increase in dietary crude protein (CP) content does not lead directly to increased microbial amino acids (AA) passage to the duodenum due to the dietary energy limitation for microbial protein synthesis. To improve microbial protein synthesis, resulting in more microbial AA reaching the duodenum, dietary protein needs to be accomplished by an energy source for the microbes to utilize in order to synthesis microbial proteins.

2.5.1 Protein requirements for maintenance

Protein requirements at maintenance represent the quantity of protein lost through urine, faeces and skin (Corbett and Ball, 2002). The majority of nitrogen is lost as urine (40–50%) (van den Borne et al., 2006) while nitrogen loss through faeces varies with the

ingested feedstuff. The loss of nitrogen through urine occurs even when animals are fed nitrogen-free diets due to amino acid catabolism during protein turnover. However, the loss is substantially less in ruminants due to the recycling of urea (Corbett and Ball, 2002).

The endogenous nitrogen losses through faeces include microbial debris, enzymes and slough cell residues. The endogenous faecal nitrogen loss of lambs is 0.29 g N /kg dry matter intake (1.81 g CP/kg dry matter intake) (Walker and Faichney, 1964). The endogenous faecal nitrogen losses increase with an increase in feed intake (Corbett and Ball, 2002). Dermal loss of nitrogen is the rate of wool growth in sheep, which use amino acids and continues even during severe undernutrition (Corbett and Ball, 2002).

Historically, CP requirements for maintenance of milk-fed lambs at 5kg, 10kg and 20 kg of body weight are reported as 10, 11 and 13 g/day (ARC, 1980). However recent studies show that crude protein requirements at maintenance of male lambs are 2.74 g CP/kg $LW^{0.75}$(Danso et al., 2016) and 2.31 g CP/kg $^{0.75}$ of shrunk body weight (i.e. weight after a period of feed and/or water withdrawal) (Galvani et al., 2009).

2.5.2 Protein requirements for growth

For every gram of protein deposited in the body, a deposition of three to four grams of water occurs while adipose tissue contains very little water (CSIRO, 2007). So, an increase in protein deposition results in a greater increase in weight gain than fat deposition. The weight gain and protein deposition of lambs increases with an increase in dietary protein intake when adequate dietary energy is also provided (Andrews and Ørskov, 1970). Nitrogen deposition in males is also higher than in female lambs (Andrews and Ørskov, 1970, Morgan and Owen, 1973, Lee et al., 1990). The crude protein requirements for the live weight gain of lambs reported by previous studies are summarised in Table 2.7.

Table 2.7 Crude protein (CP) requirements for live weight gain of pre-weaned lambs

Reference	Breed	Sex of Lamb	Indoor or Outdoor	Feeding Schedule	CP g/g of average daily gain
Danso et al. (2016)	Romney	Male	Indoor	Milk replacer and pellets	0.23 at live weight 18 kg
Galvani et al. (2009)	Texel crossbred	Male	Indoor	Solid feed	1.4*
Pires et al. (2000)	Crossbreed Texel x Ideal	Male	Indoor		0.31 – 0.48 at 5 -30 kg live weight
ARC (1980)	-	Male	-	Milk or milk replacer	0.33 – at 5kg live weight
ARC (1980)	-	Male	-	Milk or milk replacer	0.34 – at 20kg live weight

* Metabolisable protein requirement per gram of weight gain

2.5.3 Protein utilization efficiency

The protein utilization efficiency depends on the animal's physiological stage, genetic potential for protein deposition, digestion and absorption of dietary amino acids and metabolism or partitioning of absorbed amino acids (Kim and Pluske, 2016). Pre-ruminant lambs are as sensitive to dietary amino acid profiles as non-ruminant species (Rogers and Egan, 1975), thus, a balanced amino acid profile providing essential amino acids needs to be ingested. However, in mature ruminants, supplementation of diets with essential amino acids is not required due to ruminal degradation of dietary proteins and subsequent microbial protein synthesis of which contains and provides to the ruminant animal all the required amino acids (Titgemeyer, 2003).

Methionine is the most limiting amino acid for microbial protein synthesis and usage of methionine for growth seems to be inefficient in sheep and cattle (Titgemeyer, 2003). Lysine, histidine and arginine are also considered limiting amino acids in growing lambs (Titgemeyer, 2003). Today’s modern genotypes with a greater capacity to deposit lean protein, may have higher amino acid requirements than those previously reported. The efficiency of use of truly digested microbial protein for nitrogen retention (growth) was

66 % in lambs (Titgemeyer, 2003), and would have been increased by supplementation of limiting amino acids (Titgemeyer, 2003). Further, it has been reported that protein utilization efficiency for growth is reduced with body weight gain in pre-ruminant calves (van den Borne et al., 2006). The metabolisable protein utilization efficiency for body weight gain in lambs was reported by Galvani et al. (2009) as being 0.71, and Danso et al. (2016) reported that the efficiency of dietary crude protein utilization for body protein deposition is 0.68 in lambs.

2.5.4 Protein and energy interrelationship

The growth of an animal depends on both protein and energy intake. The rate of protein deposition or nitrogen retention increases linearly with increases in protein intake while the rate of fat deposition is decreased at a given energy intake (Andrews and Ørskov, 1970). Energy intake is also a major limiting factor for protein deposition (Andrews and Ørskov, 1970) (Figure 2.3), and protein deposition becomes less efficient with a further increase in protein supply (Schroeder and Titgemeyer, 2008, van den Borne et al., 2006).

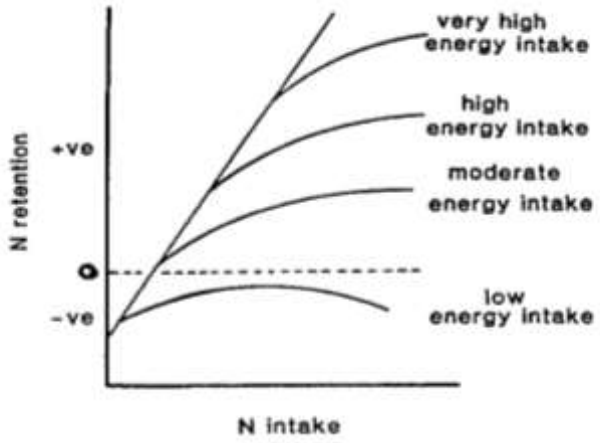

Figure 2.3 The effect of energy on nitrogen (N) retention/protein deposition (Balch, 1967), as cited by Chowdhury and Ørskov (1997)

Lambs gain weight even in a negative energy balance, by utilizing energy mobilized from adipose tissues when protein supply is adequate (Fattet et al., 1984, Chowdhury and Ørskov, 1997). However, it was reported that the mobilization of adipose tissue to supply energy for protein deposition during feed restriction only occurs in over-fat lambs (Iason and Mantecon, 1993). An intake of 14.2 g of CP per MJ ME (CP:ME ratio), resulted in the highest protein deposition (43 g/day) in pre-weaned lambs fed milk replacers (Danso et al., 2018). Further research needs to be carried out to better understand how to meet optimum dietary protein to energy ratios that maximize the growth performance of pre- and post-weaned lambs in an artificial rearing system.

2.6 Factors affecting lamb pre-weaning growth

Weaning weight of lambs is an important parameter in sheep production systems (Dahmen, 1966) having economic value (Fasae et al., 2012). The growth from birth to weaning affects weaning weight, viable weaning age and post-weaning growth performance of lambs (Selaive -Villarroel et al., 2008, Bhatt et al., 2009, Fraser and Saville, 2000). Many factors affect the pre-weaning growth performance of lambs; genetic, physiological and environmental factors can all have an impact pre-weaning growth of the lambs (Combellas et al., 1980). This review will focus on those factors that are likely to impact lamb pre-weaning growth in an artificial rearing system (i.e. in the absence of a lactating dam).

2.6.1 Genetics

Lamb growth rate is affected by the genetic make-up of the lamb and its genetic potential (Willham, 1972) for growth but its expression depends on environmental conditions (Mohammadi et al., 2010). Direct heritability of weaning weight for different sheep breeds range between 0.09 to 0.39 (Al-Shorepy, 2001). Ligda *et al*., (2000) reported that the direct heritability of genetic potential (additive effect) of weaning weight ranged

between 0.15-0.17 whilst Tosh and Kemp (1994) reported it was 0.05-0.21, suggesting that values vary with the breed. Direct additive heritability of weaning weight is higher than the heritability of maternal genetic effects for pre-weaning growth traits in lambs (Lôbo et al., 2009). Direct heritability for weight gain from birth to weaning has been reported as 0.20 (Lôbo et al., 2009), and from weaning to yearling as 0.39 (Lôbo et al., 2009).

Maternal genetics and the permanent environment (uterine environment provided by the dam) has an impact on the growth rate of the lamb (Mousa et al., 2013). The effect of the uterine environment of the dam and maternal genetic effects are half and one-third of the direct additive genetic effect (Ligda et al., 2000). Additive direct and additive maternal effects for pre-weaning growth rates are negatively correlated (correlation ranging between - 0.18 to - 0.74) (Burfening and Kress, 1993).

2.6.2 Birth weight and birth rank

Birth weight of the lamb is an economically important trait that influences pre-weaning growth (Al-Shorepy, 2001). Heavier lambs at birth typically have greater body weight at weaning (Chopra and Acharya, 1971) and had a higher average daily gain when compared to their lighter counterparts (Mavrogenis and Constantinou, 1990). New-born lambs with a low body weight take a long time to get adapted to postnatal life before gaining weight. During the early pre-weaning period, the growth rate of light lambs was lower than heavy lambs and tended to lead to a continued lower growth rate during the entire pre-weaning period (Greenwood et al., 1998). Birth rank of lambs also affects weaning weight with single born lambs reported having higher weaning weights compared to twin-and triplet-born lambs (Dahmen, 1966, Combellas et al., 1980, Fasae et al., 2012).

The efficiency of energy utilization for protein deposition (k_p) is lower in low birth weight lambs (below 2.9 kg), compared to heavy birth weight lambs (above 4.3 kg), approximately K_p -0.6 *vs* 0.8, respectively, but this difference disappears by approximately 20 kg body weight (Greenwood et al., 1998). Further, lighter birth weight lambs had lower efficiency of energy utilization for fat deposition (k_f = 0.30 *vs* 0.45, respectively for lighter and heavy lambs) to 10 kg body weight (Greenwood et al., 1998). The lighter lambs have a limited initial capacity for lean growth as their muscle tissue contains fewer myonuclei providing the necessary transcripts for protein synthesis, hence, those lambs are not able to utilize available nutrients for muscle protein deposition (Greenwood et al., 1998).

2.6.3 Sex of the lamb

The pre-weaning growth rate of male lambs is greater than that of ewe lambs (Mohammadi et al., 2010, Sidwell et al., 1964, Mousa et al., 2013, Lopez-Villalobos et al., 2017, Rashidi et al., 2008, Lee et al., 1990, Morgan and Owen, 1973, Bhatt et al., 2009). Consequently, ram lambs are typically heavier than ewe lambs at weaning (Mohammadi et al., 2010, Yilmaz et al., 2007, Lopez-Villalobos et al., 2017, Rashidi et al., 2008, Lee et al., 1990). The bone and muscle growth of male lambs occurs at a faster rate than that of female lambs, and fat deposition is also less than in male lambs compared to female lambs (Fourie et al., 1970).

2.6.4 Lamb pre-weaning nutrition

2.6.4.1 Ewe milk vs milk replacer

There are a number of published studies reporting higher growth rates of lambs fed ewe's milk compared to those fed milk replacer (Lanza et al., 2006, Ward et al., 2017). However, this higher growth rate appears to only occur during the first few weeks of life and when lambs reached weaning age, both lambs fed ewe's milk and those fed milk

replacer had the same weaning weight (Emsen et al., 2004, Hernández-Castellano et al., 2015b, Napolitano et al., 2006). The differences in growth performances of pre-weaned milk-fed lambs are likely due largely to the composition of milk replacers used and the quantities fed.

Sevi et al. (1999) reported similar pre-weaning growth rates for lambs fed ewe milk or a mix of ewe milk and milk replacer (50:50 ratio) during their first five weeks of life, however, lambs fed milk replacer only had slower pre-weaning growth rates. The gross energy content of all three types of milk used in Sevi et al. (1999) study were similar (~ 4.2 MJ/kg), while the ewe milk and the milk mix contained more crude protein than the milk replacer (55.2, 50.85 and 46.5 g CP/kg in ewe milk, the milk mix and milk replacer, respectively). The CP to ME ratios in the ewe milk, the milk mix and the milk replacer were 13.14, 12.10 and 11.07 g CP/MJ ME respectively. In addition, the ewe milk and the milk mix contained lactose, which was absent in the milk replacer.

The protein to energy ratio of the diet is a major factor affecting the growth potential of lambs. The crude protein to metabolisable energy (CP:ME) ratio of ewe's milk has been reported as being between 10-11 g CP/MJ ME (Paten *et al.*, 2017, Paten *et al.*, 2013, van der Linden, 2010). Danso et al. (2018) has reported that CP:ME of typical commercial milk replacers for lambs is approximately 11.5 g/MJ, similar to that found in ewe's milk. However, Danso et al. (2016) stated that this CP:ME may not reflect the true requirement of young lambs in the early pre-weaning period, which was reported as being closer to 13 g CP/MJ ME.

2.6.4.2 Milk from alternative animal species

Lamb fed with buffalo milk (at 10 % of their body weight) (CP 3.93 %, ME 6.04 Mcal/kg) as a substitute for ewe's milk (CP 4.6 %, ME 6.3 Mcal/kg) had a similar average daily

live weight gain and cumulative weight gain as that of lambs fed ewe's milk to 12 weeks of age, when both groups of lambs also had access to a starter ration (CP 20.5 %, ME 2.88 Mcal/kg) (Anjum et al., 2014). Lambs fed cow's milk (CP 3.21 %, ME 5.58 Mcal/kg) or milk replacer (CP 3.5 %, ME 4.96 Mcal/kg), with the same starter ration had lower average weight gain during the same period (Anjum et al., 2014). Additionally, the authors reported that there was a high milk intake and less starter pellet intake in those lambs fed ewe's milk and buffalo milk, while lambs fed cows milk or milk replacer had low milk intake and higher starter pellet intake, suggesting that the overall low energy intake of lambs fed with cows milk or milk replacer was likely the reason for the differences in growth performance.

2.6.4.3 Solid feed intake

In artificial rearing systems, introducing a pelleted or creep feed in addition to milk is common. The live weight gain of lambs fed milk replacer and pellets was greater compared to lambs that were not also offered pellets (Danso et al., 2014, Jensen et al., 2017). Restricted milk feeding will substantially increase solid feed intake of lambs, but will reduce growth rate in the early pre-weaning period (Morgan and Owen, 1973).

Lamb growth under pastoral conditions is determined by nutrient intake from both milk and pasture. Average daily gain, weaning weight and feed conversion efficiency were higher in lambs fed milk replacer over suckling lambs, when both groups were reared with access to pasture and supplemented with an *ad libitum* creep mixture (Bhatt et al., 2009). Lamb live weight at weaning and weight gain from birth to weaning are affected by the herbage type offered in pasture-based feeding systems. Twin Romney lambs grazed on lucerne had greater live weight at weaning and weight gain from birth to weaning than those grazed on plantain-clover or ryegrass-white clover pastures (Corner-

Thomas et al., 2018b). Further, higher live weights at 32 and 63 days of age were reported for lambs grazed on lucerne compared to lambs fed a herb clover-mix containing chicory, plantain, and red and white clover, while the lowest live weights recorded were for lambs on ryegrass-white clover pasture (Corner-Thomas et al., 2014, Corner-Thomas et al., 2018a).

2.7 Post-weaning lamb energy and protein requirements

2.7.1 Metabolisable energy (ME_m) and crude protein (CP_m) requirements for maintenance

Post-weaning lambs are mainly reared on pasture in New Zealand. The ME_m requirement of post-weaned lambs depends on live weight, sex, grazing environment, feed quality and climatic conditions (Nicol and Brookes, 2007). The ME_m and protein requirements of post-weaned lambs for separate sex categories and different feeding systems are presented in Table 2.8. The reported daily ME_m values range between 0.26 to 0.55 MJ/kg $LW^{0.75}$ which could be due to changes in study conditions.

According to the data presented in Table 2.8, limited research has been conducted to determine the protein requirement for maintenance of lambs post-weaning. Further research is required to investigate the protein requirement for the maintenance of lambs during the post-weaning period.

Table 2.8 Daily metabolisable energy (ME_m) and metabolisable protein (MP_m) requirements for maintenance of post-weaned lambs

Reference	Sex of Lamb	Feeding system	ME_m or MP_m requirement per day	Units
Energy requirements for maintenance				
Nicol and Brookes (2007)	Male	Pasture grazing	0.50	MJ/kg $LW^{0.75}$
Nicol and Brookes (2007)	Female	Pasture grazing	0.45	MJ/kg $LW^{0.75}$
Bellof and Pallauf (2004b)	Male and female	Concentrate and hay	0.52	MJ/kg $LW^{0.75}$
Bellof and Pallauf (2004b)	Male	Concentrate and hay	0.50	MJ/kg $LW^{0.75}$
Bellof and Pallauf (2004b)	Female	Concentrate and hay	0.55	MJ/kg $LW^{0.75}$
Early et al. (2001)	Male	Rhodes grass hay	0.53	MJ/kg $LW^{0.75}$
Ferrell et al. (1979)	Male and female	Concentrate	0.46	MJ/kg $LW^{0.75}$
Yang et al. (2020)	Male and female	Grass or grass plus concentrate	0.49	MJ/kg $LW^{0.75}$
Zhao et al. (2016)	Male	Pelleted mixed diet	0.44	MJ/kg $SBW^{0.75*}$
ARC (1980) (recommendation)	Female	Solid feed	0.26	MJ/kg $LW^{0.75}$
ARC (1980) (recommendation)	Male	Solid feed	0.30	MJ/kg $LW^{0.75}$
Protein requirements for maintenance				
Brookes and Nicol (2007)	Female	Pasture grazing	28	g/head at 20 kg LW
Brookes and Nicol (2007)	Female	Pasture grazing	42	g/head at 40 kg LW
Brookes and Nicol (2007)	Female	Pasture grazing	60	g/head at 60 kg LW
ARC (1980) [1]	Female, male	Solid feed	9	g at 20 kg LW
ARC (1980) [1]	Female, male	Solid feed	12	g at 40 kg LW

*Shrunk body weight

[1] Crude protein requirement

2.7.2 Metabolisable energy (ME_g) and crude protein (CP_g) requirements for growth

The metabolisable energy requirement for growth (ME_g) depends on the animal's growth rate, live weight, sex and breed (Nicol and Brookes, 2007). Reported ME_g values range

between 29 to 55 MJ/kg LW (Table 2.9). Lambs that reach maturity early are reported to have higher ME_g than late-maturing lambs (5.5 vs 3.5 MJ ME/100g gain in ewe lambs, respectively) at the same weight (Nicol and Brookes, 2007). This was reported to be due to higher fat content in the gain of early maturing lambs compared to late-maturing lambs (Nicol and Brookes, 2007).

Post-weaning protein requirements for growth (CP_g) are presented in Table 2.9. The CP_g includes fleece free live weight gain and wool growth. The values reported by ARC (1980) were higher than the values reported by other studies, likely due to variations in the expression of values either for empty body weight or live weight.

Table 2.9 The metabolisable energy (ME_g) and crude protein requirements (CP_g) for growth of post-weaned lambs

Reference	Live weight (kg)	Sex of lamb	Feed	ME_g or CP_g requirement per day	Units
Energy requirements for growth					
Nicol and Brookes (2007)	35	Male	Pasture (grazing)	30	MJ/kg LW
Nicol and Brookes (2007)	45	Male	Pasture (grazing)	35	MJ/kg LW
Nicol and Brookes (2007)	35	Female	Pasture (grazing)	50	MJ/kg LW
Nicol and Brookes (2007)	45	Female	Pasture (grazing)	55	MJ/kg LW
Zhao et al. (2016)	35	Male	Pelleted mixed diet	28.6	MJ/kg LW
Zhao et al. (2016)	45	Male	Pelleted mixed diet	34.3	MJ/kg LW
Protein requirements for growth					
Costa et al. (2018)	22-25	Male (Morada Nova)	Buffel grass hay and concentrates	113-109	g/kg empty body weight (EBW)
Costa et al. (2018)	28-31	Male (Morada Nova)	Buffel grass hay and concentrates	105-102	g/kg EBW
Costa et al. (2018)	22-25	Male (Santa Inês)	Buffel grass hay and concentrates	140-137	g/kg EBW
Costa et al. (2018)	28-31	Male (Santa Inês)	Buffel grass hay and concentrates	134-132	g/kg EBW
Deng et al. (2017)	-	Male	Pellets (Concentrate: roughage 44:56)	145	g/kg LW
ARC (1980)	20 - 30	Female	Solid feed	230	g/kg LW d^{-1}
ARC (1980)	20 - 30	Male	Solid feed	250	g/kg LW d^{-1}
ARC (1980)	40	Female	Solid feed	260	g/kg LW d^{-1}
ARC (1980)	40	Female, male	Solid feed	240	g/kg LW d^{-1}

2.8 Factors affect post-weaning growth

Several factors influence lamb growth post-weaning such as weaning weight, weaning age, rumen development and feed quality and quantity. The majority of previous studies report that lambs that are heavy at weaning, have greater post-weaning growth rates (Fraser and Saville, 2000, Selaive -Villarroel et al., 2008, Bhatt et al., 2009). In general, lambs are weaned on to ryegrass-based pasture in New Zealand. The nutritional quality of ryegrass is unlikely to support 250 g/d average live weight gains in lambs post-weaning during summer or in unirrigated pasture based systems (Beef + Lamb New Zealand, 2014). However, alternative summer herbage species can be used to improve lamb growth rates post-weaning. Lamb growth performance post-weaning was increased if fed with herb-clover mix containing plantain, chicory, red and white clover during the late spring and early summer period compared to ryegrass based pasture (Somasiri et al., 2016). Lamb live weight gains are also higher when fed with ryegrass based pasture containing a higher proportion of white clover (Lindsay, 2007). Golding et al. (2011) reported that herb/clover mixed sward at unrestricted feeding levels resulted in increased lamb live weight gains compared to traditional perennial ryegrass based pasture feeding.

The fibre content and the metabolisable energy content of herbage affects the digestibility of feed. Additionally, sufficient rumen development, pre-weaning, is required to ensure that post-weaning, a lamb is capable of consuming, fermenting and utilizing an adequate amount of solid, plant-based feed to maintain growth rates. A diet with a high ME content and low fibre content will improve lamb growth performance (Hodgson and Brookes, 1999), but ME concentration is generally low and fibre is higher in grass compared to concentrate-based feeds. The ME of pasture ranges from 8-12 MJ ME/kg DM (Beef + Lamb New Zealand, 2014). Post-weaned lambs need to consume an adequate amount of pasture (4-5% live weight or 1.5kg DM/day for a 30kg lamb) or be provided with

sufficient pasture to leave a residual 1400–1500kg DM/ha (3-4cm in spring) so as not to limit intake (Beef + Lamb New Zealand, 2014). Thus, a pre-weaning regimen that encourages rumen development and post-weaning pasture type and composition are important factors to be considered when rearing lambs to ensure optimum growth.

2.9 Research objectives

In summary, it would be beneficial to improve our understanding of how the protein to energy ratio of young lambs' diets impact pre-and post-weaning lamb growth and body composition in artificial rearing systems. Further, little is known about the impact of dietary fibre and age at weaning on the growth and rumen development of artificially reared lambs. The ability to accurately predict lamb growth and body composition without *in vivo* experiments would bring many benefits to farmers and researchers. However, there appears to be only one published stimulation prediction model for pre-weaned, artificially-reared lamb growth and body composition (Anim-Jnr et al., 2020) and it has not been validated against an independent dataset.

Thus, to address the above gaps in knowledge, the following objectives were investigated in this thesis:

1. The effect of dietary protein to energy ratio (CP:ME) in milk replacer (to 22 kg live weight) on growth and body composition of pre-weaned lambs reared artificially (Chapter 3).
2. The effect of dietary CP:ME ratio (to 22 kg live weight) on growth performance of pre-and post-weaned lambs (Chapter 4).

3. The influence of pellet fibre level, milk replacer composition and age at weaning (42 vs 57 days of age), on the growth and body composition of lambs reared artificially (Chapter 5).

4. The impact of varying pellet fibre levels and milk replacer composition on rumen development in artificially-reared, early-weaned lambs (Chapter 6).

5. The effect of varying pellet fibre levels on the composition of rumen bacteria in artificially-reared, early-weaned lambs (Chapter 7).

6. Validation of a mechanistic dynamic pre-weaned lamb growth and body composition simulation model (Anim-Jnr et al., 2020) based on data from Chapters 3, 4, and 5 (Chapter 8).

Chapter 3 Effect of dietary protein to energy ratio of milk replacer on growth and body composition of pre-weaned lambs reared artificially

This Chapter has been published in full elsewhere. It has been reformatted and presented here. Citation: Herath, H.M.G.P., Pain, S.J., Kenyon, P.R., Blair, H.T., Morel P.C.H., 2020. Effect of dietary protein to energy ratio of milk replacer on growth and body composition of pre-weaned lambs reared artificially. Animal Feed Science and Technology. 264, https://doi.org/10.1016/j.anifeedsci.2020.114478 (The DRC form is attached in appendices).

3.1 Abstract

This study aimed to determine the effect of changing the dietary crude protein to metabolisable energy ratio (CP:ME) of milk replacer on growth and body composition of pre-weaned lambs. Thirty-two Romney twin-born ram lambs were selected with four lambs being slaughtered at 24 h post-partum to estimate initial body composition. The remaining twenty-eight lambs were assigned to one of three nutritional treatments. Treatments consisted of either (i) a commercial milk replacer (CMR, n=10); (ii) a high protein milk replacer (HPM, n=9) or (iii) a mix of normal milk replacer and milk protein concentrate (MB, n=9). All lambs were fed at 2.1 times their maintenance requirement. The CP:ME ratio of MB was adjusted twice-weekly to match the lambs CP and ME requirements for growth and maintenance over time. All lambs were slaughtered at 22 kg live weight (LW). The LW and chemical composition of carcass and viscera plus blood were determined. The combined CP:ME ratio of milk and pellets was greater for the HPM lambs than MB lambs, which was greater ($P<0.0001$) than CMR lambs. Daily ME intake was greater ($P<0.05$) in MB compared to HPM and CMR, which did not differ ($P>0.05$). Average daily LW gain was higher ($P<0.05$) in both HPM and MB lambs than CMR lambs. The ME and CP intakes per kilogram LW gain were greater ($P<0.05$) for CMR and HPM lambs, respectively than MB lambs. Omental fat content at slaughter was lowest ($P<0.05$) and gut fill was greatest ($P<0.05$) in HPM, while CMR and MB did not differ ($P>0.05$) for either parameters. Fat content of the carcass and viscera plus blood was lowest ($P<0.05$) in HPM whilst, there was no difference ($P>0.05$) between CMR and MB. Lambs fed HPM had lower ($P<0.05$) daily fat deposition than CMR, which was highest ($P<0.05$) in MB. Daily dry matter deposition was greater ($P<0.05$) in MB than both CMR and MB, which did not differ ($P>0.05$). In conclusion, adjusting the CP:ME ratio of milk replacer to match the lambs' theoretical requirement improves growth

performance of artificially reared lambs. Further studies are required to determine the optimal CP:ME ratio effect of milk replacer, its cost effectiveness and to examine post-weaning growth effects.

Keywords: Body composition; Growth, Lamb; Milk replacer; Protein to energy ratio

3.2 Introduction

Lambs that grow faster from birth to weaning are generally heavier, have better condition at slaughter and/or can be slaughtered at a younger age (Muir et al., 2003, Beef + Lamb New Zealand, 2014). Greater lamb growth rate is associated with improved feed efficiency (Beef + Lamb New Zealand, 2014). In addition, if slaughtered at a younger age they will likely require less labour and animal health inputs.

Deposition of muscle protein is the major determinant of lean growth (Bastianelli (Bastianelli and Sauvant, 1997). Less energy is needed per unit live weight gain of muscle tissue than fat tissue due to higher water assimilation in the protein rich tissues (Oddy and Sainz, 2002). The energy and protein requirements per unit of live weight gain in lambs are between 13.8-23.34 MJ/day/kg gain and 230 – 480 g/day/kg gain, respectively (ARC, 1980, Pires et al., 2000, Silva et al., 2010, Filho et al., 2011, Danso et al., 2016). Manipulation of the protein and energy content of diet could be a means of promoting faster lean growth of lambs, which may financially benefit farmers.

Recent evidence suggests that there is a mismatch between the crude protein to metabolisable energy ratio (CP:ME) requirement and the CP:ME intake of lambs in the pre-weaning period (Danso et al., 2016). The CP:ME ratio requierements have been reported to be 13.1 and 10.9 g/MJ for lambs with live weights of 5 kg and 18 kg respectively, indicating that a greater CP:ME ratio is needed at initial stages of growth (Danso *et al.*, 2016). This relationship has been reported to decrease curvilinearly as the

lamb ages (Danso *et al.*, 2016). However, diets consumed by pre-weaned lambs typically have an increasing CP:ME ratio as the animal ages (Danso *et al*., 2016). The CP:ME ratio of ewe's milk has been reported to be 10-11 g/MJ (van der Linden, 2010, Paten et al., 2017, Paten et al., 2013), 11.0 g/MJ for milk replacer (Danso et al., 2018), 15.7 g/MJ for grain based pellets (Danso *et al.*, 2018) and between 15.3 -16.2 g/MJ for ryegrass and white clover based diet in New Zealand (Golding et al., 2011, Somasiri et al., 2016). Thus, with the present feeding system, lamb growth is limited due to low protein intake per unit ME in the early lactation stage, while protein intake per unit ME is in excess in later life (Danso *et al*., 2016).

Danso *et al.* (2018) showed that a higher (14.5 g/MJ) CP:ME ratio milk replacer fed from two days postpartum until lambs reached to 18 kg live weight improved lean growth rate, feed efficiency and resulted in less fat deposition in pre-weaned lambs than those who fed a milk replacer with 11.5 g/MJ CP:ME ratio. However, protein intake was wasted in the later pre-weaning stage. Therefore, it is possible that feeding lambs with a milk replacer adjusted to better meet their changing CP:ME requirements for growth, should result in faster lean growth at less cost. However, to date, this has not been examined during the pre-weaning growth phase of lambs. This study was designed to investigate the effect of adjusting the CP:ME in milk replacer feeding during pre-weaning on lamb growth and body composition compared to lambs fed with either a high or a low CP:ME milk replacer. It was hypothesized that feeding of a milk replacer with a changing CP:ME (16.46 g/MJ to 10.96 g/MJ over 70 days) to match lamb requirements during the pre-weaning phase would improve lamb growth and body composition compared to lambs fed with either a high or a low CP:ME milk replacer.

3.3 Materials and methods

The experiment was carried out at Massey University, Palmerston North, New Zealand from September 2017 to December 2017. The research procedures used were approved by the Massey University Animal Ethics Committee (MUAEC 17/48).

3.3.1 Experimental design and animal management

Thirty two Romney ram lambs born to 32 twin-bearing ewes were selected for the study. Lambs were allowed to suckle from their dam for 24 hours after birth and then one ram lamb from each twin set was randomly selected and separated from its dam to enter the study. The remaining lamb was left with its dam on pasture. They were not part of the study. The lambs were collected from their dams over a 7 days period (15th September 2017 to 21st September 2017).

Four lambs (6.71 ± 0.34 kg) were slaughtered 24 h post-partum to provide baseline data for body composition. The remaining 28 lambs (5.94 ± 0.86 kg) were moved in-doors and individually penned and randomly allocated to one of three treatments; a normal commercial milk replacer (CMR control, n=10), a high protein milk replacer (HPM, n=9) or a mix of normal milk replacer and milk protein concentrate (MB, n=9). The MB composition was changed twice weekly to match the optimal CP:ME based on the lamb's theoretical CP and ME requirements (Danso et al., 2016). All lambs were fed at 2.1 times their maintenance requirement. The maintenance requirement was calculated as ME_m= 0.40 MJ/kg$LW^{0.75}d^{-1}$ (Danso et al., 2016). The CMR lambs were fed with commercial milk replacer containing 240 g/kg CP and 21.89 MJ/kg ME (Milligans Feed Ltd, Oamaru, New Zealand, Table 3.1). The commercial milk replacer was mixed with additional milk protein concentrate, which contained 478.7 g/kg CP and 19.15 MJ/kg ME (Fonterra, Auckland, New Zealand, Table 3.1) at a 62:38 ratio for HPM lambs. Lambs in MB treatment were fed a milk blend prepared by mixing the commercial milk replacer used

in CMR with the milk protein concentrate and this was adjusted twice weekly to match the CP:ME ratio requirements for each lamb. The twice-weekly adjusted CP:ME ratio was calculated based on their maintenance requirement and 300 g/d live weight gain (Danso et al., 2016). The live weights (LW) of lambs were recorded twice weekly (3 and 4 day intervals) and the amount of milk they were offered, altered accordingly.

Each day, the milk powder for each lamb in all three treatments was mixed with warm tap water at a 1:4 (w/w) ratio. Lambs were bottle-fed five times daily (at 7.00 a.m., 10.30 a.m., 2.30 p.m., 6.30 p.m. and 9.00 p.m.) up to two weeks of age, then four times daily to 4 weeks of age (at 7.00 a.m., 10.30 a.m., 2.30 p.m. and 7.00 p.m.) and then finally three times (at 7.00 a.m., 1.00 p.m. and 7.00 p.m.) daily until slaughter. Pellets (180 g/kg CP and 11.5 MJ ME, Table 3.1) were provided *ad libitum* for all the lambs starting from 2 weeks of age. The lambs had free access to water.

Individual milk and pellet intakes were recorded daily. The samples of commercial milk replacer, milk protein concentrate and pellets were collected and stored at -18 ^{0}C until required for chemical analysis.

3.3.2 Slaughter

All lambs were slaughtered when they reached the target LW of 22 kg regardless of age. Lambs were weighed after being fasted for 12 hours overnight and then slaughtered via captive bolt, exsanguinated, skinned and eviscerated. Weights of the head and feet, skin, hot carcass, viscera and blood were recorded. The gut fill was determined by weighing the stomach (combined weight of rumen, reticulum, omasum and abomasum) and intestines (duodenum, jejunum, ileum, caecum, colon and rectum) before and after removal of their contents. Individual weights of some organs (liver, kidney, heart, lungs,

and testis) were also measured. The whole carcass, viscera plus blood, head, feet and skin samples were stored at -20 ^{0}C in sealed plastic bags until being further processed.

3.3.3 Body tissues sampling

Body components were separated into two fractions: 1) carcass; 2) visceral organs plus blood and frozen. The fractions were cut into small blocks and minced separately through a 10 mm grinding plate (Wolfking Industrial mincer, William Jensen, Slagelse, Denmark). Subsamples of the ground carcass, organs plus blood were collected, weighed and stored at -20 ^{0}C until analysis. The head/feet and skin samples were not analyzed. The data from Danso et al. (2018) using the same genotype sheep was used to estimate protein and fat deposition in the head/ feet and skin.

3.3.4 Proximate analysis of samples

Milk replacer, milk protein concentrate and pellet samples were analysed for crude protein content by the Dumas method (method 968.06, AOAC, 2005). The fat content of milk powder and milk protein concentrate were determined by Mojonnier extraction method (method 989.05, AOAC, 2005) and gross energy content by bomb calorimetry.

Carcass and viscera plus blood samples were analysed for dry matter content by drying the sample at 105 ^{0}C in an oven (methods 930.15 and 925.10, AOAC, 2005) and ash content in a furnace at 550 ^{0}C (method 942.05, AOAC, 2005), crude protein content by the Dumas method (method 968.06, AOAC, 2005) and fat content by the Soxtec extraction method (method 991.36, AOAC, 2005).

3.3.5 Calculations

Individual milk and pellet intakes were calculated as the daily amount offered minus refusal. Daily ME and CP intakes were calculated based on milk and pellet intake

multiplied by their respective ME and CP concentrations (Table 3.1). The cumulative ME and CP intakes were calculated by summation of daily ME and CP intakes.

Average daily lamb LW gain (g) was calculated as the difference of LW at first and last day of the experiment (slaughter) and divided by the number of days to slaughter. The ME intake per average daily gain and CP intake per average daily gain were calculated as daily ME intake and daily CP intake divided by the average daily gain over the period, respectively.

Dry matter, water, fat, protein and ash content of carcass and viscera plus blood were calculated by multiplying the weights of carcass and viscera plus blood by their respective analysed chemical composition percentage value. Whole carcass composition was calculated as the summation of compositions of carcass and viscera plus blood.

The daily deposition of dry matter, water, fat, protein and ash during the gain of each lamb was calculated as the difference between the amount of dry matter, water, fat, protein and ash in the body at slaughter and the composition of each lamb at the start of the study which was calculated based on the baseline lamb data (n=4, slaughtered at 24 h after birth).

3.3.6 Statistical Analysis

One lamb in the MB group was excluded from the experiment and data analysis due to illness. Growth performance and weights of carcass and viscera plus blood were analysed with a linear model with treatment as a fixed effect by Proc GLM, SAS 9.4 (2013), considering individual lamb as the experimental unit. Carcass composition was analysed with a linear model with treatment as a fixed effect and carcass weights as a covariate by Proc GLM, SAS 9.4 (2013) and viscera plus blood composition was analysed in the same

manner considering viscera plus blood weight as a covariate. Differences were identified, where appropriate, using the least significant difference mean comparison test.

3.4 Results

3.4.1 Chemical composition of the diet

The chemical composition of milk replacer, milk protein concentrate and pellets are presented in Table 3.1.

Table 3.1 Chemical composition of milk replacer, milk protein concentrate and pellets fed to lambs

Chemical composition	Milk replacer	Milk protein concentrate	Pellets[1]
Crude protein, g/kg as fed	240.0	478.7	180
Fat, g/kg as fed	272.0	134.3	-
Gross energy, MJ/kg as fed	22.8	19.9	-
Metabolisable energy, MJ/kg	21.89[2]	19.15[2]	11.50[3]
CP:ME ratio[4], g/MJ	10.96	25.00	15.65

[1] Composed of barley, broll, soya bean and molasses.
[2] Calculated as metabolisability = 0.96 (Danso et al., 2018).
[3] Calculated based on gross composition and metabolisability reported by Danso et al. (2018).
[4] Calculated.

3.4.2 Intake and growth performances

Initial LW and slaughter weight did not differ (P>0.05) between treatments (Table 3.2). The average daily weight gain (ADG) was higher (P<0.05) in lambs fed MB and HPM than CMR lambs. Milk powder and pellet intakes were not different (P>0.05) between treatments.

Daily CP intake was greatest (P<0.001) in HPM treatment and lowest (P>0.05) in CMR lambs with MB intermediate. Daily ME intake was higher (P<0.05) in MB lambs than both other treatment groups, which did not differ (P>0.05). The milk CP:ME intake was greater (P<0.0001) for HPM lambs than MB lambs and least (P<0.0001) in CMR lambs. The total CP:ME intake was greatest (P<0.0001) in HPM lambs and lowest in CMR lambs (P<0.0001) than CMR lambs, MB lambs were intermediate. The ME intake per kilogram

LW gain was greatest (P<0.05) in CMR lambs, HPM and MB lambs did not differ (P>0.05). The CP intake per kilogram LW gain was greater (P<0.0001) in HPM lambs than both CRM and MB lambs, which did not differ (P>0.05).

Table 3.2 Intake and growth performance of artificially reared lambs fed milk replacers with variable crude protein to metabolisable energy ratio to 22 kg live weight (Mean ± Pooled SE, CMR, Commercial milk replacer; HPM, High protein milk; MB, Milk blend).

Item	Treatment			Pooled SE	P-value
	CMR	HPM	MB		
Initial live weight (LW), kg	5.92	5.95	5.90	0.30	0.994
Final LW, kg	22.34	22.97	22.67	0.19	0.077
Age at slaughter, d	70.20	65.44	63.38	2.24	0.105
LW gain, kg	16.42	17.02	16.76	0.30	0.359
Average daily gain (ADG), g/d	236[a]	261[b]	267[b]	6.61	0.006
Total milk powder intake, kg as fed	17.23	16.32	15.65	0.56	0.154
Total pellet intake, kg as fed	8.37	7.58	9.64	0.74	0.177
Total milk CP intake, kg	4.13[a]	5.53[b]	4.06[a]	0.15	<0.001
Total pellet CP intake, kg	1.51	1.36	1.73	0.13	0.177
Total CP intake, kg	5.64[a]	6.89[b]	5.79[a]	0.19	0.0001
Milk CP intake, g/d	58.93[a]	84.50[c]	64.12[b]	1.13	<0.0001
Pellet CP intake, g/d	21.71	20.83	27.48	2.00	0.067
Daily CP intake, g/d	80.64[a]	105.33[c]	91.61[b]	1.64	<0.0001
Total milk ME intake, MJ	377.07	347.74	341.53	12.13	0.101
Total pellet ME intake, MJ	96.30	87.17	110.84	8.46	0.177
Total ME intake, MJ	473.36	434.91	452.38	13.86	0.152
Milk ME intake, MJ/d	5.37	5.32	5.40	0.08	0.789
Pellet ME intake, MJ/d	1.39	1.33	1.76	0.13	0.067
Daily ME intake, MJ/d	6.76[a]	6.65[a]	7.15[b]	0.10	0.008
Milk CP:ME intake ratio, g /MJ	10.96[a]	15.89[c]	11.88[b]	0.02	<0.0001
Pellet CP:ME intake ratio, g/MJ	15.64	15.64	15.64	-	-
Total CP:ME intake ratio, g/MJ	11.92[a]	15.84[c]	12.80[b]	0.06	<0.0001
CP intake / LW gain, g/kg	343.30[a]	405.32[b]	344.45[a]	7.81	<0.0001
ME intake / LW gain, MJ/kg	28.82[b]	25.58[a]	26.91[a]	0.58	0.002

[a,b,c] 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).

3.4.3 Slaughter parameters

There was no difference (P>0.05) between treatment groups in carcass weight or dressing out percentage (Table 3.3). Further, no differences (P>0.05) were observed in weights of head and feet, whole skin, total empty viscera, liver, kidney, empty stomach, empty large intestine, blood, heart, lung and testis. The CMR lamb empty small intestine was heavier

(P<0.05) than HPM, and MB lambs did not differ (P>0.05) from HPM or CMR lambs. The omental fat weight was lower (P<0.05) in HPM lambs than CMR and MB lambs, which did not differ (P>0.05). Gut fill was greatest (P<0.05) in HPM lambs, while CMR and MB lambs did not differ (P>0.05).

Table 3.3 Slaughter parameters of artificially reared lambs fed milk replacers with variable crude protein to metabolisable energy ratio to 22 kg live weight (Mean ± Pooled SE, CMR, Commercial milk replacer; HPM, High protein milk; MB, Milk blend).

	Treatment			Pooled	P-value
Item	CMR	HPM	MB	SE	
Final LW, kg	22.34	22.97	22.67	0.19	0.077
Carcass weight, kg	10.62	10.62	10.91	0.14	0.277
Dressing, g/kg [1]	475.34	462.44	481.35	5.90	0.098
Head and feet, kg	2.11	1.95	2.10	0.14	0.663
Whole skin, kg	2.65	2.72	2.73	0.08	0.737
Total empty viscera weight, kg	3.67	3.50	3.59	0.08	0.325
Liver, g	418.29	441.98	465.78	15.05	0.106
Kidneys, g	97.42	95.12	101.42	4.73	0.659
Empty stomach, g [2]	439.12	414.08	437.40	34.08	0.845
Empty small intestine, g	952.44[b]	786.04[a]	834.04[ab]	43.37	0.028
Empty large intestine, g	417.48	387.08	410.73	17.56	0.441
Blood, g	1072.02	1248.76	1040.63	134.51	0.515
Heart, g	137.24	128.02	137.42	4.46	0.254
Lung, g	317.87	296.45	331.74	19.66	0.470
Testis, g	46.66	36.06	40.99	4.08	0.189
Gut fill, g	1762.50[a]	2156.40[b]	1674.14[a]	126.63	0.031
Omental fat, g	146.00 [b]	88.31[a]	131.54 [b]	9.81	0.001

[1] Carcass weight as a percentage of final live weight.
[2] Includes rumen, reticulum, omasum and abomasum.
[a,b] 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).

3.4.4 Body composition

Fat percentage of the carcass and viscera plus blood was lower (P<0.05) in HPM lambs compared to both CMR and MB lambs, which did not differ (P>0.05) (Table 3.4). Lambs fed HPM had a lower (P<0.05) fat content in their carcasses, viscera plus blood and carcass with viscera plus blood, than both CMR and MB fed lambs, which did not differ (P>0.05). All the other composition parameters did not differ (P>0.05) between treatments (Table 3.4).

Table 3.4 Chemical composition of carcass and viscera plus blood of the baseline group and artificially reared lambs fed milk replacers with variable crude protein to metabolisable energy ratio to 22 kg live weight (Mean ± Pooled SE, CMR, Commercial milk replacer

Item	Baseline (Mean ±SD)	Treatment			Pooled SE	P - value
		CMR	HPM	MB		
Carcass						
Dry matter, g/kg	269.1 ± 35.0	368.7	348.8	368.2	7.8	0.149
Protein, g/kg as is	169.8 ± 7.9	187.3	192.9	182.1	3.4	0.108
Fat, g/kg as is	28.5 ± 14.2	138.6[b]	102.2[a]	137.7[b]	6.1	0.0003
Ash, g/kg as is	57.0 ± 24.3	40.7	47.9	40.2	2.9	0.151
Hot carcass weight, kg	2.94 ± 0.26	10.62	10.62	10.91	0.14	0.277
Dry matter, kg	0.79 ± 0.11	3.95	3.74	3.94	0.09	0.161
Protein, kg	0.50 ± 0.04	2.01	2.07	1.94	0.04	0.101
Fat, kg	0.08 ± 0.04	1.49[b]	1.10[a]	1.46[b]	0.07	0.001
Ash, kg	0.17 ± 0.07	0.43	0.50	0.45	0.03	0.194
Water, kg	2.15 ± 0.25	6.76	6.97	6.76	0.09	0.161
Viscera plus blood						
Dry matter, g/kg	227.8 ± 29.2	233.1	224.0	240.5	4.9	0.093
Protein, g/kg as is	156.7 ± 12.7	132.8	141.9	136.1	3.1	0.128
Fat, g/kg as is	25.8 ± 3.7	65.4[b]	47.4[a]	75.3[b]	5.6	0.007
Ash, g/kg as is	12.4 ± 3.6	9.1	9.6	9.3	0.2	0.309
Empty viscera plus blood weight, kg	1.71 ± 0.13	4.75	4.51	4.63	0.1	0.194
Dry matter, kg	0.39 ± 0.05	1.10	1.01	1.11	0.02	0.054
Protein, kg	0.27 ± 0.04	0.62	0.66	0.63	0.02	0.236
Fat, kg	0.04 ± 0.01	0.30[b]	0.23[a]	0.35[b]	0.03	0.013
Ash, kg	0.02 ± 0.01	0.04	0.04	0.04	0.00	0.446
Water, kg	1.32 ± 0.12	3.49	3.47	3.41	0.04	0.369
Carcass and viscera plus blood						
Dry matter, kg	1.18 ± 0.16	5.01	4.78	5.07	0.10	0.154
Protein, kg	0.77 ± 0.08	2.62	2.72	2.59	0.04	0.091
Fat, kg	0.13 ± 0.04	1.78[b]	1.36[a]	1.80[b]	0.07	0.001
Ash, kg	0.19 ± 0.06	0.48	0.54	0.49	0.03	0.306
Water, kg	3.47 ± 0.27	10.27	10.44	10.18	0.10	0.204

[a,b] 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).

Daily fat deposition in carcasses, empty viscera plus blood and carcass with viscera plus blood of HPM fed lambs were lower (P<0.05) than both CMR and MB treatments, which did not differ (P>0.05) (Table 3.5). Higher (P<0.05) dry matter deposition rate in carcass, viscera plus blood and carcass with viscera and blood were observed for MB lambs

compared to both CMR and HPM fed lambs, which did not differ (P>0.05). The MB lambs had higher (P<0.05) water deposition rate in the carcass with viscera plus blood than CMR, and HPM lambs did not differ (P>0.05) from MB or CMR lambs. All the other daily deposition parameters did not differ (P>0.05) between treatments (Table 3.5).

The calculated values of daily protein deposition in whole empty body of CMR, HPM and MB lambs were 33.36, 42.2 and 41.31 g/d, respectively. Daily fat deposition in whole empty body of CMR, HPM and MB lambs were 26.47, 20.78 and 30.22 g/d, respectively.

Table 3.5 Dry matter, protein, fat, ash and water deposition rates in the carcass, viscera plus blood and the whole body of artificially reared lambs fed milk replacers with variable crude protein to metabolisable energy ratio to 22 kg live weight (Mean ± Pooled SE, CMR, Commercial milk replacer; HPM, High protein milk; MB, Milk blend).

Item	Treatment			Pooled SE	P - value
	CMR	HPM	MB		
Carcass					
Dry matter, g/d	45.79[a]	45.69[a]	52.57[b]	1.79	0.021
Protein, g/d	22.09	24.49	24.53	1.13	0.223
Fat, g/d	19.96[b]	15.49[a]	22.76[b]	1.06	0.0003
Ash, g/d	4.04	5.43	4.64	0.45	0.106
Water, g/d	68.20	76.04	78.76	3.09	0.057
Viscera plus blood					
Dry matter, g/d	10.85[a]	10.19[a]	12.23[b]	0.45	0.018
Protein, g/d	5.60	6.14	6.22	0.34	0.365
Fat, g/d	3.91[b]	2.69[a]	4.86[b]	0.40	0.004
Ash, g/d	0.36	0.38	0.39	0.02	0.529
Water, g/d	34.37	33.97	35.72	1.52	0.717
Carcass and viscera plus blood					
Dry matter, g/d	56.91[a]	56.16[a]	65.05[b]	1.92	0.007
Protein, g/d	27.86	30.80	30.91	0.97	0.053
Fat, g/d	23.87[b]	18.18[a]	27.62[c]	1.25	<0.0001
Ash, g/d	4.45	5.87	5.08	0.46	0.102
Water, g/d	103.31[a]	110.76[ab]	115.18[b]	3.25	0.049

[a,b,c] 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).

3.5 Discussion

3.5.1 Intake and growth performances

The higher CP to ME ratio of milk in both the HPM and MB treatments resulted in a 10.6 % and 13.1 % ADG increase respectively, relative to CMR lambs. Further, 67.2, 63.1 and

66.6 % of ADG were explained by the daily live weight gain in carcass with viscera plus blood in CMR, HPM and MB lambs, respectively. The remaining one-third of ADG could be accounted by gut fill and the weight gain of the head, feet and skin. However, the weight gain of the head, feet and skin were not measured in the current study. Increased ADG in response to feeding milk replacers with higher CP and higher CP to ME ratios has been reported previously (Ørskov et al., 1971, Danso et al., 2018). The CP and ME intakes per kilogram live weight gain of MB lambs was lower than HPM and CMR lambs, respectively for both parameters. Farmers utilizing milk replacers with changing CP:ME ratio over time to meet the lambs' changing requirements will result in higher average daily live weight gain in the pre-weaning period which should reduce the costs associated with milk replacer, as the MB treatment replacer contained less protein compared to HPM. However, the impacts on post-weaning growth are unknown.

Body protein gain increases as protein intake increases until energy becomes limiting (Andrews and Ørskov, 1970, van den Borne et al., 2006, Schroeder and Titgemeyer, 2008). In the current study HPM and MB lambs had similar ADG. Crude protein intake of HPM lambs was higher than MB lambs, but the metabolisable energy utilised per unit ADG did not differ. Hence, lambs fed HPM were not able to utilise the extra protein intake compared to MB lambs for live weight gain, as energy became the limiting factor.

3.5.2 Slaughter parameters

In this study, empty small intestine weight was lower in HMP fed lambs compared to CMR lambs. Lower empty intestine weight was also reported previously, in which lambs fed diets with high CP to ME ratio (≥13.38 CP:ME) (Kioumarsi et al., 2008, Danso et al., 2018) supporting the current finding in HPM lambs, which had the highest CP to ME intake ratio. Nitrogen absorption in the small intestine has been reported to be 66.7 % in

lambs (at 15-17 kg live weight) (Sklan and Halevy, 1985). Sklan and Halevy (1985) have reported that nitrogen absorption occurs rapidly in the proximal small intestine, with the rate decreasing with increasing distance from the stomach in lambs. Sklan and Halevy (1985) did not report any relationship between intestine weight and nitrogen absorption. There do not appear to be any studies on the relationship between CP intake and weight of small intestine of lambs. It is possible that high nitrogen intake could improve the nitrogen absorption efficiency in the proximal small intestine, resulting in reduced relative surface area of small intestine. This could explain why HMP lambs had lighter small intestines.

Lambs fed CMR may have an inadequate CP intake compare to the energy intake for protein deposition. Andrews and Ørskov (1970) have shown that the rate of protein deposition increases with the increase in concentration of CP in the diet, until the energy becomes the limiting factor. Hence, their daily protein deposition in carcass and viscera plus blood tended to be lower than both MB and HPM lambs.

The maximum protein deposition per day has been reported as 43 g/d in empty body weights (not including blood but including head, skin and feet) of Romney male lambs reared to 18 kg (Danso et al., 2018) at 14 g/MJ CP:ME. This increases slightly when lambs had a diet containing CP:ME more than 14 g/MJ. The data from Danso et al. (2018) for protein deposition rates of head, feet and skin for the same genotype sheep, were used to estimate whole body protein depositions in the present lambs. The ADG of lambs fed high protein milk in Danso et al. (2018) were reported as 260 g/d. Therefore, the ADG of MB and HPM lambs in current study are slightly higher than value reported by Danso et al. (2018). Lambs in both the HPM and MB treatments had higher daily CP and ME intake from milk and pellets than those reported by Danso et al. (2018). Thus, daily protein

deposition in whole empty body of both HPM (42.2 g/d) and MB (41.31 g/d) lambs (including blood, head, skin and feet) likely achieved maximum protein deposition rate. Further, protein deposition rates in whole empty body of HPM and MB lambs were higher than CMR lambs (26.5 and 23.8 %, respectively for HPM and MB lambs). This indicates feeding pre-weaned lambs to meet the demand of CP:ME or in excess of CP:ME improves the protein gain. The lambs in the present study were reared to 22 kg LW vs 18 kg LW by Danso et al. (2018) and average ages at slaughter were 66 vs 57 days, respectively. The protein deposition in the body varies with animal maturity (Silva et al., 2010), therefore, the calculated daily protein deposition values may be slightly higher than the actual.

Omental fat content and fat depositions in the carcass, viscera plus blood and whole carcass were lowest in HPM fed lambs. Fat deposition is known to be lower in lambs (Andrews and Ørskov, 1970, Norton et al., 1970, Ørskov et al., 1971, Danso et al., 2018) and calves (Labussiere et al., 2008) fed high protein diets, matching the HPM findings. Nitrogen excretion of lambs, steers and calves increases as nitrogen intake increases (Labussiere et al., 2008, Hales et al., 2013, Danso, 2016). Energy is required to process the excess nitrogen to urea and excretion (Kozloski et al., 2001, Reed et al., 2017). It has been reported that due to the energy cost associated with excess nitrogen excretion in dairy cows, heat production increased which led to an increase in maintenance requirement (Reed et al., 2017). Consequently, this reduced the retained energy in dairy cattle (Reed et al., 2017, Jennings et al., 2018). The lower fat deposition found in HPM lambs could have been due to an increased energy cost associated with the excretion of excess dietary nitrogen, and possibly an increase in heat loss. However, the energy cost of excess nitrogen excretion in lambs was not measured in the current study.

Inadequate protein relative to energy intake for tissue protein deposition, may have resulted in more fat deposition in the carcass, viscera plus blood and overall in carcass with viscera plus blood of CMR lambs compared to HPM lambs, which is consistent with Labussiere et al. (2008). Lambs in MB were fed milk to meet their CP:ME requirement, which was expected to result in less fat deposition in the carcass. However, fat deposition in the carcass and viscera plus blood components in the MB lambs similar to CMR lambs. The MB lambs had higher daily ME intake from both milk and pellets compared to CMR lambs. This excess daily energy intake than maintenance and protein deposition requirements of MB lambs, could deposited as fat. Therefore, MB lambs had similar fat deposition as CMR lambs.

The fat deposition rates of head, feet and skin for the same genotype sheep reported by Danso et al. (2018) were used to estimate whole body fat deposition rate in the present lambs. Danso et al. (2018) reported fat deposition rate of 24.1 and 17.5 g/d, for normal milk replacer and high protein milk fed lambs with CP:ME ratio of 11.5 and 14.5 g/MJ, respectively. Fat deposition rate in the whole empty body of lambs in the present study were higher than the values reported by Danso *et al.* (2018). In addition, the pellet and total CP:ME intakes of lambs in present study were higher than those reported by Danso et al. (2018). Fat deposition rate increases with the maturity of lambs (Bellof and Pallauf, 2004a). Lambs in the present study were reared to 22 kg compared to 18 kg LW in Danso *et al.* (2018) and were older at slaughter (on average, 66 vs 57 days age). Hence, higher daily fat deposition could be due to the maturity of lambs and higher pellet intake which increased total CP:ME intake compared to that of Danso et al. (2018). Dry matter deposition rate of carcass and viscera plus blood in MB lambs was higher than both HPM and CMR lambs. This could be attributed to both higher fat deposition rate and observed

tendency of higher protein deposition rate in MB lambs compared to HMP and CMR lambs, respectively.

Farmers are paid premiums for lean lamb carcass production in New Zealand (Schreurs, 2012). Lambs fed a milk replacer with adjusted CP:ME ratio and HPM increased the ADG and had a tendency for higher protein deposition rate in the carcass with viscera plus blood. Further, feeding of a milk replacer with adjusted CP:ME ratio could reduce the costs of milk replacer, as the MB contained less protein compared to HPM. Hence, farmers could be financially benefited by feeding lambs with a milk replacer adjusted to change to meet their changing CP:ME requirements. The HPM fed lambs had faster growth than CMR, so HPM also could be beneficial to farmers.

Mammary gland growth of Romney and Romney cross ewe lambs has been reported to be slow from near birth (± 5 days from birth, 5.5 kg LW) to three months of age (24.4 kg LW), followed by a rapid growth at fourth month and reaches a plateau at month five with little increase during next several months (Anderson, 1975). A rapid weight gain from weaning (16 kg LW at 42 days age) to puberty has been reported to impair the mammary gland development and subsequent milk production of ewe lambs (McCann et al., 1989). Umberger et al. (1985) also found that accelerated growth from weaning (20 kg LW) to puberty reduces the milk production of lambs in their first lactation. The lambs in present study examined the pre-weaning period and lambs were slaughtered at 22 kg LW (66 days of age on average). Hence, increased ADG of HPM and MB lambs would likely to have little effect on mammary gland growth and subsequent milk production. However, no attempt was made to investigate the impacts of accelerated growth during pre-weaning on mammary gland development and milk production

3.6 Conclusion

Adjustment of the CP:ME ratio of milk replacer to meet the changing requirements of lambs pre-weaning increased average daily gain, dry matter and fat deposition rates in carcass and viscera plus blood. The CP and ME intakes per kilogram live weight gain of CP:ME adjusted milk fed lambs were lower than HPM and CMR lambs, respectively. The protein content of CP:ME adjusted milk was less than high protein milk, which could be economical for farmers. High protein milk could also be used instead of commercial milk replacer (a low CP:ME ratio) resulting in greater average daily gains and less fat deposition in the carcass and viscera plus blood.

These findings suggest lamb growth can be improved in artificial rearing systems by choosing the optimum CP:ME ratio. However, further research is warranted of the effect of pre-weaning CP:ME adjusted milk replacer feeding on post-weaning lamb performance, to determine feeding strategies to ensure less fat deposition in the carcass allowing farmers to optimize the financial output and cost effectiveness.

Chapter 4 Effect of dietary protein to energy ratio on growth performance of pre- and post-weaned lambs

This Chapter has been published in full elsewhere. It has been reformatted and presented here. Citation: H.M.G.P. Herath, Pain, SJ., Kenyon, PR., Blair, HT., Morel, PCH. 2021. Effect of dietary protein to energy ratio on growth performance of pre-and post-weaned lambs. Animal Feed Science and Technology. 272, 114787. https://doi.org /10.1016/j.anifeedsci.2020.114787 (The DRC form is attached in appendices).

4.1 Abstract

Milk replacers adjusted for crude protein to metabolizable energy (CP:ME) ratio to match the lambs' changing requirement during first few weeks post-birth have resulted in higher pre-weaning lamb growth rate compared to commercial milk replacers with a static CP:ME ratio. This study aimed to determine if milk replacer with an adjusted CP:ME ratio to meet the lambs changing CP:ME requirement over time improved lambs growth performance during both the pre-and post-weaning periods. Twenty-seven Romney twin-born ram lambs were assigned to one of two pre-weaning milk feeding treatments: i) a commercial milk replacer (CMR, n=14; CP:ME 12.28 g/MJ); or ii) a regularly adjusted blend of commercial milk replacer and milk protein concentrate (MB, n=13; CP:ME starting at 16.6 g/MJ and adjusted down to approximately 13 g/MJ). All lambs were fed at 2.1 times their maintenance energy requirement. The CP:ME ratio of the MB treatment was adjusted twice-weekly to match the changing CP:ME requirements. All the lambs had *ad libitum* access to pellets. Lambs were weaned at 22 kg live weight (LW), transferred to a ryegrass and white clover-based pasture and reared approximately to 46 kg LW. The LW during pre-and post-weaning was recorded twice weekly and fortnightly, respectively. In the pre-weaning period, total daily CP intake and daily CP intake from milk were greatest ($P<0.05$) in MB compared to CMR lambs. Daily ME intake of lambs in the pre-weaning period did not differ ($P>0.05$) between treatments. Lambs fed MB had greater total and milk CP:ME intake ($P<0.0001$), compared to CMR lambs. Average daily LW gain (ADG) during the pre-weaning phase did not differ ($P>0.05$) between treatments. The ME intake per kilogram LW gain during the pre-weaning did not differ ($P>0.05$) between treatments. The CP intake per kilogram LW gain during the pre-weaning was higher ($P<0.05$) for MB lambs than CMR lambs. In the post-weaning period

lamb LW gain and ADG did not differ (P>0.05) between treatments. There was no correlation (P>0.05) in overall ADG of lambs between pre-and post-weaning periods. The ADG during day one to ten at the start of experiment had a positive and significant effect on the overall post-weaning ADG (r=0.570, P=0.007) and ADG in first two weeks (r=0.470, P=0.032) of the post-weaning phase. Similar pre-and post-weaning growth rates were observed for lambs in both treatment groups, despite the pre-weaning adjustment of CP:ME to meet lambs requirements. The CMR feeding was cost effective as the pre-weaning feed cost per kilogram live weight gain was lower than that of milk blend feeding. As CMR lambs likely obtaining additional CP from pellet intake during pre-weaning, further research is warranted on the effect of CP:ME ratio of pellets, pellet intake and digestibility on growth performance of lambs reared artificially.

Keywords: Growth, Lamb, Milk replacer, Pre-weaning, Post-weaning, Protein to energy ratio

4.2 Introduction

A lamb's requirement for crude protein (CP) relative to metabolisable energy (ME) changes as it grows, with a greater CP:ME ratio needed at the initial stage of growth (e.g. 14.2 g/MJ at 5 kg live weight) and a lower ratio in later stages (e.g. 12.2 g/MJ at 18 kg live weight; Danso et al., 2016). Pre-weaning average live weight gain of lambs can be improved by feeding a milk replacer with a high, static CP:ME (15.89 g/MJ) or a milk replacer/milk protein concentrate blend which is adjusted regularly to match changing CP:ME requirements, compared to a commercial milk replacer with a lower, static CP:ME (CP:ME 10.96 g/MJ) (Herath et al., 2020). The rate of protein deposition in carcass and viscera tends to be higher in lambs fed a CP:ME adjusted milk replacer/milk protein concentrate blend compared to commercial milk replacer-fed lambs, whilst fat

deposition did not differ (Herath et al., 2020). Combined, these data indicate that feeding a high CP:ME milk replacer or adjusting the CP:ME content of the diet to meet the changing requirements of lambs during the pre-weaning phase, positively influences lean lamb growth up to 22 kg of live weight. Industry responded to these findings and changed the CP content of commercial milk replacer used in New Zealand from 240 g/kg to 260 g/kg while maintaining the same ME content. Consequently, the CP:ME ratio of commercial milk increased from 10.96 g/MJ to 12.28 g/MJ. Whether this change to CP:ME of commercial milk replacer, results in improved lamb growth performances is yet to be determined.

Pre-weaning growth and weaning weight are known to have an effect on post-weaning growth rate of lambs (Fraser and Saville, 2000, Selaive -Villarroel et al., 2008, Bhatt et al., 2009). In an artificial rearing system, lambs are typically fed milk replacers and concentrate pellets until weaned (at approximately 10 weeks of age) on to a pasture-based diet. Post-weaning lamb growth rates decline upon removal of concentrate meal and changing to a diet of solely pasture (Jensen et al., 2017). Greater rate of post-weaning growth is desired, which allows to slaughter lambs at a younger age with a targeted slaughter weight. Thus, farmers are benifited by reduced overall feed requirement, labour, animal health inputs and cost associated (Beef + Lamb New Zealand, 2014).

This study investigated the effect of CP:ME adjusted milk replacer on pre- and post-weaning growth when lambs were weaned on to ryegrass and white clover based pasture. It was hypothesised that feeding of a milk replacer with a changing CP:ME (16.6 g/MJ to 13.3 g/MJ over 57 days) to match lamb requirements during the pre-weaning phase would improve lamb growth in both the pre- and post-weaning phases compared to lambs fed with a constant, lower CP:ME (12.28 g/MJ) milk replacer.

4.3 Materials and methods

The experiment was carried out at Massey University, Palmerston North, New Zealand from September 2018 to March 2019. The research procedures used were approved by the Massey University Animal Ethics Committee (MUAEC 18/39).

4.3.1 Experimental design and animal management

The experiment was conducted over four different lamb growth phases including pre-weaning, milk weaning, transition to pasture and post-weaning (Table 4.1).

4.3.1.1 Pre-weaning

Twenty-seven Romney ram lambs born to twin-bearing ewes were selected for the study. Lambs were allowed to suckle from their dam for the first 24 hours after birth before a male lamb from the set was selected and separated from its dam to enter the study. The individual lambs were collected from their dams over a 19-day period (18th September 2018 to 4th October 2018) and were moved in-doors and individually penned. The average minimum and maximum temperatures of the lamb shed during the experiment were 13 ± 0.23 ^{o}C and 17 ± 0.26 ^{o}C.

The selected lambs (5.28 ± 0.59 kg) were randomly allocated to one of two treatments; a commercial milk replacer (CMR, control, n=14) or a milk replacer/milk protein concentrate blend (MB, n=13). The CP:ME ratio in the MB was changed twice weekly to best match the optimal CP:ME based on the lambs theoretical CP and ME requirements (Danso et al., 2016). All lambs were fed at 2.1 times their maintenance energy requirement (Herath et al., 2020). The maintenance energy requirement was calculated as ME_m= 0.40 MJ/kg $LW^{0.75}d^{-1}$ (Danso et al., 2016).

Table 4.1 Different phases of lamb growth during the experiment

	Growth phase			
	Pre-weaning	Milk weaning	Transition to pasture	Post-weaning
Age (days)	2 - approximately 56*	about 57 - 63	64-84	85-161
Number of weeks in each phase	approximately 8*	1	3	10
Milk	Full allowance	Reduced gradually	No	No
Pellets	*Ad libitum*	*Ad libitum*	Reduced gradually	No
Lucerne chaff	42 g/d/lamb from 42-56 d age	42 g/d/lamb	No	No
On pasture	No	2-8 hrs/day	24 hrs	24 hrs
Indoor/outdoors	Indoors	Indoors and outdoors	Outdoors	Outdoors

* The period from 2 days old until lambs reach to 22 kg live weight.

The CMR contained 260.84 g/kg CP and 21.23 MJ/kg ME (Milligans Feed Ltd, Oamaru, New Zealand, Table 4.2). The MB lambs were fed a milk replacer prepared by mixing the CMR with a milk protein concentrate which contained 569.90 g/kg CP and 17.59 MJ/kg ME (Fonterra, Auckland, New Zealand, Table 4.2). The relative quantities of CMR and milk protein concentrate were adjusted twice weekly for lambs to match the individual CP:ME ratio requirements based on their live weight, maintenance requirements and 300 g/d live weight gain (Danso et al., 2016). Lamb live weights (LW) were measured twice weekly and the amount of milk replacer/blend they were offered was altered accordingly.

Each day, the milk replacer/blend for each lamb was mixed with warm tap water in 1: 4.0 (w/w) ratios until four to six weeks age and 1:3.5 (w/w) ratio from then until weaning. The quantity of water mixed was reduced at four to six weeks of age due to two abomasal bloat incidents observed in lambs. Lambs were bottle-fed five times daily (at 7.00 a.m., 10.30 a.m., 2.30 p.m., 6.30 p.m. and 9.00 p.m.) up to two weeks of age, then four times daily to four weeks of age (at 7.00 a.m., 10.30 a.m., 2.30 p.m. and 7.00 p.m.) and then finally three times daily (7.00 a.m., 1.00 p.m. and 7.00 p.m.) until weaning (Danso et al.,

2018, Herath et al., 2020). Pellets (184.38 g/kg CP and 10.28 MJ/kg ME; Denver Stock Feeds, Palmerston North, New Zealand, Table 4.2) were provided *ad libitum* for all the lambs starting from eight days of age. The lambs had free access to water. Lucerne chaff (207.5 g/kg CP and 7.36 MJ/kg ME; Oaklane Stables Premium Chaff, Hawkes Bay, New Zealand, Table 4.2) was provided (0.2 ± 0.03 % of live weight) for lambs from six to eight weeks of age until they were weaned.

Five lambs out of 27 were excluded from the final experiment analysis of pre-weaning phase due to health reasons (n= 4 and n= 1 for CMR and MB treatments, respectively). Individual milk, pellet and lucerne chaff intakes were recorded daily. Samples of commercial milk replacer, milk protein concentrate, pellet and lucerne chaff were collected and stored at -20 ^{o}C until analysis for proximate composition.

4.3.1.2 Milk weaning and transition to pasture

The period of milk weaning began when the lambs reached an average LW of 22.18 ± 1.30 kg. Regardless of age, the quantity of milk replacer/blend offered was reduced daily by 12 % of the original milk quantity, over seven days before total removal. The lambs were gradually transitioned to a ryegrass white clover-based pasture by being placed on a ryegrass and white clover-based paddock for 2, 3, 4, 5, 6, 7 and 8 hours per day over the seven-day period. Pellets were provided *ad libitum* during this phase. All the lambs were provided with lucerne chaff (42 g/d). Milk replacer/blend, pellet and lucerne chaff intakes were recorded daily and lambs had free access to water. Pasture intake was not recorded. One lamb from the MB treatment was excluded from the final experimental analysis due to health reasons.

After total milk replacer/blend removal, during the transition to pasture phase, lambs were managed permanently outdoors on pasture. Pellets were offered to lambs as a group. The

average pellet intake of lambs during the milk weaning phase was used as the initial pellet quantity to be offered at the start of the transition to pasture phase. This was then reduced by 5 % daily over a period of 20 day. The LW of lambs was recorded weekly during the transition phase.

4.3.1.3 Post-weaning

Following the transition phase, during the post-weaning phase, lambs were offered only ryegrass and white clover-based pasture at *ad libitum* levels (> 1200 kg DM/ha pasture mass, Morris and Kenyon, 2004). Lambs from each treatment (CMR, n=10 and MB, n=11) were randomly split into two post-weaning groups (replicates), each containing five and six lambs from the CMR and MB treatments, respectively for five weeks. Then lambs were reared as a mob till the end of experiment (five weeks). Lambs had free access to water in the paddock. Eight paddocks with a total area of 2.47 ha were used. Pasture mass was determined using a plate meter (Jenquip, Feilding, New Zealand) by taking 50 measures per paddock at pre- and post-grazing. Pasture mass in paddocks was calculated by average plate meter reading multiplied by 158 plus 200 kg dry matter (Kenyon et al., 2011). Pasture grab samples from each paddock (eight samples were collected fortnightly when lambs were reared as separate treatment groups and four samples at the start of lamb grazing as a mob) were collected during the post-weaning to determine the proximate composition (Kenyon et al., 2011).

All lambs were drenched with Matrix (Merial New Zealand Limited, Auckland, New Zealand) and vaccinated with Ultravac® 7-in-1 vaccine (Zoetis New Zealand Limited, Auckland, New Zealand) at the end of first week on the pasture. Lambs were weighted fortnightly. The lambs were reared on pasture until an average of 161.5 ± 1.96 and 159.36

± 1.72 days of age, when average LWs were 46.02 ± 3.46 and 45.45 ± 2.28 kg, respectively, for CMR and MB.

4.3.2 Proximate analysis of samples

4.3.2.1 Pre-weaning and transition phases

One representative sample of milk replacer and pellets were collected from each milk replacer and pellets bags at unpacking (15 samples each) and three composite samples were prepared by pooling all the samples collected. Three representative samples of milk protein concentrate and lucerne chaff were collected. Samples were analysed at the Nutrition Laboratory, Massey University, Palmerston North, New Zealand. Three samples from each feedstuff were analysed for dry matter content by drying the sample at 105 ^{o}C in an oven (methods 930.15 and 925.10, AOAC, 2016), ash content in a furnace at 550 ^{o}C (method 942.05, AOAC, 2016), crude protein content by Dumas method (method 968.06, AOAC, 2016) and gross energy content by bomb calorimetry.

The fat content of milk replacer and milk protein concentrate was determined by the Mojonnier extraction method (method 989.05, AOAC, 2016) and the fat content of pellets was determined by Soxtec extraction method (method 2003.06, AOAC, 2016). The lactose content of milk replacer and milk protein concentrate was determined using an enzymatic method (Boehringer Mannheim/R-Biofarm Enzyme kit for Lactose/D-Galactose − enzymatic digestion colorimetric was determined at 340 nm).

The mineral content (calcium, magnesium, potassium, sodium, phosphorus and sulphur) of milk replacer, milk protein concentrate and pellets was determined by inductively coupled plasma emission spectrometry (Thermo iCAP 6000 series ICP-OES). Chloride content of milk replacer, milk protein concentrate and pellets was determined by the potentiometric titration method. Neutral detergent fibre, acid detergent fibre and lignin

content of pellets and lucerne chaff were determined using the Fibretec System (method 2002.04 and 973.18, AOAC, 2016).

4.3.2.2 Post-weaning phase

Pasture samples were analysed at the Nutrition Laboratory, Massey University, Palmerston North, New Zealand. Twelve pasture grab samples were analysed for dry matter, ash, crude protein, fat, neutral detergent fibre, acid detergent fibre, organic matter digestibility and metabolisable energy content by near-infrared spectroscopy (NIRS, Bruker PMI Ettlingen, Germany) as per previously reported studies (Kenyon et al., 2011, Kenyon et al., 2014, Corner-Thomas et al., 2015). The NIRS was calibrated for wet chemistry data obtained for New Zealand herbage (Corson et al., 1999).

4.3.3 Calculations

Individual milk replacer/blend, pellet and lucerne chaff intakes during the pre-weaning and transition phases were calculated as the daily amount offered minus refusal (Danso et al., 2018, Herath et al., 2020). Daily ME and CP intakes were calculated based on milk replacer/blend, pellet and lucerne chaff intake multiplied by their respective ME and CP concentrations (Table 4.2). The cumulative ME and CP intakes were calculated by summation of daily ME and CP intake from the different feed types. The ME and CP intakes during 1-10 d, 11-20 d, 21-30 d, 31-40 d and 41-50 d in the pre-weaning phase were calculated based on milk replacer/blend, pellet and lucerne chaff intake during each period multiplied by their respective ME and CP concentrations. The CP:ME intake of each period was calculated by CP intake during each period divided by the respective ME intakes. Average daily lamb LW gains in the overall pre-weaning, different phases of pre-weaning (1-10 d, 11-20 d, 21-30 d, 31-40 d and 41-50 d), transition to pasture and post-

weaning phases were calculated as the difference between LW at the first and last day of each period divided by the number of days in each phase.

The ME intake and CP intake per kilogram LW gain were calculated as daily ME intake and daily CP intake divided by the average daily gain over the period of gain, respectively, for pre-weaning and transition phases.

Cost of commercial milk replacer or milk blend, pellets and lucerne chaff during pre-weaning phase were calculated as total intake multiplied by their respective cost per kilogram. The total feed cost was calculated by summation of cost for milk replacer or milk blend, pellets and lucerne chaff. Feed cost per live weight gain during the pre-weaning was calculated as total feed cost divided by pre-weaning live weight gain.

4.3.4 Statistical Analysis

Growth performances during pre-weaning, transition and post-weaning phases were analysed using a linear model with treatment as a fixed effect (Proc GLM, SAS 9.4, 2013), considering individual lamb as the experimental unit. Feed, CP and ME intakes during pre-weaning, transition and post-weaning phases and feed cost analysis for pre-weaning phase were analysed using a linear model with treatment as a fixed effect (Proc GLM, SAS 9.4, 2013).

Correlations between unadjusted values of ADG in pre- and post-weaning phases and between ADGs of different periods of pre-weaning and transition, post-weaning phases were analysed using Proc CORR, SAS 9.4 (2013). Correlations between pre-weaning average daily pellet intake and ADGs in each week and overall ADG in transition phase, and between pre-weaning average daily pellet intake and ADGs in fortnightly and overall in post-weaning phase were also analysed using Proc CORR, SAS 9.4 (2013).

4.4 Results

4.4.1 Chemical composition of the diet

The chemical composition of commercial milk replacer, milk protein concentrate, pellet and lucerne chaff are presented in Table 4.2.

Table 4.2 Chemical composition of commercial milk replacer, milk protein concentrate, pellet and lucerne chaff fed to lambs on fresh matter basis.

Chemical composition	Commercial milk replacer	Milk protein concentrate	Pellet [1]	Lucerne chaff
Dry matter, g/kg	973.72	965.00	875.67	867.44
Ash, g/kg	56.58	75.23	94.80	77.79
Crude protein, g/kg	260.84	569.90	184.38	207.50
Fat, g/kg	244.2	9.36	24.76	N/A
Lactose, g/kg	365.33	271.00	-	N/A
Minerals, g/kg				
Calcium	8.77	17.03	10.23	N/A
Magnesium	8.03	1.07	2.80	N/A
Potassium	11.30	11.46	12.60	N/A
Sodium	2.60	2.10	3.20	N/A
Phosphorus	6.96	12.03	5.33	N/A
Sulphur	2.46	4.76	2.10	N/A
Chloride, mg/kg	0.64	0.53	0.15	N/A
Gross energy, MJ/kg	22.12	18.72	15.12	16.04
Neutral detergent fibre, g/kg	-	-	199.67	294.67
Acid detergent fibre, g/kg	-	-	76.33	220.33
Lignin, g/kg	-	-	16.67	53.00
Metabolisable energy, MJ/kg [2]	21.23	17.59	10.28	7.36
CP:ME ratio[3], g/MJ	12.28	32.39	17.93	28.2

[1] Composed of barley, broll, soya bean and molasses.
[2] Calculated considering metabolisability coefficient= 0.96 for milk replacer and milk protein concentrate, 0.68 for pellet (Danso et al., 2018) and 0.459 for lucerne chaff (Thomson and Cammell, 1979), so metabolisable energy =metabolisability coefficient *gross energy.
[3] Calculated.
- Absent in feed type.
N/A not analysed.

4.4.2 Pre-weaning growth and intake

Initial live weight (LW), LW at weaning, age at weaning and LW gain in pre-weaning did not differ (P>0.05) between treatments (Table 4.3). Average daily gain (ADG) (Table 4.3) and pellet intake (Figure 4.1) for the periods of 1-10 d, 11-20 d, 21-30 d, 31- 40 d

and 41-50 d did not differ (P>0.05) between treatments. Dry matter intake (DMI) of milk replacer/blend, pellet and lucerne chaff did not differ (P>0.05) between treatments (Table 4.4).

Total CP intake from all feedstuff and CP intake from milk replacer were greater (P<0.05) in the MB than the CMR group (Table 4.4). The CP intake per kilogram of LW gain was 10.6 % higher (P<0.05) in MB lambs compared with CMR lambs. Total ME intake, ME intake from milk and ME intake per kilogram LW did not differ (P>0.05) between treatments (Table 4.4).

The CP:ME intake from milk was greater (P<0.0001) in MB lambs than CMR lambs (14.73 ± 0.04 and 12.28 ± 0.00 g/MJ, respectively). The combined CP:ME intake (from milk, pellets and lucerne chaff) was greater (P<0.0001) in MB lambs than CMR lambs (15.65 ± 0.04 and 13.87 ± 0.12 g/MJ, respectively).

The CP:ME intake of CMR lambs increased as they aged, while the CP:ME intake of MB lambs declined with increasing age (Figure 4.2). The CP:ME requirement of lambs in present study ranged from 14.5 to 11.5 g/MJ. The combined CP:ME intake of individual lamb and their CP:ME requirement at each weighing during the pre-weaning period is presented in Figure 4.3. The MB lambs had higher CP:ME intake than their requirement throughout the pre-weaning phase while all CMR lambs exceeded their requirement approximately after 12 kg LW. The ADG was higher than 300 g/d, when lambs had total CP:ME intake ratio of 14.0 -15.5 g/MJ, irrespective of treatment group.

Table 4.3 Pre-weaning growth of artificially reared lambs to 22 kg live weight (day 2 to approximately 56) on commercial milk replacer (CMR) or milk blend (MB) with variable CP:ME.

Item	Treatment		Pooled SE	P – value
	CMR	MB		
Initial live weight (LW), kg	5.22	5.25	0.40	0.92
LW at weaning, kg	22.12	22.23	0.19	0.84
Age at weaning, d	57.20	56.83	1.21	0.83
LW gain, kg	16.90	16.98	0.42	0.89
Average daily gain, g/d				
1-10 days	106.6	105.7	4.33	0.88
11-20 days	174.3	188.0	14.91	0.52
21-30 days	280.3	251.3	22.63	0.37
31-40 days	312.0	371.5	23.04	0.08
41-50 days	448.6	486.6	29.13	0.36
Overall 1-50 days	267.1	280.9	11.05	0.38
Overall day 1 to weaning	297.2	299.0	8.19	0.88

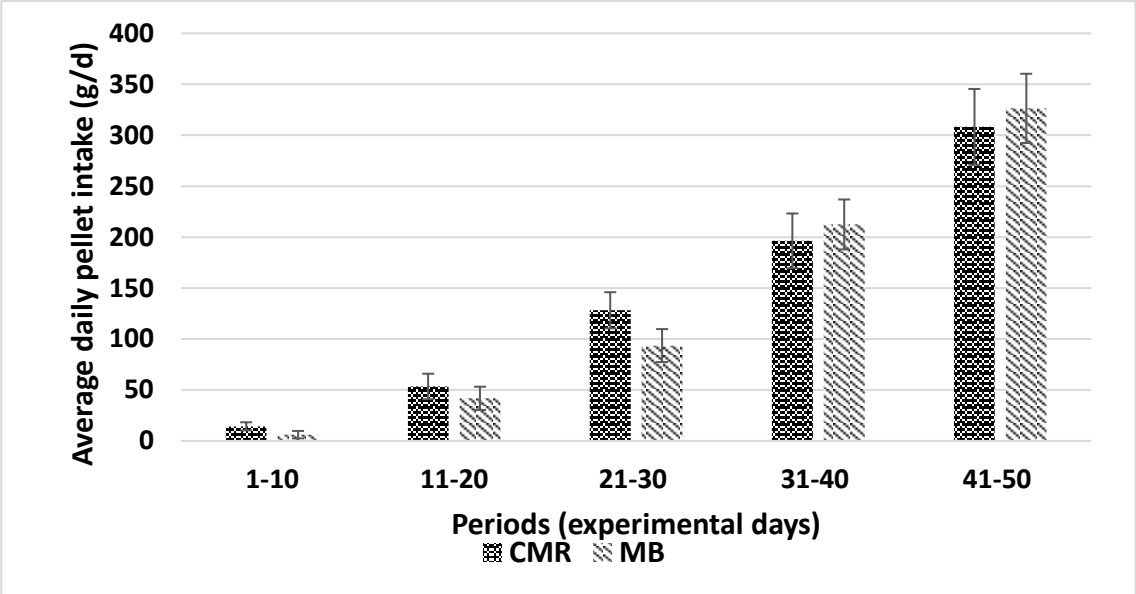

Figure 4.1 Average daily pellet intake of artificially reared lambs fed milk replacer (CMR) or milk blend (MB) with variable CP:ME during different periods in pre-weaning phase (day 2 to approximately 50)

Table 4.4 Pre-weaning feed, crude protein and metabolizable energy intake of artificially reared lambs (day 2 to approximately 56) fed commercial milk replacer (CMR) or milk blend (MB) with variable CP:ME.

Item	Treatment		Pooled SE	P - value
	CMR	MB		
Total milk replacer DMI, kg	12.27	12.34	0.34	0.89
Total pellet DMI, kg	8.55	8.49	0.66	0.95
Total lucerne chaff DMI, kg	0.16	0.16	0.05	0.95
Total DMI, kg	20.98	20.99	0.76	0.99
Total milk CP intake, kg	3.29 [a]	3.87 [b]	0.09	0.0003
Total pellet CP intake, kg	1.80	1.79	0.14	0.95
Total lucerne chaff CP intake, kg	0.04	0.04	0.01	0.95
Total CP intake, kg	5.12 [a]	5.70 [b]	0.17	0.03
CP intake / daily LW gain, g/kg	303.42 [a]	335.67 [b]	4.22	0.0012
Total milk ME intake, MJ	267.53	263.14	5.15	0.68
Total pellet ME intake, MJ	100.40	99.74	5.37	0.95
Total lucerne chaff ME intake, MJ	1.35	1.38	0.27	0.95
Total ME intake, MJ	369.29	364.26	7.45	0.74
ME intake / daily LW gain, MJ/kg	21.88	21.44	0.28	0.46

[a,b] Means in the same row with no superscripts are not significantly different (P>0.05).
DMI = dry matter intake

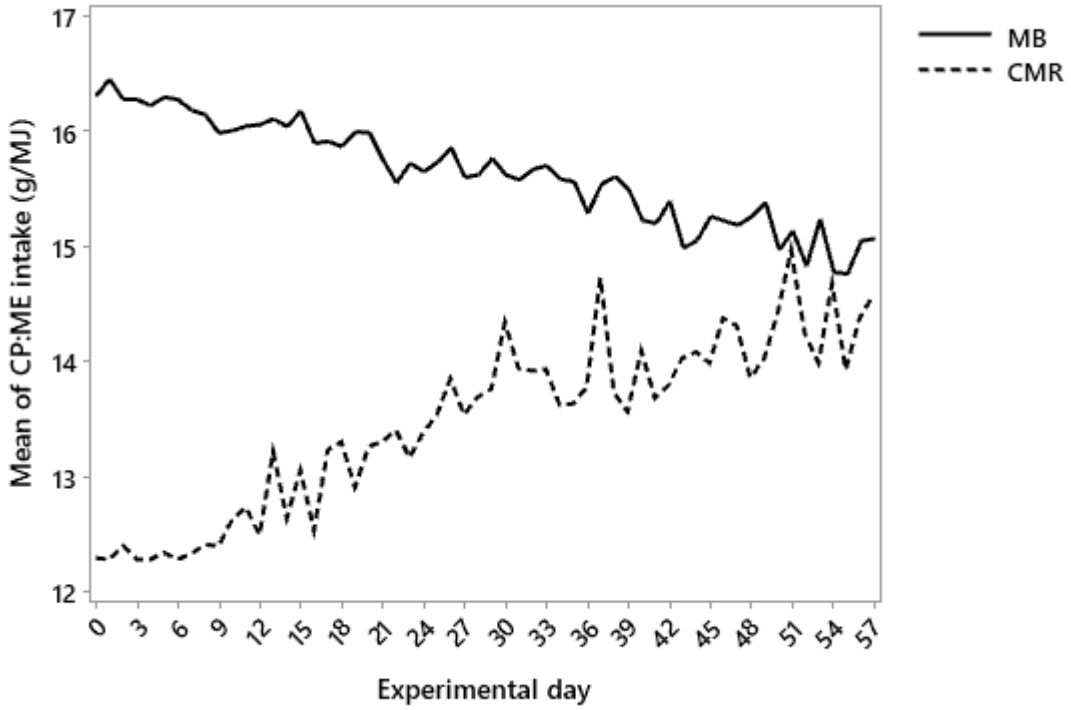

Figure 4.2 Mean total CP:ME intake ratio of ration (from milk replacer, pellets and lucerne chaff) at each point of weighing of artificially reared lambs fed commercial milk replacers (CMR - - -) or milk blend (MB ——) to 22 kg live weight during pre-weaning phase (day 2 to approximately 56).

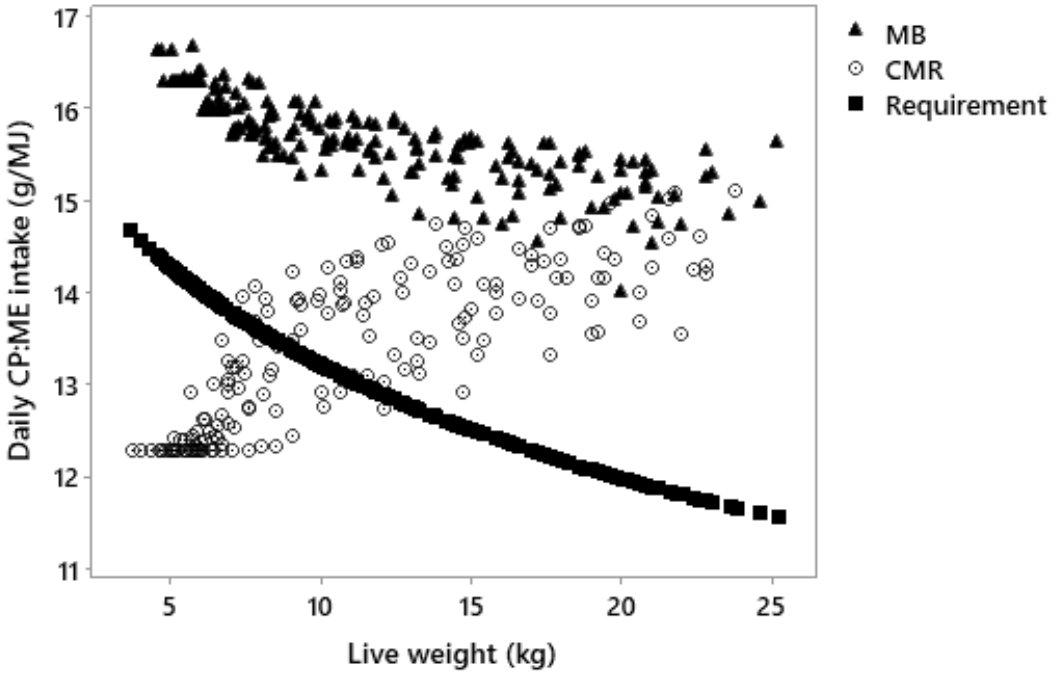

Figure 4.3 The combined CP:ME intake ratio of ration (from milk replacer, pellets and lucerne chaff) and CP:ME requirement (■) at each point of weighing of artificially reared lamb fed commercial milk replacers (CMR ⊙) or milk blend (MB ▲) to 22 kg live weight during pre-weaning phase (day 2 to approximately 56).

4.4.3 Pre-weaning feed cost analysis

Cost of milk and total feed per lamb were higher (P<0.05) for lambs fed MB compared to CMR (Table 4.5). The cost of pellets and lucerne chaff did not differ (P>0.05) between treatments. Total feed cost per kilogram live weight gain of lambs was lower (P<0.05) in CMR treatment compared to MB lambs.

Table 4.5 The cost analysis of lambs reared artificially up to weaning, fed commercial milk replacers (CMR) or milk blend (MB) with variable CP:ME ratio

Item (NZ$)	Treatment		Pooled SE	P-value
	CMR	MB		
Milk cost/lamb	69.55 [a]	78.08 [b]	1.98	0.0066
Pellet cost/lamb	7.32	7.27	0.57	0.9524
Lucerne chaff cost/lamb	0.38	0.39	0.11	0.9502
Feed cost /lamb	77.25 [a]	85.74 [b]	2.09	0.0097
Feed cost/ kg live weight gain	4.59[a]	5.06[b]	0.12	0.0090

[a,b] 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).

4.4.4 Growth and intake during transition to pasture

Lamb LW on the last day of transition to pasture, ADG (134.29 and 141.15 g/d, respectively for CMR and MB lambs) and LW gain during the transition period did not differ (P>0.05) between treatments. The overall ADGs of CMR and MB lambs during transition were reduced by 54.8% and 52.8%, respectively, compared to their overall ADGs during the pre-weaning phase. Dry matter, CP and ME intakes from pellets, ME and CP intakes per kilogram LW gain did not differ (P>0.05) between treatments.

There were no significant correlations between pre-weaning average daily pellet intake and ADG in first (r=-0.057, P=0.805), second (r=0.0833, P=0.289) and third (r =-0.365, P=0.1041) weeks of transition to pasture phase. The average daily pellet intake during pre-weaning did not influence (r=-0.042, P=0.8563) the overall ADG during the transition to pasture phase. There were no significant correlations (P>0.05) between ADGs of different periods in pre-weaning phase and the weekly ADGs in transition to pasture phase.

4.4.5 Chemical composition of pasture

The MB and CMR lambs were grazed in separate paddocks during first five weeks in post-weaning and as a mob for rest of experimental period. The chemical composition of the ryegrass and white clover pasture grazed by CMR and MB lambs separately and as a mob during post-weaning is presented in Table 4.6.

Table 4.6 Chemical composition of ryegrass and white clover pasture-fed post-weaning to lambs fed commercial milk replacer (CMR) or milk blend (MB) up to weaning.

Chemical composition on dry matter basis	Pasture CMR	Pasture MB	Pasture grazed as a mob[1]
Dry matter, g/kg	204.2	213.5	188.1
Ash, g/kg	96.8	95.5	94.0
Crude protein, g/kg	168.8	160.0	165.7
Fat, g/kg	34.8	34.6	36.5
Neutral Detergent Fibre, g/kg	497.6	498.2	508.8
Acid Detergent Fibre, g/kg	267.0	267.5	266.0
Organic matter digestibility, g/kg	779.0	771.7	722.9
Metabolisable energy, MJ/kg	10.33	10.36	9.70
CP:ME ratio[2], g/MJ	16.33	15.44	17.08

[1,] Pasture composition of the paddock when CMR and MB lambs were reared as a mob during last five weeks of the experiment.
[2,] Calculated by crude protein content divided by metabolisable energy content of pasture.

4.4.6 Post-weaning growth and pasture measurements

Lamb LW at the end of the experiment and LW gain and ADG (240.39 and 246.43 g/d, respectively for CMR and MB lambs) during the post-weaning period did not differ (P>0.05) between treatments. Overall post-weaning ADGs of both CMR and MB lambs increased by 78.9% and 74.4%, respectively, compared to their ADGs in transition to pasture phase. The ADG from the start of transition to pasture phase to the end of the experiment did not differ (P>0.05) between treatmens (Table 4.7). Average pasture mass of paddocks at pre-grazing for CMR and MB were 2899.17 ± 146.36 and 2706.41 ± 230.16 kg DM/ha, respectively. The post-grazing average pasture mass of paddocks were 2343.53 ± 96.11 and 2239.25 ± 115.99 kg DM/ha, respectively for CMR and MB.

There was no significant correlation between the overall ADG of lambs during pre-and post-weaning periods (r =- 0.045, P=0.85). The ADG during day one to ten at the start of the experiment had a positive and significant effect on the overall post-weaning ADG (r=0.570, P=0.007) and ADG in first two weeks (r=0.470, P=0.032) of the post-weaning phase. The ADG during 11-20 days of pre-weaning phase had a negative and significant effect (r=-0.484, P=0.026) on the ADG during first two weeks of the post-weaning phase.

There were no significant correlations between pre-weaning average daily pellet intake and the ADG in first two weeks (r=-0.084, P=0.717), third week (r=-0.294, P=0.196) and during fourth and fifth weeks (r =-0.163, P=0.480) of post-weaning phase. The average daily pellet intake during pre-weaning did not influence (r=-0.294, P=0.196) the overall ADG during the post-weaning phase.

Table 4.7 The effect of pre-weaning milk replacer (CMR) or milk blend (MB) with variable CP:ME feeding for artificially reared lambs on intake and growth from transition to pasture phase to post-weaning phase (day 64 to 161).

Item	Treatment		Pooled SE	P - value
	CMR	MB		
Initial LW, kg	28.06	27.73	0.63	0.71
Final LW, kg	46.02	45.45	0.89	0.66
Average daily gain, g/d	212.90	217.36	6.40	0.76
Total pellet DMI, kg	6.38	6.33	0.08	0.65
Total pellet CP intake, kg	1.34	1.33	0.02	0.65
Total pellet ME intake, MJ	74.92	74.28	0.99	0.65

4.5 Discussion

4.5.1 Pre-weaning growth and intake

This study investigated the effect of feeding a CP:ME adjusted milk replacer to lambs during the pre-weaning phase and the subsequent impact on their post-weaning growth performance. The lambs' CP:ME requirement during pre-weaning in present study ranged from 14.5 to 11.5 g/MJ. The MB lambs always had an excess of CP:ME from a combination of milk replacer, pellet and lucerne chaff intake (15.65 ± 0.04 g/MJ CP:ME) throughout the pre-weaning compared to their theoretical CP:ME requirement. The CMR lambs were offered a commercial milk replacer containing 12.28 g/MJ CP:ME and pellets containing 17.93 g/MJ CP:ME. Pellet intake increased as lambs aged and CMR lambs fulfilled their CP:ME requirement from a combination of commercial milk replacer and pellets at four to five weeks age. Thus, by increasing their intake of pellets the CMR group met the thresholds of 14 g/MJ CP:ME intake enabling higher growth. Consequently, the

ADG of the CMR and MB groups did not differ during the pre-weaning phase. Anjum et al. (2014) reported that lambs fed a milk replacer containing a CP:ME of approximately 11.8 g/MJ, increased their starter ration (CP:ME 17 MJ/kg) intake to fulfil nutrient requirements, compared to those fed milk (ewe or buffalo or cow) containing CP:ME approximately higher than 13.7 g/MJ. Sheep are able to select diets to meet their CP requirements if they are given access to a variety of feeds, which contain variable CP content (Kyriazakis and Oldham, 1993). Further, lambs reach their maximum daily protein deposition in the carcass and viscera (44 g/d) by selecting a diet to meet their CP requirement (Kyriazakis and Oldham, 1993). In the present study, CMR lambs likely supplemented their CP requirements with pellets to which they had *ab libitum* access and therefore, grew similarly to MB lambs. Higher ADG (over 300 g/d) was observed in lambs when they had intake of a diet with 14.0 -15.5 g/MJ CP:ME ratio, irrespective of treatment group. This finding is in agreement with Danso et al. (2018), who found that ADG increased by increasing protein deposition and reducing fat deposition when lambs were fed with a milk replacer having CP:ME ratio of 14 g/MJ.

The CP:ME ratio of the CMR used in current study (12.28 g/MJ) is higher than Herath et al. (2020) (CP:ME, 10.98 g/MJ). The CP:ME ratio of the MB used in current study (14.73 g/MJ) is also higher than Herath et al. (2020) (CP:ME, 11.88 g/MJ). Thus, increased CP:ME intake of lambs fed CMR and MB in the present study has resulted in higher pre-weaning ADG (297.17 and 298.95 g/d, respectively for CMR and MB lambs) compared to Herath et al. (2020) (236 and 267 g/d, respectively for CMR and MB lambs). Further, daily combined CP:ME intakes were lower for both CMR and MB lambs in the study by Herath et al. (2020) than the combined intake ratios in present study. Both CMR and MB lambs in the present study had CP:ME intakes close to 14 g/MJ, which Danso et al. (2018)

considered optimum for pre-weaning lamb growth. Hence, the lambs studied in the present study had a higher ADG than Herath et al. (2020) study. While, the CP:ME of the high protein milk replacer used in Danso et al. (2018) (15 g/MJ) is similar to the CP:ME of the MB in current study, pre-weaning ADG of lambs fed MB in the present study was higher compared to lambs fed high protein milk (ADG 260 g/d) in Danso et al. (2018). Thus, differences in ADG could also be due to variances in the genetic merit of their parents, although Herath et al. (2020), Danso et al. (2018) and the present study used Romney lambs.

Male and female Hu lambs fed ewe's milk (CP:ME, 12.9 g/MJ) for the first ten days post birth and then reared artificially on a milk replacer containing CP:ME of 14.0 g/MJ up to 60 days resulted in a lower ADG (190.5 g/d) (Chai et al., 2015) compared to both CM R and MB lambs in the present study. Although both ewe's milk and milk replacer contained higher CP:ME (Chai et al., 2015) than CMR in the current study, lamb ADG was lower compared to CMR lambs. This could be due to decreased lamb ADG in the first ten days after weaning from their dam. Additionally, female lambs could have been grew slower than males resulting a lower overall ADG.

Columbia, Hampshire and crossbred lambs reared artificially on milk replacer containing approximately 15.5 g/MJ CP:ME for four weeks period resulted in ADG of 220 g/d (Chiou and Jordan, 1973), which is higher than the ADG of both CMR and MB lambs in present study during the first four weeks. Only milk replacer was offered *ad libitum* up to 300 g DM/d for lambs in Chiou and Jordan (1973) study and their daily DMI was greater compared to total DMI from milk replacer and pellets in both CMR and MB lambs in current study during the same period. The lower DMI and differences in digestibility of

pellets and milk replacer could have been resulted in lower ADG in present study compared to Chiou and Jordan (1973) study.

Artificially reared Awassi lambs on a calf milk replacer containing approximately 14.5 g/MJ of CP:ME at *ad libitum* level twice daily, up to four weeks resulted in lower ADG (115 ± 14.5 g/d) (Emsen et al., 2004) compared to both CMR and MB lambs in current study. The CP:ME of milk used in Emsen et al. (2004) is approximately similar to MB lambs in present study. Heaney et al. (1982) reported that pre-weaning ADG of lambs reared on a calf milk replacer was lower than those reared on a lamb milk replacer irrespective of milk weaning at 21 or 28 days. Thus, higher ADG of lambs in present study than Emsen et al. (2004) study could be due to a digestibility difference of the milk replacers.

Historically, solid feed intake of lambs was considered negligible during the first three weeks of age (Owen and Davies, 1970). However, Danso et al. (2014) reported that lambs started nibbling pellets at three days of age and pellet intake at nine days of age ranged between 13.3 to 63.3 g DM/d. Chai et al. (2015) reported that artificially reared lambs had 32 g/d of starter creep intake during 15 to 20 days of age. The present study began offering pellet from eight days of age and during 11 to 20 days age, average pellet intakes were 53.5 g/d and 41.8 g/d for CMR and MB lambs, respectively. All these studies suggest that lambs will start to consume pellets at an early age if given access. The volatile fatty acid (VFA) production and rumen papillae development were increased by starter feed intake compared to lambs only fed ewe's milk or milk replacer (Danso, 2016, Sun et al., 2018). Further, early provision of a starter ration was associated with greater ruminal VFA production in lambs compared to those offered starter feed later (Zhao et al., 2016). Consequently, early initiation of rumen development could lead to greater post-

weaning growth performance due to better feed digestion in rumen, reducing weaning stress. Hence, pellet intake, its contribution to the fulfilment of lambs' nutritional requirements at early growth stages, and its impact on post-weaning growth need to be evaluated further to develop effective lamb feeding programmes.

4.5.2 Feed cost analysis for pre-weaning phase

The feeding of MB was more expensive than CMR for lambs during the pre-weaning period while the cost of pellets and lucerne chaff feeding did not differ between treatments. The higher cost of the milk blend was due to the higher cost of milk protein concentrate mixed into the milk blend to increase the CP content. This led to a higher cost per kilogram live weight gain for MB lambs as both CMR and MB treatment had a similar ADG.

The feed cost per kilogram LW gain for lambs fed the commercial milk replacer, milk blend and high protein milk (HPM) up to 22 kg LW in Herath et al. (2020) study was calculated by using the same cost per kilogram feed used in the present study. The calculated feed cost per kilogram LW gain for lambs fed the commercial milk replacer, milk blend and high protein milk (HPM) up to 22 kg LW in Herath et al. (2020) study were 6.18, 5.94 and 7.26 NZ $, respectively (unpublished data). Feed cost per kilogram LW gain of both CMR and MB lambs in present study were lower than all the treatment costs in Herath et al. (2020). This was due to the lower ADG observed in Herath et al. (2020), resulting in lambs take long to reach 22 kg LW (70.2, 63.4 and 65.4 days, respectively for CMR, MB and HMP lambs), compared to present study (57.2 and 56.8 days, respectively for CMR and MB lambs). Thus, increased crude protein level of commercial milk replacer from 240 g/kg (Herath et al., 2020) to 260 g/kg (present study) improved the pre-weaning lamb growth performance and reduced overall feed cost.

Feeding of CMR with 260 g/kg (12.28 g/MJ CP:ME) was more cost effective than feeding the milk blend adjusted for changing CP:ME requirement during the pre-weaning phase.

4.5.3 Growth during transition to pasture and post-weaning

As the weaning weight and DMI from pellets did not differ between CMR and MB lambs at transition, ADG was unaffected by the treatment. However, pasture intake during the transition phase was not measured and was not considered for the calculations of total CP:ME ratio of intake. The overall ADGs of CMR and MB lambs during transition were reduced compared to their overall ADGs during the pre-weaning phase. This could be attributed to inadequate pasture intake and/or lower digestibility of pasture for higher ADG as they were weaned from pellets gradually in the same period.

Post-weaning, lambs had *ad libitum* access to ryegrass and white clover based pasture as both CMR and MB lambs were grazed in paddocks which had >1200 kg DM/ha pasture mass (Morris and Kenyon, 2004). Post-weaning ADG did not differ between CMR and MB lambs which suggests that there was no effect of pre-weaning dietary CP:ME treatment on post-weaning lamb growth. Post-weaning lamb growth check was reported previously after concentrate meal removal and being fed pasture only diet (Jensen et al., 2017). In contrast, overall post-weaning ADGs of both CMR and MB lambs of present study were increased compared to their respective ADGs in transition to pasture phase, in which lambs gradually removed from pellets. Hence, lambs in present study could have been fully adapted to pasture diet.

Pre-weaning growth and weaning weight have been reported to positively influence post-weaning growth of lambs (Fraser and Saville, 2000, Selaive -Villarroel et al., 2008, Bhatt et al., 2009). Although there was no correlation in overall ADG of lambs between pre- and post-weaning periods, ADG in first ten days at the start of experiment has an

influence the overall post-weaning ADG and ADG in first two weeks of the post-weaning phase. Thus, assessment of nutritional interventions that improves the growth of lamb during first few days of life could benefit the weaned lambs management followed by an artificial lamb rearing system.

4.6 Conclusion

Feeding of lambs with a milk replacer containing CP:ME ratio of 12.28 g/MJ with *ad libitum* access to pellets (CP:ME 17.93 g/MJ) results in similar pre- and post-weaning average daily live weight gains to lambs fed a milk replacer adjusted for CP:ME ratio to meet the changing lambs' requirement. This was due to CMR lambs likely obtaining additional CP from pellet intake during the pre-weaning phase. Thus, fulfilment of CP:ME requirements at an early stage of life depends on both milk replacer and pellet intakes. The feeding of CMR with 12.28 g/MJ CP:ME during pre-weaning phase was more cost effective than feeding of the milk blend adjusted for changing CP:ME requirement. However, further research is warranted on the effect of CP:ME ratio of pellets, pellet intake and digestibility on growth performance of lambs reared artificially.

Forward to Chapter 5, 6 and 7

The studies present in Chapters 5, 6, and 7 are based on a lamb rearing experiment designed to investigate the influence of pellet fibre level, milk replacer composition and time of weaning on growth and body composition (Chapter 5), rumen development (Chapter 6) and bacterial composition of rumen (Chapter 7) of lambs reared artificially. Therefore, Chapter 5, 6, and 7 shares common methodology section on experimental animal management. Thus, general methodology of experiment is presented in Chapter 5, and Chapter specific methodology is presented within the text of Chapters 6 and 7 as necessary.

Chapter 5 Growth and body composition of artificially-reared lambs exposed to three different rearing regimens

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5.1 Abstract

This study was designed to investigate the influence of pellet fibre level, milk replacer composition and age at weaning on growth and body composition of lambs reared artificially. Romney ram lambs were randomly allocated to one of three rearing treatments; HFP57: commercial milk replacer to 57 days of age, and high fibre concentrate pellets; HFP42: commercial milk replacer with early weaning at 42 days of age, and high fibre concentrate pellets; LFP42: high protein milk replacer from 2-16 days of age followed by commercial milk replacer with early weaning at 42 days of age, and low fibre concentrate pellets. Lambs were slaughtered at 57 days of age. Overall average daily liveweight gain of lambs did not differ ($P>0.05$) between treatments. Dressing out percentage, carcass weight, empty small intestine and omental fat were higher ($P<0.05$) in HFP57 than in both HFP42 and LFP42 lambs. HFP42 and LFP42 lambs had heavier ($P<0.05$) empty rumen weights. Whole body protein content was higher ($P<0.05$) in HFP42 lambs compared to both HFP57 and LFP42 lambs. Fat content and daily fat deposition were greater ($P<0.05$) in HFP57 lambs than HFP42 and LFP42 lambs. Weaning lambs at 42 days of age with provision of either low or high fibre concentrate pellets, resulted in similar growth rates, reduced whole body fat deposition and was a more cost-effective rearing regimen.

Key words; carcass and viscera composition, commercial milk replacer, cost analysis, early weaning, fat deposition, neutral detergent fibre, pellet fibre level, stomach components

5.2 Introduction

Artificial rearing of lambs is necessary in cases of orphaned and mismothered lambs and is becoming an increasing requirement in the sheep dairy industry (Caroprese et al., 2016, Urbano et al., 2017). Traditionally, fulfillment of a lamb's nutrient requirement was

considered to depend on milk intake due to negligible solid feed intake in the first few weeks of life (Owen and Davies, 1970). Consequently, many studies have focused on optimisation of milk composition to improve lamb growth (Danso et al., 2018, Herath et al., 2020). However, lambs have higher crude protein to metabolisable energy ratio (CP:ME) requirement during their first few weeks of life than current typical commercial lamb milk replacers, with static CP:ME, provide (Danso et al., 2018). When CP:ME requirements are either met or exceeded, improved lamb growth rates are observed (Herath et al., 2020). Moreover, lambs fed commercial milk replacers could not compensate for the imbalance of CP:ME during their first two weeks of age through solid feed, as little or no intake occurs and the rumen is not yet sufficiently developed. However, pellet intake has been reported to reach considerable levels (approximately 200 g/d/lamb) by 30-40 days of age in lambs fed a diet of milk replacer and pellets (Herath et al., 2021a).

Early solid feed intake improves lamb growth and rumen development (Danso et al., 2014, Bhatt et al., 2009, Ward, 2008, Sun et al., 2018). This prepares lambs for a smoother transition to their post-weaning diet (Khan et al., 2016) and minimises potential post-weaning growth check due to better nutrient utilization through a more developed rumen and increased fermentative capacity. Thus, if pellet intake is substantial, it may be possible to wean lambs from milk replacer earlier without compromising their growth. Early milk weaning of artificially reared lambs would benefit farmers by shortening the milk feeding phase, reducing milk replacer costs and the labour costs associated with milk feeding. Previous studies have focused on artificial rearing regimens for calves to a greater extent than lambs (Kertz et al., 2017). Optimal and cost-effective weaning and nutritional regimens (milk and solid feed) for artificially reared lambs require further

investigation to ensure not only optimum growth and development pre-weaning occurs, but also appropriate post-weaning growth.

Roughage supplementation is reported to improve growth and rumen development in young ruminants (Nemati et al., 2016, Yang et al., 2015). Although roughage inclusion dilutes the energy density of lambs' rations, which could impair early-life growth. Thus, provision of low levels of fibre in a concentrate diet could be a strategy to improve growth performance pre- and post-weaning, whilst also ensuring adequate rumen development. Porter et al. (2007) reported that digestibility of a calf diet was higher when they were fed a low fibre starter compared to high fibre starter. Further, Castells et al. (2013) also showed that average daily liveweight gains of pre-weaned calves tended to be greater when fed a low fibre pelleted starter diet, compared to calves fed a high fibre starter diet. However, there is limited research on pellet fibre level and growth performance of lambs. Xie et al. (2020) reported that lambs grew at a similar rate from 21 to 60 days of age when they were provided a pelleted diet containing either 14 or 18% neutral detergent fibre (NDF) in combination with milk replacer feeding. Increasing the NDF level of the pellets to 22 or 26% increased the starter intake of lambs and resulted in greater average daily gain (ADG) during the same period compared to lambs fed the low NDF pellets (14% NDF). Pellet NDF level did not have any effect on feed efficiency of lambs in the same study from days 21 to 60 of age.

The objective of this study was investigate the effect of three different artificially rearing regimens (HFP57: commercial milk replacer to 57 days of age, and high fibre concentrate pellets; HFP42: commercial milk replacer with early weaning at 42 days of age, and high fibre concentrate pellets; LFP42: high protein milk replacer from 2-16 days of age followed by commercial milk replacer with early weaning at 42 days of age, and low fibre

concentrate pellets) on the growth and body composition of lambs. Additionally, the cost effectiveness of each rearing regimen was determined.

5.3 Materials and method

The experiment was carried out at Massey University, Palmerston North, New Zealand from August 2019 to October 2019. The research procedures used were approved by the Massey University Animal Ethics Committee (MUAEC 19/64).

5.3.1 Animal management

The experiment encompassed three different phases: pre-weaning, milk weaning, and post-weaning as shown in Table 5.1.

5.3.1.1 Pre-weaning

Twenty-seven Romney ram lambs born to twin-bearing ewes were selected for the study. Lambs were allowed to suckle from their dam for the first 24 hours after birth before one of the lambs' in the twin set was separated from its dam to enter the study. The 27 individual lambs were collected from their dams over a four-day period (28th August 2019 to 31st August 2019).

The selected lambs (mean live weight (LW) 4.93 ± 0.22 kg) were moved in-doors, individually penned and randomly allocated to one of three rearing treatments (Table 5.1); (i) HFP57 (n=9): commercial milk replacer, high fibre concentrate pellets to 57 days of age; (ii) HFP42 (n=9): commercial milk replacer, high fibre concentrate pellets and early weaning from the milk replacer at 42 days of age; (iii) LFP42 (n=9): high protein milk replacer from 2-16 days of age followed by commercial milk replacer, low fibre pellets and early weaning from milk replacer at 42 days of age. All lambs were fed milk replacer at 2.1 times their maintenance energy requirement based on their LW as per previous

studies by our group (Herath et al., 2020, Herath et al., 2021a). The maintenance requirement was calculated as ME_m= 0.40 MJ/kg $LW^{0.75}d^{-1}$ (Danso et al., 2016). The commercial milk replacer contained 262.9 g/kg crude protein (CP) and 21.8 MJ/kg metabolisable energy (ME) (Milligans Feed Ltd, Oamaru, New Zealand, Table 5.3). The high protein milk replacer contained 324.1 g/kg CP and 20.84 MJ/kg ME (CP:ME 15.55 g/MJ) and was an 80:20 blend of the commercial milk replacer powder with a powdered milk protein concentrate (Fonterra, Auckland, New Zealand, Table 5.3). To prepare the milk, the powdered milk replacer was mixed with warm tap water at a ratio of 1/4 (w/w) (Danso et al., 2016, Danso et al., 2018, Herath et al., 2020). Lambs were bottle-fed five times daily (at 8.00 a.m., 11.00 a.m., 2.30 p.m., 6.30 p.m. and 9.00 p.m.) up to two weeks of age, then four times daily (at 8.00 a.m., 11.00 a.m., 2.30 p.m. and 6.00 p.m.) up to the milk-weaning phase (days 38 to 42) for HFP42 and LFP42 lambs and until the end of experiment for HFP57 lambs.

Lambs were provided ad libitum access to either high fibre concentrate pellets (HFP57 and HFP42 lambs), or low fibre concentrate pellets (for LFP42 lambs) from four days of age to slaughter at approximately 57 days of age. The high fibre concentrate pellet was a blend of broll, soya bean, molasses, barley and limestone (acid detergent fibre (ADF) 69.35 g/kg, NDF 208.56 g/kg; Denver Stock Feeds, Palmerston North, Table 5.2 and 5.3). The low fibre concentrate pellet was a blend of skim milk powder, soya bean, molasses, barley and limestone (ADF 44.40 g/kg, NDF 116.76 g/kg; Denver Stock Feeds, Palmerston North, Table 5.2 and 5.3). The lambs had free access to water at all times. Individual milk, pellet and lucerne chaff intakes were recorded daily. Lamb LW was recorded twice weekly.

Table 5.1 Treatments and different phases of the experiment

Lambs' age (days)/ treatment	Days 2-37: Pre-weaning	Days 38-42: Weaning	Days 43-57: Post-weaning
HFP57	Commercial milk replacer – full allowance, *Ad libitum* high fibre concentrate pellets	Commercial milk replacer - full allowance, *Ad libitum* high fibre concentrate pellets, Lucerne chaff	Commercial milk replacer - full allowance, *Ad libitum* high fibre concentrate pellets, Lucerne chaff
HFP42	Commercial milk replacer – full allowance, *Ad libitum* high fibre concentrate pellets	Commercial milk replacer – 50 % of milk allowance, *Ad libitum* high fibre concentrate pellets, Lucerne chaff	No commercial milk replacer, *Ad libitum* high fibre concentrate pellets, Lucerne chaff
LFP42	High protein milk replacer - full allowance from day 2 to 16, followed by commercial milk replacer - full allowance only till day 37, *Ad libitum* low fibre concentrate pellets	Commercial milk replacer – 50 % of milk allowance, *Ad libitum* low fibre concentrate pellets, Lucerne chaff	No commercial milk replacer, *Ad libitum* low fibre concentrate pellets, Lucerne chaff

5.3.1.2 Milk weaning (days 38 to 42) and post-weaning (days 43 to 57) phases for HFP42 and LFP42 treatments

Milk weaning (38 to 42 days of age) and post-weaning (43 to 57 days of age) phases were applicable only for HFP42 and LFP42 treatments (Table 5.1), while HFP57 lambs continued to be provided with their full milk allowance until slaughter at 57 days of age. During the milk-weaning phase, lambs in HFP42 and LFP42 treatments were offered 50 % of their milk allowance for five days from day 37. Lambs in HFP42 and LFP42 treatments were bottle-fed twice daily at 8.00 a.m. and 6.00 p.m. and fully weaned from milk at 42 days. All lambs were provided with *ad libitum* access to either high (HFP57 and HFP42 lambs) or low fibre concentrate pellets (LFP42 lambs) during both the milk weaning and post weaning phases. Lucerne chaff (115 g/kg CP and 7.14 MJ/kg ME; Oaklane Stables Premium Chaff, Hawkes Bay, New Zealand, Table 5.3) was offered (40 g/d) for lambs from 38 to 57 days of age. All lambs were reared until approximately 57 days of age.

Samples of commercial milk replacer (n=4) and concentrate pellets (n=5 high fibre and n=3 low fibre concentrate pellets) were collected by sub-sampling each milk replacer/pellet bag at unpacking. Three composite samples of milk replacer, high fibre and low fibre concentrate pellets were prepared by pooling all the samples collected during the experiment. Three samples from high protein milk replacer and lucerne chaff were collected. All collected feed samples were stored at -20 ^{o}C until analysis for proximate composition.

Table 5.2 Pellet composition in fresh matter basis

Ingredient	Low fibre concentrate pellet (LFP)	High fibre concentrate pellet (HFP)
Barley, g/kg	0.270	0.390
Broll, g/kg[1]	0.000	0.351
Soya bean meal, g/kg	0.225	0.218
Wheat, g/kg	0.389	0.000
Molasses, g/kg[2]	0.030	0.030
Skim milk powder, g/kg	0.075	0.000
Limestones, g/kg	0.010	0.010
Sheep premix, g/kg[3]	0.001	0.001

[1] Broll is a mixture of wheat bran and wheat pollard, crude protein 153 g/kg and neutral detergent fibre 359 g/kg.
[2] Source of molasses is sugar beet
[3] Cobalt 0.2 g/kg, Iodine 0.2 g/kg, Magnesium 0.14 g/kg, Selenium 0.04 g/kg, Sodium 0.14 g/kg, Zinc 4 g/kg, and vitamin E 1 IU/g.

5.3.1.3 Faecal sample collection

Fresh faecal samples were collected from each lamb at slaughter and were stored at -20 ^{o}C until analyse for dry matter, gross energy and acid insoluble ash contents. Details on chemical analysis methods are included in the analysis of sample section.

5.3.2 Slaughter

Three lambs out of 27 were excluded from the experiment due to health reasons (n=1 and n=2 from HFP57 and LFP42 treatments, respectively). All lambs were weighed and their crown to rump length, rib length and abdominal girth measured, after being fasted for 12 hours.

The lambs were slaughtered via captive bolt, exsanguinated, skinned and eviscerated. Weights of the head, feet and tail (combined), whole skin, hot carcass, total viscera, blood and full stomach were recorded. Weights of the liver, kidneys, heart, lungs, and testis, spleen and omental fat were also measured for each individual.

Length, width, circumference and height measurements of the rumen, reticulum, omasum and abomasum were recorded (Figure 5.1) after placing the whole full stomach on a flat surface before separating each stomach component. Individual weights of the full and empty rumen, reticulum, omasum, reticulum, small intestine and large intestine were recorded.

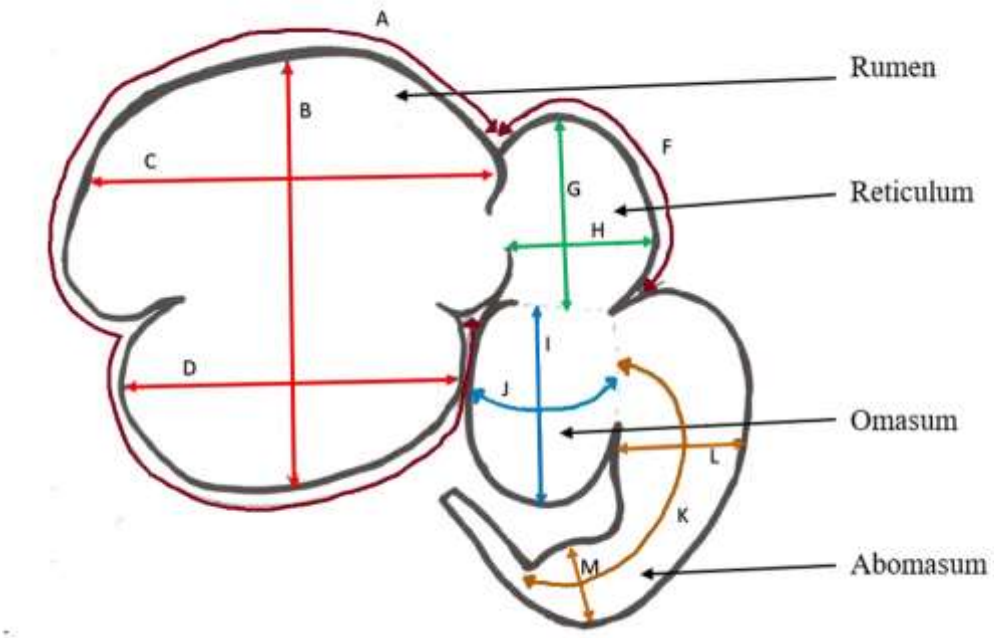


Figure 5.1 Length, width and circumference measurements of rumen, reticulum, omasum and abomasum recorded at slaughter (A, circumference of rumen; B, length of rumen; C, width of dorsal rumen; D, width of ventral rumen; F, circumference of reticulum; G, length of reticulum; H, width of reticulum; I, length of omasum; J, circumference of omasum; K, length of abomasum; L, width at proximal end of abomasum; M, width at distal end of abomasum; red, green, blue and brown lines represent measurements recorded for rumen, reticulum, omasum and abomasum, respectively and dark brown lines represent circumference of rumen and reticulum, respectively).

Carcasses were cut in half longitudinally in the middle of the spine and the weights of each half recorded. A half from each carcass, total viscera, blood, head, feet and whole skin and skin samples were stored at -20 ^{o}C in sealed plastic bags until being further processed. Prior to processing, the frozen carcass and viscera plus blood were cut into small blocks by a band saw and ground separately through a 3 mm grinding plate (Hobart Manufacturing Company, Troy, Ohio, USA). Subsamples of the ground carcass and viscera plus blood were collected and stored at -20 ^{o}C until analysis for proximate composition. The head, feet, tail and skin samples were not analysed.

5.3.3 Analysis of samples

Feed, body tissue and faecal samples were analysed at the Nutrition Laboratory, Massey University, Palmerston North, New Zealand.

5.3.3.1 Feed samples

Commercial milk replacer, high protein milk replacer, low and high fibre concentrate pellets and lucerne chaff samples were analysed for dry matter content by drying the sample at 105 ^{o}C in an oven (methods 930.15 and 925.10, (AOAC, 2016)) and ash content in a furnace at 550 ^{o}C (method 942.05, (AOAC, 2016)), crude protein content using the Dumas method (method 968.06, (AOAC, 2016)) and gross energy content by bomb calorimetry.

Fat content of commercial milk replacer and high protein milk replacer were determined by the Mojonnier extraction method (method 922.06, (AOAC, 2016)) and fat content of concentrate pellets was determined by the Soxtec extraction method (method 2003.06, (AOAC, 2016)). Lactose content of commercial milk replacer and high protein milk replacer was determined using an enzymatic method (Boehringer Mannheim/R-Biofarm

Enzyme kit for Lactose/D-Galactose − enzymatic digestion colorimetric was determined at 340 nm). Calcium and phosphorous content of commercial milk replacer and high protein milk replacer were determined by colorimetric method (sample preparation according to method 968.08D, (AOAC, 2016)). The mineral content (calcium, magnesium, potassium, sodium, phosphorus, sulphur and chloride) of pellets was determined by inductively coupled plasma emission spectrometry (Thermo iCAP 6000 series ICP-OES). The NDF, ADF and lignin content of pellets and lucerne chaff were determined using the Fibretec System (method 2002.04 and 973.18, (AOAC, 2016)).

Acid insoluble ash content of faecal samples were analysed using the method described by Sullivan and Carpenter (1993). Briefly, ashed samples were digested in acid, filtered through an ash-less filter paper and re-ashed, and the acid insoluble ash determined. The acid insoluble ash was used as an indigestible marker (Sales and Janssens, 2003).

5.3.3.2 Body tissue samples

Carcass and viscera plus blood samples were analysed for dry matter content by drying the sample at 105 ^{o}C in an oven (methods 950.46B, (AOAC, 2016)) and ash content in a furnace at 550 ^{o}C (method 920.153 and 923.03, (AOAC, 2016)), crude protein content by the Dumas method (method 968.06, (AOAC, 2016)), fat content by the Soxtec extraction method (method 991.36, (AOAC, 2016)) and gross energy content by bomb calorimetry.

5.3.3.3 Faecal samples

Faecal samples of five lambs, which had the highest pellet intakes from each treatment were freeze dried (Cuddon FB18, Cuddon Freeze dry, McArteny street, Blenheim, New Zealand). The freeze-dried samples were ground by Variable Speed Rotor Mill (Fritsch Pulverisette 14, Fritsch GmbH, Idar-Oberstein, Germany) at 16,000 rpm and sieved through a 0.5 mm sieve. Those samples were analysed for dry matter content by drying

the sample at 105 ^{o}C in an oven (methods 930.15 and 925.10, (AOAC, 2016)), and gross energy content by bomb calorimetry.

Acid insoluble ash content of faecal samples were analysed using the method described by Sullivan and Carpenter (1993). The acid insoluble ash was used as an indigestible marker (Sales and Janssens, 2003).

5.3.4 Calculations

Individual milk replacer, pellet and lucerne chaff intakes during the experiment were calculated as the daily amount offered minus refusal. Metabolisable energy content of pellets and lucerne chaff were calculated as gross energy multiplied by their metabolisability coefficient, considering metabolisability coefficient of 0.68 for pellet (Danso *et al.*, 2018) and 0.459 for lucerne chaff (Thomson and Cammell, 1979). Daily ME and CP intakes were calculated based on milk replacer, pellet and lucerne chaff intake multiplied by their respective ME and CP concentrations (Table 5.2). The cumulative ME and CP intakes were calculated by summation of daily ME and CP intake from the different feed types. Daily NDF and ADF intakes were calculated based on pellet and lucerne chaff intake multiplied by their respective NDF and ADF concentrations (Table 5.2). The cumulative NDF and ADF intakes were calculated by summation of daily NDF and ADF intake from the different feed types.

Energy digestibility of feed was calculated using following equation considering acid insoluble ash as an indigestible marker.

$$Energy\ digestability\ of\ feed = \frac{\left\{\left(\frac{gross\ energy\ feed}{indicator\ in\ feed}\right) - \left(\frac{gross\ energy\ faeces}{indicator\ in\ faeces}\right)\right\}}{\left(\frac{gross\ energy\ feed}{indicator\ in\ feed}\right)}$$

Average daily lamb live weight gain during the overall experimental period and 2-37, 38-42 and 43-57 day periods were calculated as the difference between LW at the first and last day of each period divided by the number of days in each phase. The LW gain per kilogram dry matter intake (DMI) was calculated as LW gain during experiment divided by total DMI.

Dressing percentage of carcass was calculated as carcass weight divided by LW at the slaughter (approximately after 12 hours since last feed) and multiplied by 100 and presented as g/kg. The gut fill was determined by weighing the stomach (combined weight of rumen, reticulum, omasum and abomasum) and intestines (duodenum, jejunum, ileum, caecum, colon and rectum) before and after removal of their contents. Empty stomach weight was determined by summation of empty rumen, reticulum, omasum and abomasum weights. Percentage of empty stomach components relative to LW at slaughter were calculated as weight of empty stomach and stomach components divided by LW at slaughter and multiplied by 100 and expressed as g/kg.

The volume of each stomach component was calculated as follows, where B; length of rumen, C; width of dorsal rumen, D; width of ventral rumen, G; length of reticulum, H; width of reticulum, I; length of omasum, J; circumference of omasum, K; length of abomasum, L; width at proximal section of abomasum, M; width at distal section of abomasum (Figure 5.1).

Rumen volume was calculated assuming dorsal and ventral rumen sections as two ellipsoids, reticulum as an ellipsoid, omasum as a cylinder and abomasum as one-half of a cone.

Rumen volume was calculated assuming dorsal and ventral rumen sections as two ellipsoids, reticulum as an ellipsoid, omasum as a cylinder and abomasum as one-half of a cone.

$$Rumen\ volume = \left[\frac{B}{4} * \frac{C}{2} * \frac{rumen\ height}{2} * \frac{4}{3} * \pi\right] + \left[\frac{B}{4} * \frac{D}{2} * \frac{rumen\ height}{2} * \frac{4}{3} * \pi\right]$$

$$Reticulum\ volume = \left[\frac{G}{2} * \frac{H}{2} * \frac{reticulum\ height}{2} * \frac{4}{3} * \pi\right]$$

$$Omasum\ volume = \pi * \left[\frac{J}{\pi * 2}\right]^2 * I$$

$$Abomasum\ volume = \left[\left(\frac{L}{2}\right)^2 + \left(\frac{L}{2} * \frac{M}{2}\right) + \left(\frac{M}{2}\right)^2\right] * \pi * \frac{K}{3}$$

The total stomach volume was calculated as summation of volumes of rumen, reticulum, omasum and abomasum.

The body volume was calculated as follows.

Body volume = π*(Girth/2π)*(Girth/2π)*rib cage width along spine

Dry matter, water, fat, protein and ash content of carcass and viscera plus blood were calculated by multiplying the weights of carcass and viscera plus blood by their respective analysed chemical composition percentage value. Whole carcass composition was calculated as the summation of compositions of carcass and viscera plus blood. Dry matter, water, fat, protein and ash content of carcass and viscera plus blood of lambs at the start of study were calculated as initial live weight times the average percentage chemical composition at the start of the trial using baseline data from (Herath et al., 2020) which were the same genotype lambs and slaughtered after 24 hours from birth (Herath et al., 2020). The daily deposition of dry matter, water, fat, protein and ash during the growth of each lamb was calculated as the difference between the amount of dry matter,

water, fat, protein and ash in the body at slaughter and the calculated composition of each lamb at the start of the study, divided by number of days to slaughter (Danso et al., 2018, Herath et al., 2020).

The cost of commercial milk replacer or high protein milk replacer, pellets and lucerne chaff for the experimental period were calculated as total intake multiplied by their respective cost per kilogram (commercial milk replacer, high protein milk replacer, high fibre pellets, low fibre pellets and lucerne chaff were 5.52, 6.41, 0.75, 0.85 and 2.06 NZ$/kg, respectively). The total feed cost for the experimental period was calculated by summation of cost for milk replacer or high protein milk replacer, pellets and lucerne chaff. Feed costs per kg liveweight gain was calculated as total feed cost divided by total liveweight gain.

5.3.5 Statistical analysis

Growth performance, feed and nutrient intakes data collected throughout the experiment were used to calculate average daily live weight gain, or feed and nutrient intake values as mentioned in 5.3.4 calculation section and those daily average values were analysed using a linear model with treatment as a fixed effect (Proc GLM, SAS 9.4 (SAS 9.4, 2013)), considering individual lamb as the experiment unit. Slaughter parameters, volumes of stomach components, daily nutrient deposition in carcass and viscera plus blood samples and feed cost were analysed using a linear model with treatment as a fixed effect (Proc GLM, SAS 9.4 (SAS 9.4, 2013)), considering individual lamb as the experiment unit. Carcass composition was analysed with a linear model with treatment as a fixed effect and carcass weight as a covariate by Proc GLM, SAS 9.4 (SAS 9.4, 2013). Viscera plus blood composition was analysed with a linear model with treatment as a fixed effect considering viscera plus blood weight as a covariate by Proc GLM, SAS 9.4

(2013). Differences were identified identified considering confidence level as 0.05, where appropriate, using the least significant difference (LSD) mean comparison test.

5.4 Results

5.4.1 Proximate composition of feed samples

The chemical composition of the commercial milk replacer, high protein milk replacer, high fibre concentrate pellet, low fibre concentrate pellet and lucerne chaff is presented in Table 5.2. The diet combination of commercial milk replacer, high fibre concentrate pellets and lucerne chaff fed to HFP57 lambs had a higher digestibility ($P<0.05$; 0.93) compared to the combinations of high fibre concentrate pellets and lucerne chaff fed to HFP42 lambs (0.78) and low fibre concentrate pellet, lucerne chaff fed to LFP42 lamb (0.81), which did not differ ($P>0.05$) (Table 5.4).

5.4.2 Intake and growth performance

5.4.2.1 Pre-weaning phase (2-37 days)

The dry matter intake (DMI) from either milk replacer or pellets, daily ME, CP, ADF and NDF intakes and DMI from lucerne chaff and total DMI did not differ ($P>0.05$) between treatments at 2 to 37 days of age (Table 5.4). The combined CP:ME intake was higher ($P<0.05$) in LFP42 lambs compared to both HFP57 and HFP42 lambs, which did not differ ($P>0.05$). The initial live weight, ADG and LW gain per kilogram DMI did not differ ($P>0.05$) between treatments.

5.4.2.2 Early weaning phase (38-42 days)

The DMI from milk replacer at 38 to 42 days of age was higher ($P<0.05$) in HFP57 lambs than both HFP42 and LFP42 lambs because HFP42 and LFP42 lambs were being weaned off milk during this period (Table 5.4). The lambs in HFP42 had higher ($P<0.05$) DMI from pellets at 38 to 42 days of age than HFP57 lambs while LFP42 lambs did not differ ($P>0.05$) from either HFP57 or HFP42 lambs. The ME intake of lambs at 38 to 42 days

of age did not differ (P>0.05) between treatments. The CP intake of HFP57 lambs at 38 to 42 days of age was greater (P<0.05) than HFP42 and LFP42 lambs, which did not differ (P>0.05). The combined CP:ME intake did not differ (P>0.05) between treatments.

The HFP42 lambs had higher (P<0.05) daily ADF and NDF intakes at 38 to 42 days of age compared to HFP57 and LFP42 lambs, which did not differ (P>0.05). The DMI from lucerne chaff and total DMI did not differ (P>0.05) between treatments. The ADG of lambs did not differ (P>0.05) between treatments. Live weight gain per kilogram DMI tended to be higher (P=0.055) in HFP57 lambs compared to HFP42 and LFP42 lambs.

5.4.2.3 Post-early weaning phase (43-57 days)

The lambs in HFP42 and LFP42 treatments had higher (P<0.05) DMI from pellets at 43 to 57 days of age than HFP57 lambs (Table 5.4). Daily ME and CP intakes of lambs at 43 to 57 days of age were greater (P<0.05) in HFP57 lambs compared to HFP42 and LFP42 lambs, which did not differ (P>0.05). The combined CP:ME intake was higher (P<0.05) in both HFP42 and LFP42 compared to HFP57 lambs. The HFP42 lambs had higher (P<0.05) daily NDF intake during the same period than HFP57 and LFP42 lambs, which did not differ (P>0.05). Daily ADF intakes of HFP42 and LFP42 lambs were higher (P<0.05) than HFP57 lambs at 43 to 57 days of age. The DMI from lucerne chaff and total DMI did not differ (P>0.05) between treatments.

The ADG of HFP57 lambs at 43 to 57 days of age was greater (P<0.05) than HFP42 and LFP42 lambs, which did not differ (P>0.05). The HFP57 lambs had higher (P<0.05) LW gain per kilogram DMI at 43 to 57 days of age compared both HFP42 and LFP42 lambs, which did not differ (P>0.05).

Table 5.3 Chemical composition of commercial milk replacer, high protein milk replacer, high fibre pellet, low fibre concentrate pellet and lucerne chaff fed to lambs on fresh matter basis.

Chemical composition	Commercial milk replacer	High protein milk replacer	High fibre concentrate pellet [1]	Low fibre concentrate pellet[2]	Lucerne chaff
Dry matter, g/kg	969.8	967.4	880.5	891.2	873.8
Ash, g/kg	56.2	61.2	53.5	60.4	66.0
Crude protein, g/kg	262.9	324.1	185.6	180.0	114.7
Fat, g/kg	275.0	215.4	47.4	34.4	0.00
Lactose, g/kg	350.4	343.64	N/A	N/A	N/A
Minerals, g/kg					
Calcium	11.4	13.6	8.6	6.9	N/A
Magnesium	N/A	N/A	2.0	3.0	N/A
Potassium	N/A	N/A	10.6	12.2	N/A
Sodium	N/A	N/A	0.6	0.3	N/A
Phosphorus	6.1	7.2	4.5	5.8	N/A
Sulphur	N/A	N/A	2.0	2.2	N/A
Chloride, mg/kg	N/A	N/A	0.21	0.16	N/A
Gross energy, MJ/kg	22.73	21.71	15. 97	15. 66	15.56
Neutral Detergent Fibre, g/kg	-	-	208.6	116.8	543.3
Acid Detergent Fibre, g/kg	-	-	69.3	44.4	431.4
Lignin, g/kg	-	-	14.3	10.2	92.2
Metabolisable energy, MJ/kg [3]	21.82	20.84	10.86	10.65	7.14
CP:ME ratio[4], g/MJ	12.05	15.55	17.10	16.91	16.06

[1] Composed of barley, broll, soya bean, molasses and limestone
[2] Composed of skim milk powder, soya bean, molasses, barley and limestone
[3] Calculated as metabolisable energy = metabolisability coefficient*gross energy, considering metabolisability coefficient = 0.96 for milk replacer and milk protein concentrate, 0.68 for pellet (Danso *et al.*, 2018), 0.459 for lucerne chaff (Thomson and Cammell, 1979).
[4] Calculated as dividing crude protein content by metabolisable energy content of feed.
- Absent in feed type
N/A not analysed.

5.4.2.4 Overall experimental period (2-57 days)

Feed intake

Daily milk replacer intake was highest (P<0.05) in HFP57 lambs compared to both HFP42 and LFP42 lambs, which did not differ (P>0.05) (Table 5.5). Daily pellet intake was higher (P<0.05) in both HFP42 and LFP42 than HFP57 lambs. Total lucerne and dry matter intakes (DMI) did not differ (P >0.05) between treatments.

Crude protein (CP) intake

Daily CP intake from milk replacer was higher (P<0.05) in HFP57 than HFP42 and LFP42 lambs (Table 5.5). Daily CP intake from pellets was higher (P<0.05) in both early-

weaned lamb groups (HFP42 and LFPF) compared to HFP57 lambs. The CP intake from lucerne chaff did not differ (P>0.05) between treatments. Daily CP intake from milk replacer, pellet and lucerne chaff did not differ (P>0.05) between treatments.

Metabolizable (ME) and digestible energy intake

Daily ME intake from milk replacer was higher (P<0.05) in HFP57 compared to HFP42 and LFP42 lambs (Table 5.5). Daily ME intake from pellet was higher (P<0.05) in both early-weaned groups (HFP42 and LFP42) than HFP57 lambs. Daily ME intake from lucerne chaff did not differ (P<0.05) between treatments. Total ME intake was greater (P<0.05) in HFP57 compared to LFP42 lambs and HFP42 lambs did not differ (P>0.05) either from HFP57 or LFP42 lambs. The digestible energy intake was higher (P<0.05) in HFP57 lambs compared to HFP42 and LFP42 lambs, which did not differ (P>0.05). The combined total CP:ME intake from milk replacer, pellet and lucerne chaff did not differ (P>0.05) between treatments (12.82 ± 0.30, 13.79 ± 0.38 and 14.23 ± 0.13 g/ MJ, respectively for HFP57, HFP42 and LFP42 lambs).

Table 5.4 Intake and growth performance during different phases (2-37, 38-42 and 43-57 days of age) of artificially reared lambs using three different rearing regimens: HFP57, commercial milk replacer, high fibre concentrate pellets and milk feeding to 57 days of age; HFP42, commercial milk replacer, high fibre concentrate pellets and early weaning at 42 days of age; LFP42, high protein milk replacer from 2-16 days of age followed by commercial milk replacer, low fibre concentrate pellets and early weaning at 42 days of age (ADF, Acid detergent fibre; ADG, average daily live weight gain; CPI, crude protein intake; DMI, dry matter intake; LW, live weight; MEI, metabolisable energy intake; NDF, neutral detergent fibre; SE, standard error)

	Pre-weaning phase Lambs: 2-37 days of age					Milk weaning phase Lambs: 38-42 days of age					Post- early weaning phase Lambs: 43-57 days of age				
Measurement	HFP57	HFP42	LFP42	Pooled SE	P-value	HFP57	HFP42	LFP42	Pooled SE	P-value	HFP57	HFP42	LFP42	Pooled SE	P-value
Initial LW, kg	5.0	4.9	4.9	0.37	0.97	10.8	10.9	10.3	0.81	0.88	12.1	11.9	11.1	0.96	0.75
Final LW, kg	10.8	10.9	10.3	0.81	0.88	12.1	11.9	11.1	0.96	0.75	17.1	14.7	14.3	1.14	0.20
ADG, g/d	165.7	171.3	156.0	16.33	0.81	257.8	193.0	147.4	38.99	0.17	355.8^{b}	215.6^{a}	228.6^{a}	0.02	<.001
Milk replacer DMI, g/d	200.6	196.6	193.4	10.83	0.90	281.9^{b}	138.7^{a}	138.1^{a}	11.95	<.001	340.7	0.0	0.0	-	-
Pellet DMI, g/d	55.2	60.7	43.8	14.07	0.70	108.9^{a}	269.6^{b}	193.8^{ab}	36.3	0.015	192.6^{a}	448.0^{b}	429.8^{b}	33.42	<.001
Lucerne chaff DMI, g/d	-	-	-	-	-	5.4	5.8	8.2	1.84	0.54	19.8	14.5	22.7	3.62	0.28
Total DMI, g/d	249.2	250.1	230.6	20.60	0.77	396.2	424.4	340.2	46.49	0.45	553.1	462.5	452.5	40.59	0.18
MEI from milk, MJ/d	4.5	4.4	4.3	0.2	0.83	6.3^{b}	3.1^{a}	3.1^{a}	0.27	<.001	7.7	0.0	0.0	-	-
MEI from pellets, MJ/d	0.7	0.8	0.5	0.2	0.66	1.4^{a}	3.5^{b}	2.4^{ab}	0.46	0.014	2.5^{a}	5.8^{b}	5.4^{b}	0.4	<.001
MEI from lucerne chaff, MJ/d	-	-	-	-	-	0.04	0.05	0.07	0.01	0.54	0.2	0.1	0.2	0.03	0.14
MEI, MJ/d	5.2	5.2	4.8	0.4	0.73	7.8	6.6	5.6	0.67	0.10	10.3^{b}	5.9^{a}	5.5^{a}	0.57	<.001
CPI from milk, g/d	54.4	53.3	56.1	2.96	0.80	76.4^{b}	37.6^{a}	37.4^{a}	3.05	<.001	92.4	0.0	0.0	-	-
CPI, g/d	66.0	66.1	65.0	5.0	0.99	100.1	95.2	77.7	10.0	0.30	135.5^{b}	96.3^{a}	89.8^{a}	8.7	0.003
Combined CP:ME ratio, g/MJ	12.6^{a}	12.6^{a}	13.4^{b}	0.12	<.001	12.8	14.2	13.8	0.18	<.001	13.1^{b}	16.4^{b}	16.2^{b}	0.05	<.001
NDF intake, g/d	13.1	14.3	5.7	3.22	0.17	29.1^{a}	69.4^{b}	30.5^{a}	8.86	0.005	57.9^{a}	115.2^{b}	70.5^{a}	9.0	<.001
ADF intake, g/d	4.3	4.8	2.2	1.07	0.23	11.2^{a}	24.8^{b}	13.7^{a}	3.26	0.015	24.9^{a}	42.4^{b}	32.6^{b}	3.8	0.01
Feed digestibility coefficient	-	-	-	-	-	-	-	-	-	-	0.93^{b}	0.78^{a}	0.81^{a}	0.02	<.001
Feed efficiency[1]	0.65	0.66	0.66	0.02	0.93	0.65	0.41	0.41	0.07	0.055	0.65^{b}	0.47^{a}	0.50^{a}	0.03	<.001

[a,b] 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).
[1]Feed efficiency calculated as LW gain /DMI

Acid detergent fibre (ADF) and Neutral detergent fibre (NDF) intake

The ADF and NDF intakes from pellets were greater (P<0.05) in HFP42 lambs compared to both HFP57 and LFP42 lambs, which did not differ (P>0.05) (Table 5.5). The ADF and NDF intakes from lucerne chaff did not differ between treatments (P>0.05). Daily ADF intake was tended to be higher (P<0.069) in HFP42 lambs compared to both HFP57 and LFP42 lambs. Daily NDF intake was greater (P<0.05) in HFP42 lambs compared to both HFP57 and LFP42 lambs, which did not differ (P>0.05).

Growth performance

The LW at slaughter and average daily gain (ADG) did not differ (P>0.05) between treatments (Table 5.5). The LW gain per kilogram DMI was greater (P<0.05) in HFP57 lambs compared to both HFP42 and LFP42 lambs.

5.4.3 Slaughter parameters

The crown to rump length, abdominal girth and ribs length did not differ (P>0.05) between treatments (Table 5.6). Carcass weight, dressing, full and empty weights of small intestine and omental fat were greater (P<0.05) in HFP57 lambs compared to HFP42 and LFP42 lambs, which did not differ (P>0.05). All the other slaughter parameters did not differ (P>0.05) between treatments.

Empty rumen weight was higher (P<0.05) in HFP42 and LFP42 lambs compared to HFP57 lambs (Table 5.7). Full omasum was heavier (P<0.05) in HFP42 lambs compared to HFP57 lambs. The LFP42 lambs did not differ (P>0.05) either from HFP57 or HFP42 lambs.

The weights of rumen, reticulum and omasum relative to live weight of lambs at slaughter were greater (P<0.05) in both HFP42 and LFP42 lambs compared to HFP57 lambs. The

abomasum weight relative to the live weight at slaughter was higher ($P<0.05$) in both HFP57 and HFP42 lambs compared to LFP42 lambs (Table 5.7). Lambs in HFP42 and LFP42 treatments had heavier ($P<0.05$) rumens relative to their total empty stomach weight, compared to HFP57 lambs. The HFP57 lambs had a heavier ($P<0.05$) abomasum relative to the total empty stomach weight compared to both HFP42 and LFP42 lambs, which did not differ ($P>0.05$). The calculated reticulum volume tended to be higher ($P=0.077$) in HFP42 and LFP42 lambs compared to HFP57 lambs. The abomasum volume tended to be higher ($P=0.063$) in HFP57 and HFP42 lambs compared to LFP42 lambs. All the other stomach parameters did not differ ($P>0.05$) between treatments (Table 5.7).

Table 5.5 Overall intake and growth performance of lambs reared artificially (2-57 days of age) using three different rearing regimens: HFP57, commercial milk replacer, high fibre concentrate pellets and milk feeding to 57 days of age; HFP42, commercial milk replacer, high fibre concentrate pellets and early weaning at 42 days of age; LFP42, high protein milk replacer from 2-16 days of age followed by commercial milk replacer, low fibre concentrate pellets and early weaning at 42 days of age (ADF, Acid detergent fibre; ADG, average daily live weight gain; CP, crude protein; DMI, dry matter intake; LW, live weight; ME, metabolisable energy; NDF, neutral detergent fibre; SE, standard error)

	Whole trial period; lambs 2 to 57 days of age				
Measurement	HFP57	HFP42	LFP42	Pooled SE	P-value
Initial LW, kg	5.0	4.9	4.9	0.37	0.97
Final LW, kg	17.1	14.7	14.3	1.07	0.20
ADG, g/d	220.9	184.1	171.3	16.71	0.13
Milk replacer DMI, g/d	241.8[b]	143.0 [a]	136.0 [a]	10.13	<.001
Pellet DMI, g/d	94.4[a]	172.8 [b]	155.3[b]	17.80	0.012
Lucerne chaff DMI, g/d	5.5	3.9	6.6	0.98	0.18
Total DMI, g/d	341.7	319.7	297.8	25.45	0.51
ME intake from milk, MJ/d	5.4 [b]	3.6 [a]	3.0 [a]	0.30	<.001
ME intake from pellets, MJ/d	1.2 [a]	2.2 [b]	1.9 [b]	0.23	0.013
ME intake from lucerne chaff, MJ/d	0.04	0.03	0.05	0.01	0.22
ME intake, MJ/d	6.7[b]	5.9 [ab]	5.0 [a]	0.40	0.029
CP intake from milk, g/d	65.5 [b]	38.8 [a]	39.2 [a]	2.76	<.001
CP intake from pellets, g/d	19.9 [a]	36.4 [b]	31.4 [b]	3.74	0.013
CP intake from lucerne chaff, g/d	0.7	0.5	0.9	0.13	0.18
CP intake, g/d	86.1	75.7	71.4	5.80	0.22
Combined CP:ME ratio, g/MJ	12.8	13.1	14.2	0.51	0.16
NDF intake from pellet, kg	1.225[a]	2.186 [b]	1.116[a]	0.224	0.004
NDF intake from lucerne chaff, kg	0.189	0.130	0.223	0.033	0.160
NDF intake, g/d	25.8[a]	43.4[b]	24.4[a]	4.47	0.01
ADF intake from pellet, kg	0.407[a]	0.726 [b]	0.424[a]	0.075	0.008
ADF intake from lucerne chaff, kg	0.150	0.104	0.177	0.027	0.160
ADF intake, g/d	10.2	15.5	11.0	1.71	0.069
Feed efficiency[1]	0.65 [b]	0.57 [a]	0.57 [a]	0.02	0.009

[a,b] 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).

[1]Feed efficiency calculated as LW gain /DMI

Table 5.6 Slaughter parameters of lambs reared artificially using three different rearing regimens: HFP57, commercial milk replacer, high fibre concentrate pellets and milk feeding to 57 days of age; HFP42, commercial milk replacer, high fibre concentrate pellets and early weaning at 42 days of age; LFP42, high protein milk replacer from 2-16 days of age followed by commercial milk replacer, low fibre concentrate pellets and early weaning at 42 days of age (SE, standard error)

	Treatment			Pooled	P-value
Item	HFP57	HFP42	LFP42	SE	
Crown to rump length, cm	74.5	70.6	71.5	1.9	0.32
Girth, cm	66.0	61.1	62.7	1.96	0.20
Ribs length, cm[1]	27.1	27.2	26.1	0.89	0.63
Carcass weight, kg	8.0 [b]	6.4[a]	6.3 [a]	0.53	0.051
Dressing, g/kg [2]	470.9 [b]	431.1 [a]	440.8 [a]	6.43	<.001
Head, feet and tail, kg	1.8	1.7	1.6	0.11	0.38
Whole skin, kg	1.9	1.6	1.6	0.13	0.43
Total viscera weight, kg	4.2	4.0	3.8	0.32	0.64
Liver, g	316.7	287.2	291.7	24.67	0.66
Kidneys, g	70.3	74.3	56.1	7.34	0.22
Full stomach, kg	1.4	1.6	1.5	0.15	0.82
Full small intestine, g	960.3 [b]	646.9 [a]	706.3 [a]	73.91	0.015
Empty small intestine, g	776.4 [b]	543.1 [a]	605.4 [a]	54.36	0.016
Full large intestine, g	413.7	516.8	429.2	42.16	0.18
Empty large intestine, g	221.6	237.0	221.1	19.17	0.79
Blood, g	602.4	579.0	540.5	0.08	0.87
Heart, g	100.1	85.9	82.5	5.77	0.10
Lung, g	229.7	220.9	201.8	18.73	0.59
Testis, g	27.7	21.3	21.1	2.76	0.18
Spleen, g	29.4	25.0	22.8	2.04	0.10
Gutfill, kg	1.5	1.5	1.4	0.16	0.92
Omental fat, g	69.0 [b]	35.4 [a]	30.7 [a]	7.47	0.003

[1] Rib cage width along spine

[2] Carcass weight as a propotion of final live weight.

[a,b] 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).

Table 5.7 Stomach parameters of lambs reared artificially using three different rearing regimens: HFP57, commercial milk replacer, high fibre concentrate pellets and milk feeding to 57 days of age; HFP42, commercial milk replacer, high fibre concentrate pellets and milk replacer, low fibre pellets and early weaning at 42 days of age ((SE, standard error)

Item	Treatment			Pooled SE	P-value
	HFP57	HFP42	LFP42		
Stomach weight					
Full stomach, kg[1]	1.2	1.6	1.5	0.14	0.82
Empty stomach, g[1]	338.4	421.6	381.0	27.73	0.12
Full rumen, g	972.2	1155.0	1165.8	110.89	0.40
Empty rumen, g	189.8[a]	274.2[b]	248.5 [b]	20.13	0.019
Full reticulum, g	52.9	63.6	69.6	6.11	0.18
Empty reticulum, g	32.4	37.7	37.1	2.38	0.24
Full omasum, g	21.7 [a]	33.6[b]	25.8 [ab]	3.27	0.043
Empty omasum, g	18.4	23.0	21.4	1.81	0.20
Full abomasum, g	378.6	310.6	245.3	50.10	0.21
Empty abomasum, g	97.8	86.6	73.9	6.34	0.057
Stomach component weights relative to live weight, g/kg					
Rumen	11.0[a]	19.1[b]	17.4[b]	0.89	<.001
Reticulum	2.0 [a]	2.6[b]	2.6[b]	0.09	<.001
Omasum	1.1[a]	1.6[b]	1.5[b]	0.10	0.003
Abomasum	5.8[b]	5.9[b]	5.2[a]	0.20	0.036
Stomach component weights relative to empty stomach weight, g/kg					
Rumen	554.9[a]	648.9[b]	651.6[b]	14.56	<.001
Reticulum	96.5	90.7	98.5	4.36	0.42
Omasum	55.4	55.0	55.2	3.30	0.99
Abomasum	293.1[b]	205.4[a]	194.7[a]	10.54	<.001
Body volume, L[2]	9.5	8.3	8.3	0.68	0.36
Stomach volume					
Rumen, L[3]	1.01	1.16	1.27	0.124	0.37
Reticulum, L[4]	0.05	0.08	0.08	0.010	0.077
Omasum, L[5]	0.03	0.03	0.03	0.003	0.22
Abomasum, L[6]	1.33	1.09	0.67	0.179	0.063
Total stomach, L[7]	2.42	2.36	2.06	0.282	0.64

[1] Includes rumen, reticulum, omasum and abomasum.

[2] Calculated as body volume = $\pi * \left(\frac{Girth}{2\pi}\right) * \left(\frac{Girth}{2\pi}\right) *$ ribs width along spine

[3] Calculated as $\text{Rumen volume} = \left[\frac{B}{4} * \frac{C}{2} * \frac{\text{rumen height}}{2} * \frac{4}{3} * \pi\right] + \left[\frac{B}{4} * \frac{D}{2} * \frac{\text{rumen height}}{2} * \frac{4}{3} * \pi\right]$, where B; length of rumen, C; width of dorsal rumen, D; width of ventral rumen

[4] Calculated as $\text{Reticulum volume} = \left[\frac{G}{2} * \frac{H}{2} * \frac{\text{reticulum height}}{2} * \frac{4}{3} * \pi\right]$, where G; length of reticulum, H; width of reticulum

[5] Calculated as $\text{Omasum volume} = \pi * \left[\frac{J}{\pi * 2}\right]^2 * I$, where I; length of omasum, J; circumference of omasum

[6] Calculated as $\text{Abomasum volume} = \left[\left(\frac{L}{2}\right)^2 + \left(\frac{L}{2} * \frac{M}{2}\right) + \left(\frac{M}{2}\right)^2\right] * \pi * \frac{K}{3}$ where, K; length of abomasum, L; width at proximal section of abomasum, M; width at distal section of abomasum

[7] Calculated as summation of volumes of rumen, reticulum, omasum and abomasum

[a,b] 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).

5.4.4 Body composition

5.4.4.1 Nutrient content

Carcass

Protein content of the carcass was greater (P<0.05) in HFP42 lambs compared to both HFP57 and LFP42 lambs, which did not differ (P>0.05) (Table 5.8). Fat content of the carcass was greater (P<0.05) in HFP57 lambs compared to both HFP42 and LFP42 lambs, which did not differ (P>0.05). Dry matter, ash, water and gross energy contents of carcass did not differ (P>0.05) between treatments.

Viscera plus blood

Fat and gross energy contents of viscera plus blood were higher (P<0.05) in HFP57 lambs compared to both HFP42 and LFP42 lambs, which did not differ (P>0.05) (Table 5.8). Dry matter, protein, ash and water contents of viscera plus blood did not differ (P>0.05) between treatments.

Whole body (carcass and viscera plus blood)

Protein content of whole body was higher (P<0.05) in HFP42 lambs compared to HFP57 and LFP42 lambs, which did not differ (P>0.05) (Table 5.8). Fat content of whole body was greater (P<0.05) in HFP57 lambs compared to both HFP42 and LFP42 lambs, which did not differ (P>0.05). Dry matter, ash, water and gross energy contents of whole body did not differ (P>0.05) between treatments.

5.4.4.2 Nutrient deposition rate

Daily fat deposition in the carcass, viscera plus blood and whole body were greater in HFP57 lambs compared to both HFP42 and LFP42 lambs, which did not differ (P>0.05) (Table 5.9). Daily dry matter, protein, ash and water depositions in carcass did not differ (P>0.05) between treatments.

Table 5.8 Chemical composition of carcass, viscera plus blood and the whole body of lambs reared artificially using three different rearing regimens: HFP57, commercial milk replacer, high fibre concentrate pellets and milk feeding to 57 days of age; HFP42, commercial milk replacer, high fibre concentrate pellets and early weaning at 42 days of age; LFP42, high protein milk replacer from 2-16 days of age followed by commercial milk replacer, low fibre concentrate pellets and early weaning at 42 days of age (SD, standard deviation; SE, standard error)

Measurement	Baseline	Treatment			Pooled	P -
	(Mean ± SD)*	HFP57	HFP42	LFP42	SE	value
Carcass						
Dry matter, g/kg	269.1 ± 35.0	324.2	304.3	303.8	6.2	0.51
Protein, g/kg as is	169.8 ± 7.9	182.2[a]	190.1[b]	185.5[a]	2.3	0.016
Fat, g/kg as is	28.5 ± 14.2	97.6[b]	66.4[a]	66.3[a]	6.3	0.002
Ash, g/kg as is	57.0 ± 24.3	40.5	44.8	45.7	1.9	0.060
Water, g/kg as is	730.9 ± 35.0	683.5	691.1	694.4	6.2	0.51
Gross energy, MJ/kg	-	7.84	7.23	7.24	0.2	0.16
Dry matter, kg	0.79 ± 0.11	2.18	2.14	2.11	0.05	0.51
Protein, kg	0.50 ± 0.04	1.26[a]	1.32[b]	1.26[a]	0.01	0.007
Fat, kg	0.08 ± 0.04	0.80[b]	0.44[a]	0.42[a]	0.08	0.005
Ash, kg	0.17 ± 0.07	0.28	0.30	0.32	0.01	0.13
Water, kg	2.15 ± 0.25	4.72	4.75	4.79	0.05	0.51
Gross energy, MJ	-	53.93	50.63	49.95	1.99	0.14
Viscera plus blood						
Dry matter, g/kg	227.8 ± 29.2	208.0[b]	191.5[a]	191.7[a]	4.7	0.030
Protein, g/kg as is	156.7 ± 12.7	136.5	139.4	138.5	1.7	0.45
Fat, g/kg as is	25.8 ± 3.7	57.2[b]	35.2[a]	37.1[a]	3.6	<.001
Ash, g/kg as is	12.4 ± 3.6	9.8	10.0	10.1	0.2	0.55
Water, g/kg as is	772.2 ± 29.2	792.0	808.5	808.3	4.7	0.030
Gross energy, MJ/kg as is	-	5.62[b]	4.94[a]	4.90[a]	0.2	0.009
Dry matter, kg	0.39 ± 0.05	0.65	0.61	0.60	0.016	0.11
Protein, kg	0.27 ± 0.04	0.43	0.44	0.44	0.006	0.37
Fat, kg	0.04 ± 0.01	0.18[b]	0.12[a]	0.12[a]	0.012	0.002
Ash, kg	0.02 ± 0.01	0.03	0.03	0.03	0.001	0.59
Water, kg	1.32 ± 0.12	2.47	2.52	2.52	0.016	0.10
Gross energy, MJ	-	17.72[b]	15.76[a]	15.52[a]	0.553	0.034
Carcass and viscera plus blood						
Dry matter, kg	1.18 ± 0.16	2.9	2.7	2.7	0.08	0.28
Protein, kg	0.77 ± 0.08	1.7[a]	1.8[b]	1.7[a]	0.01	0.003
Fat, kg	0.13 ± 0.04	0.8[b]	0.6[a]	0.6[a]	0.06	0.018
Ash, kg	0.19 ± 0.06	0.3	0.3	0.3	0.01	0.16
Water, kg	3.47 ± 0.27	7.2	7.3	7.3	0.08	0.28
Gross energy, MJ	-	72.5	66.1	65.3	2.73	0.08

[a,b] 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)

* Adapted from Herath *et al*., 2020.

Table 5.9 Dry matter, protein, fat, ash and water deposition rates in the carcass, viscera plus blood and the whole body of lambs reared artificially using three different rearing regimens: HFP57, commercial milk replacer, high fibre concentrate pellets and milk feeding to 57 days of age; HFP42, commercial milk replacer, high fibre concentrate pellets and early weaning at 42 days of age; LFP42, high protein milk replacer from 2-16 days of age followed by commercial milk replacer, low fibre concentrate pellets and early weaning at 42 days of age (SE, standard error)

Item	Treatment			Pooled SE	P - value
	HFP57	HFP42	LFP42		
Carcass					
Dry matter, g/d	29.6	28.3	26.7	1.34	0.25
Protein, g/d	16.7	17.3	15.5	0.05	0.18
Fat, g/d	13.3 [b]	7.1 [a]	6.4[a]	0.001	0.004
Ash, g/d	3.0	3.3	3.4	0.25	0.55
Water, g/d	58.9	56.9	55.5	1.73	0.39
Viscera plus blood					
Dry matter, g/d	6.8	5.9	5.6	0.45	0.22
Protein, g/d	4.3	4.4	4.2	0.29	0.71
Fat, g/d	2.7 [b]	1.6 [a]	1.5 [a]	0. 22	0.002
Ash, g/d	0.29	0.30	0.28	0.03	0.85
Water, g/d	27.6	28.7	27.3	1.25	0.67
Carcass and viscera plus blood					
Dry matter, g/d	37.1	34.0	32.1	1.88	0.17
Protein, g/d	24.4	25.4	23.4	0.63	0.13
Fat, g/d	13.1 [b]	9.7 [a]	8.8 [a]	0.11	0.017
Ash, g/d	3.4	3.5	3.6	0.25	0.58
Water, g/d	86.7	85.6	82.8	3.05	0.60

[a,b] 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).

5.4.5 Cost Analysis

The cost of milk replacer per lamb and total cost of feed per lamb were highest (P<0.05) in HFP57 treatment compared to both HFP42 and LFP42 treatments (Table 5.10). The cost of pellets was higher (P<0.05) in both HFP42 and LFP42 lambs compared to HFP57 lambs. The cost of lucerne chaff did not differ (P>0.05) between treatments.

Table 5.10 Feed cost analysis of lambs reared artificially using three different rearing regimens: HFP57, commercial milk replacer, high fibre concentrate pellets and milk feeding to 57 days of age; HFP42, commercial milk replacer, high fibre concentrate pellets and early weaning at 42 days of age; LFP42, high protein milk replacer from 2-16 days of age followed by commercial milk replacer, low fibre concentrate pellets and early weaning at 42 days of age.

Cost Item (NZ$)	Treatment			Pooled SE	P-value
	HFP57	HFP42	LFP42		
Milk cost/lamb	75.1[b]	43.1[a]	46.66[a]	3.09	<.001
Pellet cost/lamb	4.41[a]	7.86[b]	8.12[b]	0.85	0.009
Lucerne chaff cost/lamb	0.72	0.52	0.82	0.13	0.260
Feed cost /lamb	80.3[b]	51.4[a]	55.60[a]	3.69	<.001
Feed cost/ kg live weight gain	6.76[b]	5.51[a]	5.99[ab]	0.32	0.018

[a,b] 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).

5.5 Discussion

The aim of the present study was to investigate the effect of pellet fibre level (high vs low), milk replacer composition and age at weaning (early weaning at 42 days of age vs continued milk replacer feeding to 57 days age) on the growth and body composition of lambs reared artificially. Additionally, the cost effectiveness of each artificial lamb rearing regimen was determined.

5.5.1 Intake and growth performance

5.5.1.1 Pre-weaning phase (2-37 days of age)

There was no difference in ADG between treatments up until the early weaning of HFP42 and LFP42 lambs. During this period, all the lambs had access to commercial milk replacer/ high protein milk replacer and low or high fibre concentrate pellets. There was no difference between treatments for either milk replacer or pellet intake. This explains the lack of difference in ADG and live weight (LW) gain per kilogram DMI.

Higher CP:ME intake early in a lambs' life has been reported to improve their growth rate (Danso et al., 2018, Herath et al., 2020). During the first two weeks of the study, LFP42 lambs were fed a high protein milk replacer (CP:ME 15.55 g/MJ), with their CP intake from milk being higher. When milk intake and pellet intake were combined, LFP42 lambs had a higher CP:ME intake compared to both HFP57 and HFP42 lambs. However,

the higher CP:ME intake of LFP42 lambs did not result in improved growth rate compared to HFP57 and HFP42 lambs. This suggests that lambs may require a higher CP:ME intake for a longer period (63 and 57 days, for Herath et al. 2020 and Herath et al. 2021a studies, respectively) to have a notable growth difference. Alternatively, the commercial milk replacer (CP:ME ratio 12.2 MJ ME/kg) used in this study provided an adequate CP:ME for lamb growth, resulting in the lack of difference.

5.5.1.2 Early weaning phase (38-42 days of age)

The HFP42 and LFP42 lambs were weaned from milk over a period of five days (38-42 days of age). During this period, ADG between early weaned and un-weaned lambs did not differ. Early weaned lambs were offered only half an allowance of 2.1 times of their maintenance energy requirement via the commercial milk replacer and so to meet their nutrient requirements for growth, they increased their pellet intake. Daily pellet DMI intake of HFP42 and LFP42 lambs was higher than HFP57 lambs during this period. Pellet DMI relative to initial LW in the weaning period increased in HFP42 and LFP42 lambs compared to their HFP57 counterparts, resulting in all lambs having similar total daily DMI, ME and CP intakes. The boosted pellet intake compensated the nutrient requirements of HFP42 and LFP42 lambs, leading to similar ADG between treatments, despite the tendency of higher LW gain per kilogram of DMI by HFP57 lambs compared to HFP42 and LFP42 lambs.

5.5.1.3 Post-early weaning phase (43-57 days of age)

The ADG of HFP42 and LFP42 lambs was lower than HFP57 over this period. The HFP57 lambs were still being offered commercial milk replacer during this period while HFP42 and LFP42 lambs were not. Daily pellet DMI of HFP42 and LFP42 lambs was more than twice that of HFP57 lambs, however, there was no difference between treatments for total daily DMI. The HFP57 lambs had greater combined CP and ME

intake from milk replacer, pellets and lucerne chaff than the early weaned lambs. This led to higher ADG and LW gain per DMI in HFP57 lambs compared to both HFP42 and LFP42 lambs during this period. The main CP sources were milk replacer and pellets, respectively, for HFP57 and early weaned lambs. Nitrogen utilization efficiency for growth is higher in milk than pellet diets (73 and 30 %, respectively (Danso et al., 2016)), meaning that the digestibility of the combined diet for the HFP57 treatment was higher than that of HFP42 and LFP42 treatments. The HFP57 lambs ME intake largely from milk, while the ME intake of HFP42 and LFP42 lambs was from pellets. The gross energy retained for growth of lambs from milk is higher than from pellets (96 and 71 %, respectively (Danso et al., 2016)). Combined, this meant that HFP57 lambs had higher efficiency of both nitrogen and energy utilization for growth, resulting in a higher ADG and LW gain per kilogram DMI compared to early weaned lambs.

Pellet DMI at 42 days of age relative to LW at the start of the experiment was 2.2, 5.5 and 3.9 %, respectively for HFP57, HFP42 and LFP42 lambs. Greenwood et al. (1997a) reported no significant post-weaning growth check in calves if they reached a pellet intake of 1.0-2.0 % of their birth weight, at weaning. Although HFP42 and LFP42 lambs in the present study had higher pellet intakes than the range suggested by Greenwood et al. (1997a), a post-early weaning growth check was observed. Pellet compositions (crude protein, ash and fat content) of the present study and that of Greenwood et al. (1997a) were approximately similar. Unfortunately, Greenwood et al. (1997a) did not report the energy content and digestibility of the pellet used in their study, making it difficult to explain the growth check observed in lambs in the present study, despite their higher pellet intake. It may simply be there is a difference between calves and lambs.

5.5.1.4 Overall experimental period (2-57 days of age)

Lambs weaned at 42 days of age and fed pellets containing either low or high fibre had similar overall ADG and LW at slaughter (57 days) as their counterparts fed milk replacer and high fibre pellets. Early weaned lambs (HFP42 and LFP42) had consumed approximately half the quantity of milk replacer consumed by HFP57 lambs. The pellet intake of HFP42 and LFP42 lambs over this period was 83 and 64 %, respectively, higher than that of HFP57 lambs, resulting in similar total DMI between the treatments. This resulted in similar ADG in the three treatment groups. The higher LW gain per kilogram DMI in HFP57 lambs was due to the higher digestibility of their diet and higher ME intake compared to both HFP42 and LFP42 lambs. This indicates increased diet digestibility would likely increase the LW gain per kilogram DMI of early weaned lambs.

5.5.2 Slaughter parameters

Carcass weight and dressing out percentage were greater in HFP57 lambs compared to early weaned lambs (i.e. HFP42 and LFP42). This was most likely caused by heavier weights of the rumen, reticulum and omasum relative to LW in both HFP42 and LFP42 lambs compared to HFP57 lambs. Muir et al. (2008) reported a higher dressing out percentage for unweaned lambs compared to weaned lambs and suggested the difference was due to the less rumen development of unweaned lambs, which in consistent with findings of the present study.

Empty small intestine weight was heavier in HFP57 lambs compared to HFP42 and LFP42 lambs in the present study. Attaix and Meslin (1991) reported that progressive intestinal maturity of ruminants was observed during the milk feeding phase, not the milk weaning phase. In addition, they observed that the villus was longer in the distal parts of small intestine (jejunum and ileum) of unweaned lambs at eight weeks of age compared to weaned lambs. These results are consistent with the current study which found the

small intestine was heavier in lambs fed milk replacer until slaughter compared to those early weaned from milk replacer.

The percentage contribution of the abomasum to the combined stomach weight is higher (60%) at birth and progressively becomes less as a lamb transitions from a milk-based diet to a solid plant-based diet (20%, in adult sheep) due to rumen development and the establishment of fermentation functionality (Hynd, 2019). The HFP42 and LFP42 lambs had lighter abomasal weight (20% vs 19%, respectively) compared to HFP57 lambs (29 %) at slaughter, confirming that early weaning promotes rumen growth and development.

At birth, the reticulo-rumen is about 35% of the whole stomach weight and its' contribution increases to 60-65% in adult sheep (Hynd, 2019). In the present study, the percentage weight of rumen relative to whole stomach of HFP42 and LFP42 lambs was approximately 65% while it was 55% for HFP57 lambs. Moreover, HFP42 and LFP42 lambs had heavier rumens relative to their LW at slaughter compared to HFP57 lambs, suggesting that early weaning positively affected their rumen growth and development.

The greater ME intake of HFP57 lambs, and higher digestibility of their diet compared to the diets of HFP42 and LFP42 lambs, resulted in higher omental fat deposition. Gastrointestinal tract (GIT) protein synthesis increases as a lamb's rumen develops, being 11.5% in a pre-ruminant lamb vs 18-35% in ruminant lamb (Caton et al., 2000). McBride and Kelly (1990) suggested this alters the animal's energy use due to its marked contribution to the animal's energy cost. Therefore, HFP42 and LFP42 lambs may have had higher energy expenditure than HFP57 lambs, leading to less energy available for omental fat deposition which could reduce the visceral fat content of lambs.

5.5.3 Body composition

Early weaned lambs had reduced fat content and reduced rates of fat deposition in the carcass, viscera plus blood and whole body compared to lambs fed milk replacer until slaughter (HFP57). The HFP57 lambs had higher ME and higher digestible energy intake than HFP42 and LFP42 lambs. As mentioned above, HFP42 and LFP42 lambs may have had higher energy expenditure for GIT development and protein synthesis compared to HFP57 lambs. Combined, these differences could have resulted in a lower fat deposition and consequently more lean growth of HFP42 and LFP42 lambs compared to HFP57 lambs.

Protein content of both the carcass and whole body (carcass and viscera plus blood) was higher in HFP42 lambs compared to both HFP57 and LFP42 lambs. The HFP42 lambs would have gained LW mainly by protein deposition rather than fat deposition, which reflects the findings of previous studies on restricted lamb feeding (Morgan and Owen, 1972, Santos et al., 2018, Hornick et al., 2000). Interestingly, the protein content of both the carcass and whole body of the LFP42 lambs was similar to HFP57 lambs, although they were weaned early. Thus, further investigations on effect of pellet fibre level and early weaning on protein content of lamb carcass and whole body are required to clarify the relationships.

Gross energy content of viscera plus blood was reduced in early weaned lambs (HFP42 and LFP42) compared to those fed milk and pellet until slaughter (HFP57). This is due to the higher fat content of viscera plus blood of HFP57 lambs, which contained more energy compared to protein. Although HFP57 carcasses had a higher fat content, the higher protein deposition in HFP42 carcasses increased the energy content, resulting in no difference between treatments for carcass gross energy content.

5.5.4 Cost analysis

The feed costs of early weaned lambs were lower compared to HFP57 lambs, as early weaned lambs were offered approximately half of the more expensive milk replacer (compared to pellets) quantity of HFP57 lambs. The HFP42 and LFP42 lambs required an additional 4.0 and 4.7 kg/lamb of high and low fibre pellets, respectively, to match the weight of HFP57 lambs. There are no previous studies on lamb feed cost comparisons between early and late-weaned artificial lamb rearing systems. In calf rearing systems, early weaning has been reported to reduce the feed cost compared to late weaned calves (Jones and Heinrichs, 2007), which supports the findings of the present study.

5.6 Conclusion

Lambs early weaned at 42 days of age and fed concentrate pellets containing either a low or high fibre level showed similar overall growth to 57 days of age, as that of lambs feed milk replacer and pellets for the whole period. However, the early weaning rearing regimen showed overall reduced feed costs. Additionally, early weaning reduced the fat content and rate of fat deposition in the carcass, viscera plus blood and whole body, and was more cost effective than feeding lambs with milk replacer and pellets to 57 days. Thus, early weaning of lambs may benefit the farmers by receiving premium price for a leaner carcass at market and reduced feed cost under artificial lamb rearing conditions. However, the effect of early weaning on growth performance of lambs and rumen development subsequent to 57 days of age deserves further study.

Chapter 6 Rumen development of artificially-reared, early-weaned lambs fed pellets of varying fibre level and milk replacer composition

This chapter has been published elsewhere. Citation: Herath, H. M. G. P., Pain, S. J., Kenyon, P. R., Blair, H. T. & Morel, P. C. H. 2021. Rumen development of artificially-reared lambs exposed to three different rearing regimens. *Animals*, 11 (12), 3606; DOI: 10.3390/ani11123606 (The DRC form is attached in appendices).

6.1 Abstract

The objective of this study was to examine the effect of three different rearing regimens on rumen development in lambs reared artificially. Romney ram lambs were randomly allocated to one of three treatments; commercial milk replacer fed to 57 days of age and high fibre concentrate pellets (HFP57); commercial milk replacer, high fibre concentrate pellets and early weaned from milk replacer at 42 days of age (HFP42); high protein milk replacer from 2-16 days of age followed by commercial milk replacer, low fibre concentrate pellets and early weaned from milk replacer at 42 days of age (LFP42). Lambs were slaughtered at 57 days of age. Volatile fatty acid content in rumen fluid at slaughter was analysed and rumen tissue samples were collected for histological examination. The rumen n-butyric content was greater ($P<0.05$) in both LFP42 and HFP42 treatment lambs compared to HFP57 lambs. The n-valeric content was greater ($P<0.05$) in LFP42 lambs compared to both HFP57 and HFP42 treatment lambs. Thickness of the rumen dorsal wall determined by ultrasound scanning at 49 days was greater ($P<0.05$) in both HFP42 and LFP42 lambs compared to HFP57 lambs. There was an interaction ($P<0.05$) between treatment and site of rumen tissue sampling on papillae width, density and rumen muscular layer thickness. Collectively, early weaning and the provision of a low fibre pellet leads to improved rumen function and physical development.

Keywords; milk replacer; neutral detergent fibre; papillae development; pellet composition, rumen site; ultrasound scan; volatile fatty acids; age at weaning

6.2 Introduction

At birth, the rumen is anatomically and physiological underdeveloped in lambs (Baldwin et al., 2004b). The development of the pre-stomach in a young ruminant undergoes three phases: the non-ruminant phase (birth to 3 weeks), transition phase (from non-ruminant

to ruminant stage) and the ruminant phase (Membrive, 2016). Appropriate rumen development is an important physiological change that facilitates alterations in nutrient metabolism and absorption (Jiao et al., 2015b) and consequently, it influences the early growth of young ruminants. A young ruminant's dietary regimen is the key factor affecting solid feed intake, establishment of rumen micro-flora, rumen fermentation and rumen papillae development, all of which contribute to nutrient digestion and absorption (Baldwin and Connor, 2017).

Unrestricted feeding, or a higher quantity of milk replacer feeding, reduces starter solid feed intake of calves (Jasper and Weary, 2002, Terré et al., 2007). This can delay rumen development and reduce performance during the transition period to a pasture-based diet. In contrast, early milk weaning or restricted milk feeding increases solid feed intake in lambs (Wang et al., 2019). Early solid feed in-take results in earlier establishment of rumen fermentation capacity (Liu et al., 2016) and greater utilisation of nutrients from solid feed (Terré et al., 2007). This helps facilitate a smoother transition from the non-ruminant to ruminant stage, with less impact on post-weaning growth in young ruminants (Khan et al., 2016).

In general, fibre level in a diet affects the mass and volume of the rumen rather than papillary development (Diao et al., 2019). Further, it has been reported that digestion of a roughage-based diet in the rumen does not provide adequate butyrate, which is required for rumen papillae development (Coverdale et al., 2004). Grain-based concentrate diets result in higher butyrate through rumen fermentation, which helps stimulate papillae development in young ruminants (Hynd, 2019). Thus, a combination of a nutrient-balanced concentrate diet with fibre should improve overall rumen development. However, increased fibre level in a concentrate diet can reduce feed digestibility,

especially in the early life period of young ruminants who have an underdeveloped rumen (Porter et al., 2007). Thus, early weaned lamb rearing systems highlight the importance of feeding highly digestible concentrate feed with adequate fibre before milk weaning occurs, to optimise lamb growth and maximise cost effectiveness (Zhong et al., 2014, Herath et al., 2021b).

There are knowledge gaps regarding the effect of both grain-based pellet fibre level and early weaning on lamb rumen development. This study aimed to investigate the effect of three rearing regimens on rumen development of artificially reared lambs. The three rearing regimens are different combinations of pellet fibre level, milk replacer composition and age at weaning (Herath et al., 2021b).

6.3 Materials and method

The experiment was carried out at Massey University, Palmerston North, New Zealand from August 2019 to October 2019. The research procedures used were approved by the Massey University Animal Ethics Committee (MUAEC 19/64).

6.3.1 Animal management

The experimental rearing regimens and lambs utilised for this study have been previously described by Herath et al. (2021b). The experiment was conducted with lambs between 2 and 57 days of age as described below.

Twenty-seven, twin-born, Romney ram lambs were allowed to suckle from their dam for the first 24 hours after birth before one lamb per set was randomly selected for the study (Herath et al., 2021b). The lambs were then individually penned and randomly allocated to one of three rearing treatments; (i) HFP57 (n=9): commercial milk replacer fed to 57 days of age plus high fibre concentrate pellets; (ii) HFP42 (n=9): commercial milk replacer, high fibre concentrate pellets and early weaned from milk replacer at 42 days of

age; (iii) LFP42 (n=9): high protein milk replacer from 2-16 days of age followed by commercial milk replacer, low fibre concentrate pellets and early weaned from milk replacer at 42 days of age. All lambs were fed milk replacer at 2.1 times their maintenance energy requirement (Herath et al., 2021b), considering maintenance energy requirement (ME_m) as 0.40 MJ/kgLW0.75d^{-1} (Danso et al., 2016). The crude protein (CP) and metabolisable energy (ME) contents (expressed as fed basis) of commercial milk replacer powder were 262.9 g/kg and 21.8 MJ/kg, respectively (Milligans Feed Ltd, Oamaru, New Zealand). The high protein milk replacer powder was an 80:20 blend of the commercial milk replacer powder with a powdered milk protein concentrate (Fonterra, Auckland, New Zealand). The CP and ME contents of high protein milk replacer powder were 324.1 g/kg and 20.8 MJ/kg, respectively.

Milk replacer and high protein milk replacer were mixed with warm tap water at a ratio of 1:4 (w/w). Bottle feeding of lambs was done five times daily up to two weeks of age, then four times daily up to milk weaning phase at day 38-42 for HFP42 and LFP42 lambs and until the end of the experiment for HFP57 lambs (57 days).

Lambs were offered either high fibre concentrate pellet (69.35 g/kg acid detergent fibre (ADF) and 208.56 g/kg neutral detergent fibre (NDF), for HFP57 and HFP42 lambs) or low fibre concentrate pellet (44.40 g/kg ADF and 116.76 g/kg NDF for LFP42 lambs) *ad libitumly* from 4 to 57 days of age (Table 6.1). The lambs had free access to water at all the times. Lu-cerne chaff contained 114.69 g/kg CP and 7.14 MJ/kg ME (Oaklane Stables Premium Chaff, Hawkes Bay, New Zealand).

Lambs in HFP42 and LFP42 treatments were provided 50 % of their milk allowance during the five days of milk weaning phase (38-42 days of age), while HFP57 lambs were given their full milk allowance until 57 days of age. The HFP42 and LFP42 lambs were

bottle-fed twice daily during the milk weaning phase and fully weaned from milk at 42 days. During the milk weaning phase, lambs in HFP57 and HFP42 were offered *ad libitum* access to high fibre concentrate pellets, while LFP42 lambs were offered *ad libitum* access to low fibre concentrate pellets. All the lambs were provided 40 g/d of lucerne chaff through the day 38-57 period. Pellet, milk replacer and lucerne chaff intakes were recorded daily. All lambs remained in the study until 57 days of age.

Table 6.1 Pellet composition in fresh matter basis (Adapted from Herath et al. (2021b))

Ingredient	Low fibre concentrate pellet (LFP)	High fibre concentrate pellet (HFP)
Barley, g/kg[1]	0.270	0.390
Broll, g/kg	0.000	0.351
Soya bean meal, g/kg	0.225	0.218
Wheat, g/kg	0.389	0.000
Molasses, g/kg[2]	0.030	0.030
Skim milk powder, g/kg	0.075	0.000
Limestones, g/kg	0.010	0.010
Sheep premix, g/kg[3]	0.001	0.001

[1] Broll is a mixture of wheat bran and wheat pollard, crude protein 153 g/kg and neutral detergent fibre 359 g/kg.
[2] Source of molasses is sugar beet.
[3] Cobalt 0.2 g/kg, Iodine 0.2 g/kg, Magnesium 0.14 g/kg, Selenium 0.04 g/kg, Sodium 0.14 g/kg, Zinc 4 g/kg, and vitamin E 1 IU/g.

6.3.2 Ultrasound scanning of rumen

Ultrasound scanning of each lamb's rumen was performed at experimental week five (36 ± 1.2 days of age) and seven (49 ± 1.2 days of age). Scanning was undertaken with the lamb laying on its right side, using a Clarius portable ultrasound scanner with a 3.0- 7.0 MHz convex transducer and maximum penetration depth of 20 cm (Clarius, Gilmore way, Canada). The wool was clipped from the area on the dorsal left side, just caudal to the last rib, to the end of the transverse process of the last lumbar vertebrae. The rumen was examined caudal to the last rib by placing the ultrasound scanner parallel to the last rib with approximately a 30^{o} angle from the zenith (Figure 6.1), after application of

transmission gel (Aquasonic, Parker laboratories Inc, Fairfield, New Jersey). Images were then captured and the thickness of the rumen wall was measured (9 measurements per lamb) by FIJI imageJ software (Schindelin et al., 2012).

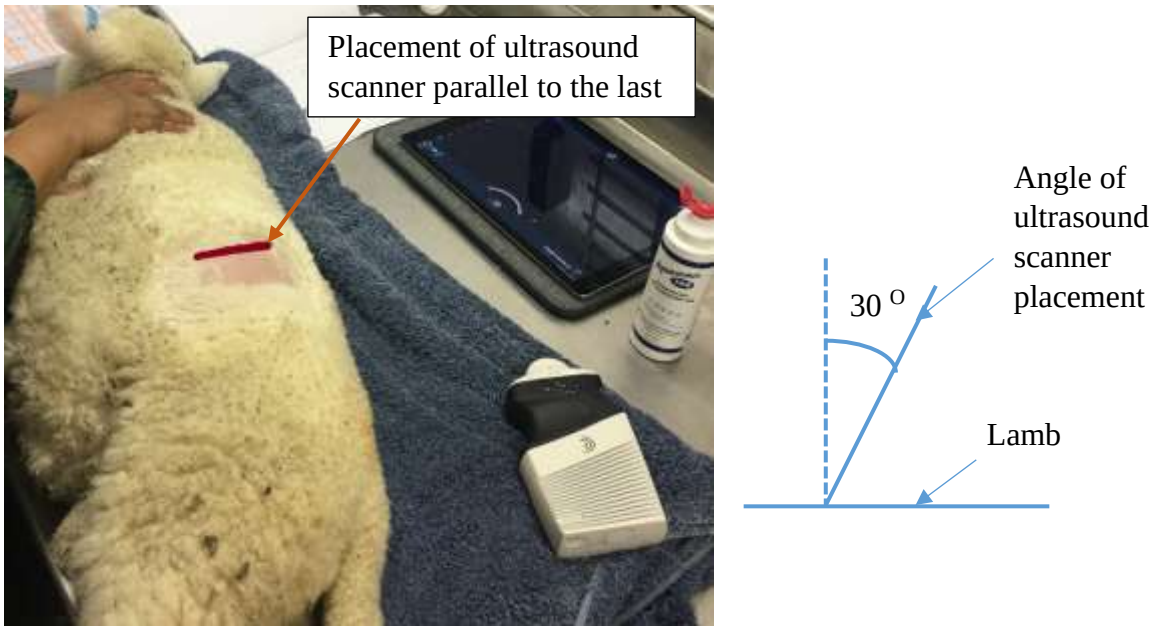


Figure 6.1 The angle and site of positioning of ultrasound scanner to capture rumen images.

6.3.3 Slaughter

Lambs were slaughtered at 57 days of age regardless of their live weight. The lambs were slaughtered via captive bolt, after being fasted for approximately 12 hours, exsanguinated, skinned and eviscerated. Rumen fluid samples were collected into cryovials with phosphate buffer saline (PBS) immediately after opening the rumen, for the analysis of volatile fatty acid (VFA) content. All rumen fluid samples were stored in liquid nitrogen immediately after collection and then at -80 ^{o}C until further analysis.

Tissue samples, approximately 1.5 cm by 1.5 cm, were collected at slaughter from each lamb, from the rumen dorsal and ventral walls and placed in histology cassettes with a tissue sponge. The tissues were fixed in 4 % paraformaldehyde for 24 hours and transferred to 70 % ethanol solution until blocks were prepared for histology slides.

6.3.4 Analysis of rumen fluid samples

Rumen fluid samples were analysed at the Nutrition Laboratory, Massey University, Palmerston North, New Zealand.

Volatile fatty acid (VFA) content of rumen fluid collected at slaughter was analysed by gas chromatography as described by Sukhija and Palmquist (1988). Briefly, rumen fluid samples were suspended in toluene and fatty acids were methylated by using methanolic hydrochloride (a mixture of methanol and acetyl chloride) in culture tubes. Samples were vortexed and heated at 70 °C for methylation for 2 hours. After methylation, samples were cooled on ice and potassium carbonate and toluene were added. Samples were vortexed and centrifuged at 2500 rpm for 7 minutes at room temperature to separate the solvent layer containing methyl esters and the aqueous layer. The VFA content was determined by Shimadzu GC-2010 Plus Gas Chromatograph (Shimadzu, Japan) equipped with a Supelco^{TM}-2560 Capillary Column (100 m x 0.25 mm x 0.2 µm film thickness). The acetic, propionic, n-butyric, iso-butyric, iso-valeric, n-valeric and n-caproic acid contents were determined.

6.3.5 Histology

Histology slides of rumen dorsal and ventral tissue samples were prepared by the Histopathology Laboratory of the School of Veterinary Science, Massey University, Palmerston North, New Zealand. Rumen tissue samples were dehydrated overnight in graded alcohol and embedded in wax (Histostar Embedding, Thermo Fisher Scientific, USA). Tissue samples were cut at 5 µm thickness (Rotary microtome cut 4055, Microtec, Germany) and mounted on slides (Paraffin section mounting bath, Electrothermal, UK). Two slides were prepared from each sample leaving 500 µm distance between sampling points. Slides were stained with hematoxylin and eosin stain (Leica auto strainer XL,

Germany, Leica CV 5040 cover slipper, Germany). The images from each slide were captured by QImaging micropublisher six color camera (Teledyne QImaging, Canada) using OCULAR^{TM}, advanced scientific camera control software (Digital Optics Limited, Auckland, New Zealand) fixed to a Leica DMRBE fluorescent microscope (Leica Microsystems, Wetzlar, Germany) under 5 X 10 magnification at Manawatu Microscopy and Image Center, Massey University, Palmerston North, New Zealand. FIJI ImageJ software (Schindelin et al., 2012) was then used to measure rumen histomorphometry. Rumen papillae length, width and thickness of the muscular layer of all complete rumen papillae across each image captured were measured. The number of papillae, the surface length of papillae (Dieho et al., 2016) and the respective straight length of rumen tissue in the images were measured (Figure 6.2).

6.3.6 Calculations

Acetate to propionate ratio of rumen fluid was calculated as acetate content divided by propionate content. Total VFA content of rumen fluid was calculated as the sum of acetic, propionic, n-butyric, iso-butyric, iso-valeric, n-valeric and iso-caproic acid contents.

Papillae density of the rumen wall was calculated as the number of papillae divided by the respective length of rumen tissue. Papillae surface length per unit rumen tissue length was calculated as the measured papillae surface length divided by respective straight rumen tissue length (Figure 6.2). Thickness of the rumen wall was calculated as the summation of mean papillae height and the thickness of muscular layer of rumen wall measured from histology slides.

Total organic matter intake of lambs from pellet and lucerne chaff was calculated as total dry matter intake minus total ash intake from each feedstuff during the experiment. Total hemicellulose intake of lambs was calculated as total NDF intake minus total ADF intake.

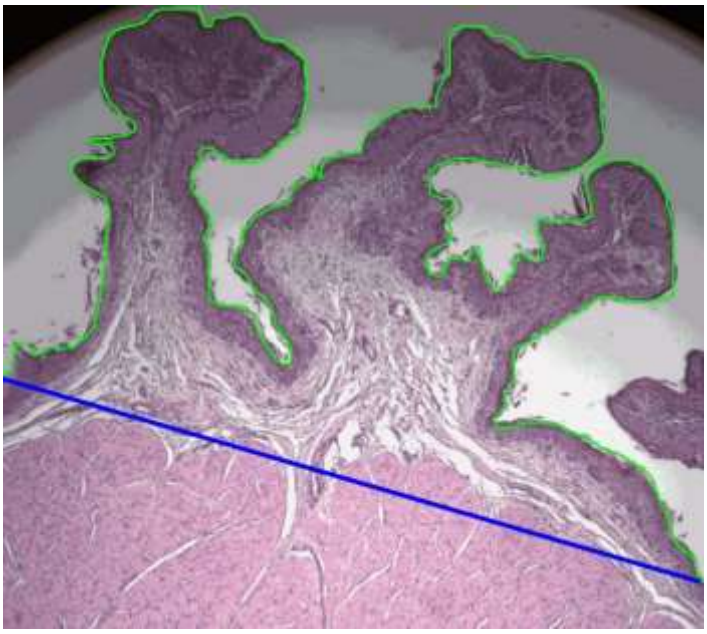


Figure 6.2 The length of papillae outer boundary of the stratum corneum (——) and the respective straight length of rumen tissue (——) measured in dorsal and ventral sites of rumen by FIJI ImageJ software to calculate the ratio between length of papillae surface/ straight length of rumen tissue (PSL/STL) of lambs

6.3.7 Statistical analysis

Three lambs out of 27 were excluded from statistical analysis of VFA content, rumen histology and ultrasound image analysis due to health issues (1 and 2 from HFP57 and LFP42 treatments, respectively). In addition, two lambs from the HFP42 treatment which were not deprived of feed 12 h before slaughter were excluded from the VFA and correlation analyses between dietary parameters and rumen development parameters, as their VFA values would have been affected by the different treatments.

Volatile fatty acid content of rumen fluid samples was analysed using a linear model with treatment as a fixed effect (Proc GLM, SAS 9.4 (SAS 9.4, 2013)), considering individual lamb as the experiment unit. Papillae height, width, rumen wall muscle layer thickness, papillae density and papillae surface length per rumen tissue length were analysed using a linear model with treatment, rumen tissue sampling sites and their interaction as a fixed

effect (Proc mixed, SAS 9.4 (SAS 9.4, 2013)) after log (base 10) transformation of the data. Rumen dorsal wall thickness measured by ultrasound scanning was analysed using a linear model with treatment as a fixed effect (Proc GLM, SAS 9. (SAS 9.4, 2013)), and lamb and replicate ultrasound image were nested within treatment. Differences ($P<0.05$) were identified, where appropriate, using the least significant difference (LSD) mean comparison test.

An overall summary of pellet, lucerne chaff and nutrient intakes, empty rumen weight and rumen volume data of all lambs from the study that was used for correlation analysis is presented in Table 6.2. A detailed analysis of those parameters is presented in Herath et al (Herath et al., 2021b). Correlations between ADF, NDF, ME, CP, organic matter, pellet and lucerne chaff intakes and rumen morphology development parameters (empty rumen weight, rumen papillae length, papillae width and rumen wall muscular layer thickness) and metabolic development parameters (VFA content of rumen fluid) were estimated using Proc CORR, SAS 9.4 (2013).

Principal component analysis was carried out for rumen morphology development parameters (empty rumen weight, rumen papillae length, papillae width and rumen wall muscular layer thickness) and rumen metabolic development parameters by Minitab 19 statistical software (Minitab, 2020). Correlations between total dry matter, ME, CP, organic matter, ADF, NDF and hemicellulose intakes from pellet and lucerne chaff and the first two estimated principal components for rumen morphology and metabolic development parameters were analysed using Proc CORR, SAS 9.4 (SAS 9.4, 2013).

Correlations between the thickness of dorsal rumen wall recorded from ultrasound image analysis at seven weeks of age and rumen papillae height, rumen wall thickness and

muscular layer thickness at the dorsal site as recorded from histology image analysis (at slaughter) were estimated using Proc CORR, SAS 9.4 (SAS 9.4, 2013).

Table 6.2 Overall means, standard deviation (SD) and range for pellet, lucerne chaff and nutrients intakes, empty rumen weight and rumen volume of the lambs included in the study (DMI, dry matter intake; ME, metabolisable energy; CP, crude protein; ADF, acid detergent fibre; NDF, neutral detergent fibre)

Parameter	Mean (n=22)	SD	Range
Total DMI from pellets, kg	7.60	3.31	1.76-12.98
Total DMI from lucerne chaff, kg	0.29	0.16	0.02-0.58
Total DMI, kg	7.89	3.37	2.01-13.47
Total ME intake, MJ	99.17	42.72	24.74-171.10
Total CP intake, g	1616.97	697.34	404.56-2800.56
Combined CP:ME intake, g/MJ	16.31	0.08	16.19-16.38
Organic matter intake from pellets and lucerne chaff[1], kg	7.45	3.19	1.90-12.74
Total ADF intake, g	661.24	287.05	197.08-1254.23
Total NDF intake, g	1698.45	825.25	525.26-3380.44
Total hemicellulose intake[2], g	1037.21	547.57	312.37-2126.21
Empty rumen weight, g	238.46	68.10	125.30-404.60
Rumen volume, ml	1099.00	327.88	552.59-1917.81

[1] Calculated as total dry matter intake from pellet and lucerne chaff minus ash intake of respective feedstuff.
[2] Calculated as total NDF intake minus total ADF intake.

6.4 Results

6.4.1 Volatile fatty acid content of rumen fluid

The n-butyric content of rumen fluid at slaughter was greater (P<0.05) in both LFP42 and HFP42 treatment lambs compared to HFP57 lambs, which did not differ (P>0.05, Table 6.3). The n-valeric content of rumen fluid at slaughter was greater (P<0.05) in LFP42 lambs compared to both HFP57 and HFP42 lambs, which did not differ (P>0.05). The n-caproic content of rumen fluid at slaughter was tended to be higher (P=0.06) in LFP42 lambs compared to both HFP57 and HFP42 lambs. The remaining VFA measures did not differ (P>0.05) between treatments.

6.4.2 Rumen Morphology

6.4.2.1 Histology

The average number of measurements recorded (mean ± SD) for rumen papillae length, width and muscular layer thickness from dorsal rumen site per lamb were 22 ± 6, 22 ± 7 and 26 ± 7, respectively. From the ventral rumen site the numbers of measurements (mean ± SD) per lamb were 31 ± 10, 31 ± 10 and 27 ± 7, respectively. There was a significant effect of sampling site (Table 6.4), whereby papillae on the dorsal rumen wall were shorter but wider than those on the ventral rumen wall. Papillae density (number of papillae per cm) was also lower at the dorsal sampling site, whilst dorsal muscle layer thickness was greater than that of the ventral rumen samples. The ratio between the length of papillae outer boundary of the stratum corneum and corresponding straight length of rumen tissue (PSL:STL) was greater ($P<0.05$) on the ventral site of the rumen.

There was a significant ($P<0.05$) interaction between rearing regimen/treatment and site of rumen tissue sampling for papillae width, papillae density and muscle layer thickness (Table 6.4). Whilst dorsal rumen papillae were significantly ($P<0.05$) wider than ventral rumen papillae for HFP57 lambs, papillae width was comparable between dorsal and ventral rumen sites for those lambs weaned early; HFP42 and LFP42, and also similar to papillae width at dorsal rumen site of HFP57 lambs. Consequently, papillae density (number of papillae per cm) at the dorsal sampling site was significantly ($P<0.05$) lower than that at the ventral site for HFP57 lambs. Papillae density was comparable between dorsal and ventral rumen sites for those lambs weaned early; HFP42 and LFP42, and also similar to papillae density at dorsal rumen site of HFP57 lambs. The dorsal rumen of HFP57 lambs had a thicker ($P<0.05$) muscle layer compared to both the dorsal and ventral rumen sampling sites of HFP42 and LFP42 lambs and ventral site of HFP57 lambs.

Papillae height, percentage of papillae longer than 500 µm and the ratio between the length of papillae outer boundary of the stratum corneum and corresponding straight length of rumen tissue (PSL:STL) did not differ (P>0.05) between treatments (Table 6.4). Papillae height, percentage of papillae longer than 500 µm and the PSL:STL ratio were greater (P<0.05) in the ventral site of the rumen than dorsal site.

Table 6.3 Effect of pellet fibre level, milk replacer composition and time of weaning on volatile fatty acid content of rumen fluid from artificially reared lambs at slaughter (HFP57, high fibre concentrate pellets and conventional weaning; HFP42, high fibre concentrate pellets and early weaning; LFP42, low fibre concentrate pellets and early weaning).

	Treatment LS means			Pooled SE	P-value
Item	HFP57*	HFP42*	LFP42*		
Acetic, mg/g	0.85 (1.41)	0.78 (1.29)	0.93 (1.56)	0.100	0.33
Propionic, mg/g	0.38 (0.51)	0.38 (0.52)	0.42 (0.57)	0.041	0.48
n-Butyric, mg/g	0.13[a] (0.15[)]	0.17[b] (0.20)	0.19[b] (0.22)	0.021	0.019
iso-Butyric, mg/g	0.06 (0.073)	0.07 (0.080)	0.08 (0.090)	0.008	0.18
n-Valeric, mg/g	0.057[a] (0.056)	0.063[a] (0.063)	0.087[b] (0.085)	0.0106	0.018
iso-Valeric, mg/g	0.08 (0.075)	0.08 (0.081)	0.10 (0.097)	0.021	0.29
n-Caproic, mg/g	0.03 (0.026)	0.03 (0.031)	0.05 (0.046)	0.011	0.06
Acetic to propionic ratio[1]	2.22 (2.75)	1.99 (2.46)	2.19 (2.74)	0.116	0.11
Total VFA, mg/g[2]	1.58 (22.9)	1.58 (22.6)	1.86 (26.7)	0.172	0.19

[1] Calculated as acetic acid content divided by propionic acid content of rumen fluid
[2] Calculated as summation of all volatile fatty acid contents of rumen fluid
* Individual VFA values in mmol/100ml and total VFA values in mmol/L are presented in parentheses

Table 6.4 Papillae height (µm), width (µm), density (Number/cm), thickness of muscle layer (µm, MLT), longer papillae percentage and papillae surface length to straight tissue length (PSL/STL) ratio at the dorsal and ventral sites of rumen from artificially reared lambs fed different pellet fibre levels, milk replacer composition and weaned early (HFP57, high fibre concentrate pellets and conventional weaning; HFP42, high fibre concentrate pellets and early weaning; LFP42, low fibre concentrate pellets and early weaning; D, Dorsal rumen site; V, Ventral rumen site).

Variable	Back transformed log LSmeans (logLSmeans)[1]			Pooled log SE	Back transformed log LSmeans (logLSmeans) [1]		Pooled log SE	Back transformed log LSmeans (logLSmeans) [1]						Pooled log SE	P value		
	Treatment				Rumen site			Treatment * rumen site									
								HFP57		HFP42		LFP42					
	HFP57	HFP42	LFP42		D	V		D	V	D	V	D	V		Treat-ment	Rumen site	Treatment*rumen site
Papillae height	648.3 (2.8)	748.7 (2.9)	796.5 (2.9)	0.04	649.2^{a} (2.8)	817.3^{b} (2.9)	0.02	569.4 (2.8)	738.2 (2.9)	675.8 (2.8)	829.5 (2.9)	711.4 (2.9)	891.9 (3.0)	0.04	0.23	<0.0001	0.8651
Papillae width	419.6 (2.6)	421.4 (2.6)	437.7 (2.6)	0.02	442.7^{b} (2.7)	410.2^{a} (2.6)	0.01	478.0^{b} (2.68)	368.3^{a} (2.57)	414.0^{ab} (2.62)	428.7^{b} (2.57)	438.3^{b} (2.64)	437.1^{b} (2.64)	0.02	0.81	0.0006	<0.0001
MLT	1112.8 (3.1)	834.1 (2.9)	874.0 (2.9)	0.04	1006.0^{b} (3.0)	864.6^{a} (2.9)	0.02	1224.1^{c} (3.09)	1011.6^{a} (3.00)	913.3^{a} (2.96)	761.9^{b} (2.88)	910.8^{ab} (2.96)	838.7^{ab} (2.92)	0.04	0.08	<0.0001	0.0033
Papillae density	16.2 (1.2)	13.7 (1.1)	14.2 1.2)	0.04	13.4^{a} (1.1)	16.1^{b} (1.2)	0.02	13.5^{a} (1.1)	19.5^{b} (1.3)	13.3^{a} (1.1)	14.1^{a} (1.2)	13.3^{a} (1.1)	15.1^{a} (1.2)	0.04	0.33	<.0001	0.0055
Longer Papillae %[2]	63.0 (1.8)	64.5 (1.8)	68.1 (1.8)	0.03	59.0^{b} (1.2)	71.9^{a} (1.9)	0.02	57.0 (1.76)	69.6 (1.84)	58.8 (1.77)	70.7 (1.85)	61.3 (1.79)	75.7 (1.88)	0.03	0.67	<.0001	0.9696
PSL/STL[3]	4.0 (0.6)	4.2 (0.6)	4.3 (0.6)	0.04	3.6 ^{a} (0.6)	4.9^{b} (0.7)	0.03	3.2 (0.51)	4.9 (0.69)	3.8 (0.58)	4.7 (0.67)	3.7 (0.57)	5.0 (0.70)	0.04	0.82	<.0001	0.1232

[1] Value in bracket after LS mean represents respective log LS mean
[2] Percentage of papillae longer >500 µm
[3] Length of papillae surface/ straight length of rumen tissue.

6.4.2.2 Rumen wall thickness as measured by ultrasound scanning

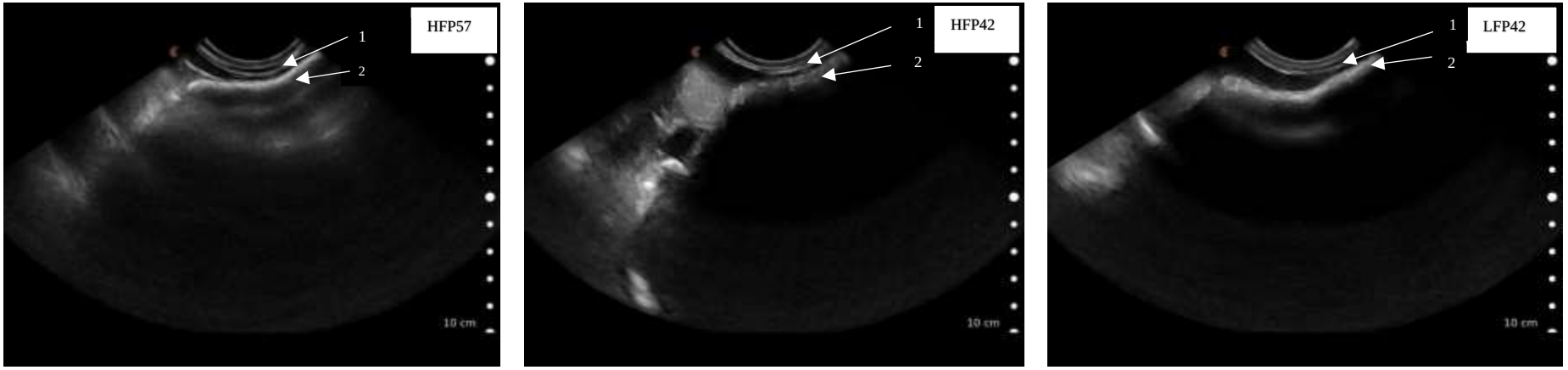
Rumen dorsal wall thickness of lambs at five weeks (36 ± 1.2 days) of age as captured by ultrasound scanning did not differ (P>0.05) between treatments (Table 6.5). Rumen wall thickness of lambs at seven weeks (49 ± 1.2 days) of age was greater (P<0.05) in both HFP42 and LFP42 lambs compared to HFP57 lambs (Table 6.5, Figure 6.3). The variations in lambs within treatment and ultrasound scanned images captured from the same lamb were significant (P<0.05) for thickness of the rumen wall at both five and seven weeks (Table 6.5).

There were no significant correlations between rumen wall thickness measured via ultrasound at 36 ± 1.2 days and papillae height at dorsal site (r=0.03, P=0.89), muscle layer thickness at dorsal site (r=0.34, P=0.10) and rumen wall thickness (r=0.34, P=0.11) obtained from histology measurements recorded at 57 days of age. There was a significant positive correlation (r=0.42, P=0.04) between rumen wall thickness measurement obtained from ultrasound scanned images captured at 49 ± 1.2 days of age and the papillae height of dorsal rumen site, measured using histology slides prepared from samples collected at slaughter (56 ± 2.1 days of age). There were no significant correlations between rumen wall thickness measurements obtained from ultrasound scanned images (49 ± 1.2 days of age) and muscle layer thickness (r=-0.23, P=0.27), thickness of rumen wall at dorsal site (r=-0.04, P=0.87) measured from histology slides based on samples collected at slaughter.

Table 6.5 Effect of three lamb rearing treatments (HFP57, high fibre concentrate pellets and milk feeding to 57 days of age; HFP42, high fibre concentrate pellets and early weaning at 42 days of age; LFP42, low fibre concentrate pellets and early weaning at 42 days of age) on lamb rumen dorsal wall thickness at five and seven weeks of age as measured from ultrasound scanned images (SE, standard error).

	Treatment			Pooled SE	P-value		
Lambs age	HFP57	HFP42	LFP42		Treatment	Lamb[1]	Image[2]
5 weeks (36 ± 1.2 days of age), cm	0.25	0.20	0.22	0.04	0.7299	<.0001	0.0014
7 weeks (49 ± 1.2 days of age), cm	0.33[a]	0.48[b]	0.56[b]	0.05	0.0068	<.0001	0.0204

[1] Effect of individual lamb in each treatment.
[2] The effect of replicate Ultrasound scanned image captured from each lamb.



(a) (b) (c)

Figure 6.3 Ultrasound scanned images showing rumen wall thickness captured caudal to the last rib by placing the Clarius ultrasound scanner (3.0-7.0 MHz) parallel to the last ribs of seven weeks (49 ± 1.2 days) old lambs ((a) HFP57, high fibre concentrate pellet and milk feeding to 57 days of age; (b) HFP42, high fibre concentrate pellet and weaning at 42 days of age; (c) LFP42, low fibre concentrate pellet and weaning at 42 days of age) laying on its right side, 1. Abdominal wall, 2. Rumen wall.

6.4.3 Correlations between dietary factors and rumen development parameters

The correlation analysis was carried out between the dietary intake and rumen development using data generated Herath et al 2021b study (Chapter 5). Pellet intake of lambs varied between treatments, where HFP42 and LFP42 lambs had greater pellet intake compared to late weaned lambs (Table 6.2). Further, there were variations in pellet intake of individual lamb within the treatment. Consequently, the CP, ME and organic matter intakes from pellet and lucerne chaff of lambs fluctuated within a huge range (Table 6.2).

6.4.3.1 Morphology development

Eigenvectors with an eigenvalue greater than 1.0 in the principal components analy-sis on rumen physical development are presented in Table 6.6. Approximately 85 % of the total variation was explained by the first four principal components and approximately 55 % by the first two principal components. Empty weight of rumen and rumen papillae height at the dorsal site had positive contribution ($r \geq 0.5$) on the first principal component ($PC1_{(pd)}$). Rumen papillae width at the dorsal site had a greater and negative contribution for the second principal component ($PC2_{(pd)}$).

The first two principal components did not allow complete discrimination between the three treatments (Figure 6.4), but HFP57 lambs were mostly discriminated from HFP42 and LFP42 lambs based on the $PC1_{(pd)}$.

Figure 6.5 shows the significant positive correlations and tendencies in correlations between dietary factors and rumen development parameters. Empty rumen weight had significant ($P<0.05$) positive correlations with ME, CP, total and pellet dry matter, organic matter intakes from pellets and lucerne chaff, ADF, NDF and hemicellulose intakes (Table 6.7, Figure 6.5). Rumen volume had positive significant ($P<0.05$)

correlations with the ME, CP, total dry matter, organic matter intakes from pellets and lucerne chaff and ADF intake. Rumen papillae height of both the dorsal and ventral rumen sites had positive significant (P<0.05) correlations with ME, CP, organic matter intake from pellets and lucerne chaff and dry matter intakes (Table 6.7, Figure 6.5). Principal component one ($PC1_{(pd)}$) had significant (P<0.05) correlations with ME, CP, total dry matter, organic matter from pellets and lucerne chaff, ADF, NDF and hemicellulose intakes. There were no significant correlations (P>0.05) between all the other dietary measurements and principal component two $PC2_{(pd)}$). There were no significant correlations (P>0.05) between all the other dietary and rumen physical development parameters.

Empty rumen weight had positive significant correlation with papillae height at the ventral (r=0.46, P= 0.03), and dorsal sites (r=0.52, P=0.01) and papillae width at the ventral site (r= 0.47, P=0.03) of the rumen (Figure 6.5).

Table 6.6 Eigenvectors on the first four principal components determined by principal component analysis of rumen physical development parameters ($PC1_{(pd)}$, $PC2_{(pd)}$, $PC3_{(pd)}$ and $PC4_{(pd)}$) of artificially reared lambs exposed to three rearing treatments

Variable	$PC1_{(pd)}$*	$PC2_{(pd)}$*	$PC3_{(pd)}$*	$PC4_{(pd)}$*
Empty rumen weight, g	0.517	-0.230	-0.272	-0.026
Rumen volume, ml	0.240	-0.332	-0.474	0.529
Rumen papillae height – Dorsal site, µm	0.528	0.134	0.153	-0.233
Rumen papillae height – Ventral site, µm	0.415	0.364	-0.167	-0.356
Rumen papillae width – Dorsal site, µm	-0.166	-0.618	0.048	-0.173
Rumen papillae width – Ventral site, µm	0.350	-0.434	0.195	-0.094
Rumen wall muscle layer thickness – Dorsal site, µm	-0.226	-0.262	-0.338	-0.707
Rumen wall muscle layer thickness – Ventral site, µm	-0.151	0.219	-0.704	-0.012
Eigen value	2.514	1.869	1.345	1.034
Variation explained, %	31.4	23.4	16.8	12.9
Cumulative variation explained, %	31.4	54.8	71.6	84.5

* $PC1_{(pd)}$, $PC2_{(pd)}$, $PC3_{(pd0}$ and $PC4_{(pd)}$; the first, second, third and fourth principal components, respectively, which estimated for rumen physical development parameters by principal component analysis.

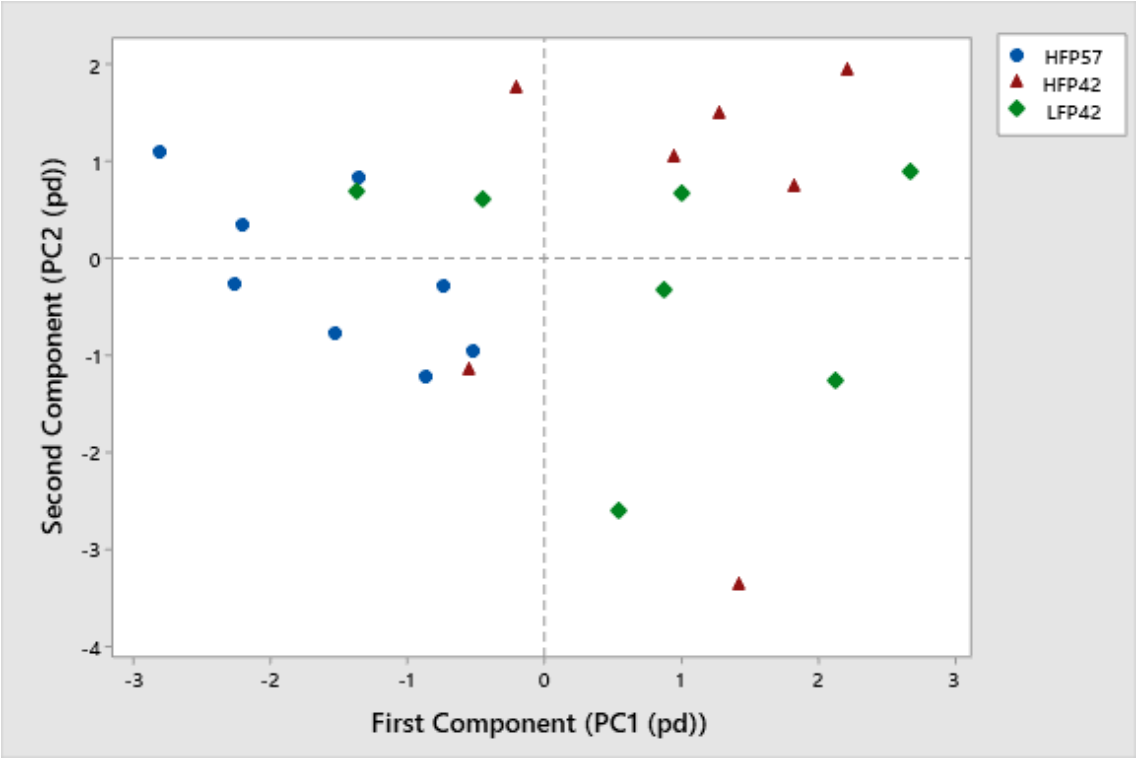


Figure 6.4 Principal component analysis of rumen morphology development of lambs given three rearing treatments (HFP57, high fibre concentrate pellet and milk feeding to 57 days of age ●; HFP42, high fibre concentrate pellet and early weaning ▲; LFP42, low fibre concentrate pellet and early weaning ◆)

Table 6.7 Pearson correlation coefficients of dietary factors and rumen physical development parameters, principal components of rumen physical development ($PC1_{(pd)}$ and $PC2_{(pd)}$) of artificially reared lambs exposed to three rearing treatments (DMI, dry matter intake; ME, metabolisable energy; CP, crude protein; ADF, acid detergent fibre; NDF, neutral detergent fibre)

Parameter	Empty rumen weight, g	Rumen volume, ml	Papillae height – Dorsal, µm	Papillae height - Ventral, µm	Papillae width – Dorsal, µm	Papillae width – Ventral, µm	Muscle layer thickness - Dorsal, µm	Muscle layer thickness – Ventral, µm	$PC1_{(pd)}$ [3]	$PC2_{(pd)}$ [3]
Total DMI from pellets, kg	0.85**	0.51*	0.58*	0.53*	-0.05	0.30	-0.19	-0.16	0.76**	0.05
Total DMI from lucerne chaff, kg	0.35	0.55*	-0.03	-0.05	0.31	0.24	0.21	-0.14	0.12	-0.49
Total DMI, kg	0.86**	0.53*	0.54*	0.51*	-0.03	0.30	-0.18	-0.19	0.75**	0.03
Total ME intake, MJ	0.86**	0.51*	0.53*	0.52*	-0.03	0.29	-0.18	-0.16	0.75**	0.06
Total CP intake, g	0.86**	0.51*	0.53*	0.52*	-0.03	0.29	-0.18	-0.15	0.74**	0.04
Combined CP:ME intake, g/MJ	-0.07	-0.36	-0.25	-0.09	0.08	-0.26	0.20	0.23	-0.33	0.08
Organic matter intake from pellets and lucerne chaff[1], kg	0.86**	0.53*	0.54*	0.51*	-0.03	0.30	-0.18	-0.16	0.75**	0.03
Total ADF intake, g	0.81**	0.46*	0.39	0.39	0.10	0.20	-0.05	-0.07	0.56*	-0.05
Total NDF intake, g	0.77**	0.34	0.38	0.41*	0.05	0.13	-0.07	-0.03	0.53*	0.04
Total hemicellulose intake[2], g	0.74**	0.28	0.36	0.42	0.03	0.09	-0.08	-0.004	0.50*	0.09

**Significant at confidence level of <0.0001, * Significant at confidence level of 0.05.

[1] Calculated as total dry matter intake from pellet and lucerne chaff minus ash intake of respective feedstuff.

[2] Calculated as total NDF intake minus total ADF intake.

[3] $PC1_{(pd)}$ and $PC2_{(pd)}$; the first and second principal components, which estimated for rumen physical development parameters by principal component analysis.

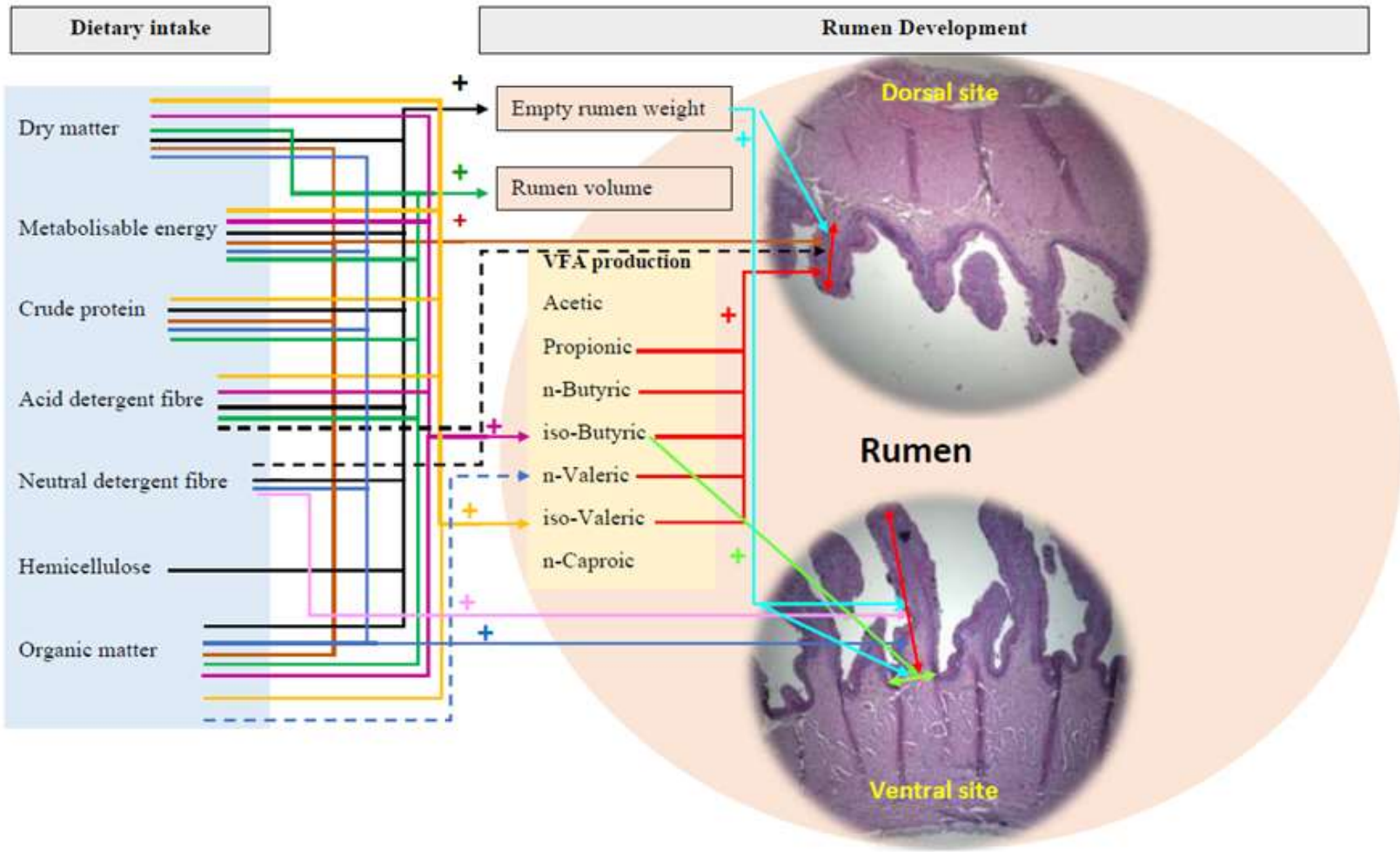


Figure 6.5 Schematic diagram of correlations between dietary factors and rumen development parameters of artificially reared lambs fed different pellet fibre levels, milk replacer composition and weaned early (significant positive influence —— and tended positive influence - - -). The parameters combined to a same colour line influence the respective rumen development parameter (e.g. all parameters joined to black colour solid line positively influenced the empty rumen weight).

6.4.3.2 Metabolic development

Eigenvectors with an eigenvalue greater than 1.0 of principal components analysis on rumen metabolic development are presented in Table 6.8. Approximately 94 % of the total variation was explained by the first three principal components while approximately 79 % was explained by the first two principal components. However, none of the metabolic parameters had a greater contribution (correlation above 0.5) for the first principal component while iso-butyric and iso-valeric acid had greater (correlation above 0.5) contributions for the second principal component (Table 6.8).

Iso-butyric and iso-valeric contents of rumen fluid had positive significant ($P<0.05$) correlations with the ME, ADF, dry matter and organic matter intakes from pellets and lucerne chaff (Table 6.9, Figure 6.5). Iso-valeric content of rumen fluid had positive significant ($P<0.05$) correlations with the CP intake (Table 6.9, Figure 6.5). There were no significant correlations ($P>0.05$) between all the other dietary and rumen metabolic development parameters and principal components one and two (Appendix 1).

6.4.3.3 Morphology vs metabolic rumen development

Papillae height at the dorsal rumen site had significant ($P<0.05$) positive correlations with propionic ($r=0.49$), iso-butyric ($r=0.48$), n-butyric ($r=0.61$), iso-valeric ($r=0.42$) and n-valeric ($r=0.48$) contents of rumen fluid (Figure 6.5). Papillae width at the ventral rumen site had a significant ($P<0.05$) positive correlation with iso-butyric ($r=0.43$) content of the rumen fluid. All the other rumen physical development parameters did not significantly ($P>0.05$) correlate with the volatile fatty acid content of the rumen fluid (Appendix 2).

Table 6.8 Eigenvectors on the first three principal components ($PC1_{(md)}$, $PC2_{(md)}$ and $PC3_{(md)}$) determined by principal component analysis of rumen metabolic development parameters of artificially reared lambs exposed to three rearing treatments

Variable	$PC1_{(md)}$ *	$PC2_{(md)}$ *	$PC3_{(md)}$ *
Acetic, mg/g	0.40	0.37	0.29
Propionic, mg/g	0.45	0.24	0.21
iso-Butyric, mg/g	0.36	-0.56	0.05
n-Butyric, mg/g	0.42	0.30	-0.10
iso-Valeric, mg/g	0.32	-0.62	-0.01
n-Valeric, mg/g	0.47	0.02	-0.15
n-Caproic, mg/g	0.12	0.12	-0.91
Eigen value	4.05	1.47	1.07
Variation explained, %	57.8	21.0	15.3
Cumulative variation explained, %	57.8	78.9	94.2

* $PC1_{(md)}$, $PC2_{(md)}$ and $PC3_{(md)}$; the first, second and third principal components, respectively, which estimated for rumen metabolic development parameters by principal component analysis.

Table 6.9 Pearson correlation coefficients of dietary factors and iso-butyric, and iso-valeric content of rumen fluid, principal components determined for rumen metabolic development parameters ($PC1_{(md)}$ and $PC2_{(md)}$) of artificially reared lambs exposed to three rearing treatments (DMI, dry matter intake; ME, metabolisable energy; CP, crude protein; ADF, acid detergent fibre; NDF, neutral detergent fibre)

	Iso-Butyric	Iso-Valeric	$PC1_{(md)}$[3]	$PC2_{(md)}$[3]
Total DMI from pellets	0.54*	0.49*	0.21	-0.03
Total DMI from lucerne chaff	0.45*	0.43*	0.27	0.10
Total DMI	0.55*	0.50*	0.22	-0.02
Total ME intake	0.54*	0.49*	0.21	-0.03
Total CP intake	0.53	0.49*	0.21	-0.03
Organic matter intake from pellets and lucerne chaff[1], kg	0.55*	0.50*	0.23	-0.02
Total ADF intake	0.48*	0.45*	0.20	-0.01
Total NDF intake	0.39	0.37	0.12	-0.06
Hemicellulose intake	0.34	0.32	0.08	-0.08

* Significant at confidence level of 0.05.

[1] Calculated as total dry matter intake from pellet and lucerne chaff minus ash intake of respective feedstuff.

[3] $PC1_{(md)}$ and $PC2_{(md)}$; the first and second principal components, respectively, which estimated for rumen metabolic development parameters by principal component analysis.

6.5 Discussion

The objective of this study was to investigate the effect of pellet fibre level, time of weaning and milk composition on rumen development of lambs reared artificially.

6.5.1 Volatile fatty acid content of rumen fluid

Lambs weaned early (at 42 days of age) had greater n-butyric content in their rumen fluid at 57 days of age irrespective of their pellet fibre level (HFP vs LFP). Early-weaned lambs consumed more pellets than their unweaned counterparts (HFP57 lambs) (Herath et al., 2021b). The increased pellet intake is likely responsible for the greater n-butyric content observed. Additionally, the low fibre pellet fed to LFP42 lambs contained 7.5 % skim milk powder which is a source of lactose. Inclusion of lactose in the diet is known to increase butyric acid content of rumen fluid in both adult sheep (Chamberlain et al., 1993) and cows (Doreau et al., 1987, DeFrain et al., 2006, DeFrain et al., 2004). Therefore, the higher pellet intake and inclusion of milk powder in pellets fed to LFP42 lambs would both have contributed to the higher rumen fluid n-butyric acid content compared to HFP57 lambs.

The increased fibre content of the high fibre pellets could have increased acetic acid in the rumen fluid of HFP42 lambs compared to LFP42 lambs. High fibre diets have been reported to favour the production of acetic acid (Dijkstra, 1994). Acetic and butyric acid are interconvertible to a considerable extent, and acetic and butyric acids show little incorporation into propionic acid (Bergman et al., 1965). It has been reported that 61 % of butyric acid carbon is in equilibrium with approximately 20 % of acetic acid in sheep (Bergman et al., 1965). Hence, the higher n-butyric acids content in HFP42 lambs could be due to the maintenance of the butyric and acetic acid equilibrium in the rumen. Although HFP57 lambs were also fed the high fibre containing diet, they had lower n-butyric acid, due to their lower pellet intake compared to HFP42 lambs. Therefore, the overall higher pellet intake of both HFP42 and LFP42 lambs, the high fibre content of the high fibre pellets and the inclusion of lactose in the low fibre pellets, likely account for

the greater n-butyric acid content in rumen fluid of HFP42 and LFP42 lambs in the present study.

Although comprising a smaller proportion of the VFA pool, branched-chain fatty acids (BCFA: iso-butyrate and iso-valerate) are vital growth factors for cellulolytic bacteria which degrade the structural carbohydrates in feed and are produced from deamination of valine and leucine by proteolytic bacteria (Van Soest, 1994, Membrive, 2016). The CP intake of both HFP42 and LFP42 lambs from pellets was higher compared to HFP57 lambs. However, the iso-butyric and iso-valeric acid content of rumen fluid did not differ between treatments, suggesting that net turnover of these BCFA were not impacted by diet or weaning age.

The LFP-fed lambs had higher n-valeric content in their rumen fluid than HFP-fed lambs, irrespective of weaning age. The n-valeric acid in rumen fluid is synthesised from propionyl-CoA and acetyl-CoA, as a product of rumen fermentation (Van Soest et al., 1991). Similarly to n-butyric (Chamberlain et al., 1993), n-valerate concentration of rumen fluid in cows can be increased by feeding lactose (Doreau et al., 1987, DeFrain et al., 2004). The low fibre pellets fed to LFP42 lambs contained lactose (in the form of skim milk powder) which was absent in high fibre pellet. Thus, the higher n-valeric acid content in LFP42 lambs compared to HFP42 and HFP57 lambs may be explained by the presence of milk powder in their pellets.

In the presence of BCFA, n-valeric acid satisfies the straight carbon chain requirement of cellulolytic rumen bacteria, *Bacteroides succinogenes* (Dehority et al., 1967, Bryant and Doetsch, 1954) and n-caproic is required for growth of *Bacteroides succinogenes* (Bryant and Doetsch, 1954). Further, n-valerate, iso-butyrate and iso-valerate levels are significantly correlated with the abundance of rumen bacterial genera:

Ruminococcaceae_NK4A214, Erysipelotrichaceae_UCG.004, Olsenella, Rikenellaceae_RC9_gut_group, Syntrophococcus, Prevotellaceae_UCG.001, Treponema_2, Megasphaera, Succinivibrio, Prevotella_1 (Li et al., 2020a). Higher n-valeric and a tendency for higher n-caproic content in rumen fluid was observed in LFP42 lambs, compared to both HFP42 and HFP57 lambs. The low fibre fed LFP42 lambs may have had a lower proportion of cellulolytic bacteria in their rumen compared to the high fibre fed lambs, potentially causing reduced utilization of n-valeric and n-caproic acids. There is limited research on the impact of dietary fibre level on branched and straight-chain fatty acids content of young lambs' rumen fluid, and the impact this may have on rumen bacterial composition and rumen function. Future research examining rumen bacteria composition and gene expression would better elucidate the impact of altered fatty acid composition on rumen function.

6.5.2 Rumen morphology

Rumen morphology parameters, measured histologically, were influenced by an interaction between treatment and rumen tissue sampling site. The HFP57 lambs had reduced papillae width and density at the ventral site compared to the other treatments, suggesting that HFP57 lambs may have a lower surface area at the ventral rumen site for nutrient absorption. The papillae height, percentage of papillae longer than 500 µm and PSL/STL in both the dorsal and ventral rumen sites were not influenced by treatment, but those parameters were greater for ventral rumen tissue compared to dorsal rumen tissue irrespective of rearing treatment. Hynd (2019) reported greater papillae development in the ventral sac of the rumen and proposed that it allowed greater VFA absorption compared to the dorsal sac, as ruminal fluid is in greater contact with the rumen ventral wall compared to the dorsal wall. This is consistent with the increased rumen morphology development (papillae height, percentage of papillae longer than 500 µm and PSL/STL)

observed on the ventral rumen tissue from all lambs in the present study. The principal component analysis of rumen physical development parameters did not allow complete discrimination between the three treatments. However, HFP57 lambs were mostly discriminated from HFP42 and LFP42 lambs based on the principal component one estimated for physical development parameters.

Ultrasonographic scanning is a real-time and non-invasive method (Crilly et al., 2017) that has potential to be used to follow rumen development during the transition from non-ruminant to ruminant phase of young ruminants. The lack of differences observed via ultrasound at 36 days suggest that prior to early weaning rumen development in HFP57, HFP42 and LFP42 lambs was similar. Both HFP42 and LFP42 lambs, early weaned off milk at 42 days of age, had increased rumen dorsal wall thickness at day 49. The early weaned lambs likely had thicker dorsal rumen walls due to their increased solid feed intake positively impacting their rumen development. The positive correlation between rumen wall thickness measured via ultrasound at 49 days of age and papillae height at the dorsal site recorded at slaughter (57 days of age) suggests that the rumen image visualised via ultrasound could be used as an indicator of papillae growth. Additional research is warranted on ultrasonographic examination of morphological changes in the rumen, such as size and wall thickness in different sacs of the rumen, at different ages, adapted feeding programs, early weaning strategies and rearing systems.

6.5.3 Correlations between dietary factors and rumen development

Nutrients from milk replacer are directed to the abomasum through the oesophageal groove, thereby bypassing the rumen in bottle-fed lambs (Ørskov et al., 1970), thus milk feeding has a little effect on the rumen development (Sander et al., 1959). Therefore, dry matter and nutrient intake from pellets and lucerne chaff were only considered for the

correlation analyses between dietary factors and rumen development parameters. It is worth noting that due to the treatment effect of early weaning, there was significant variation in the dry matter intake from pellets, CP, ME and organic matter intake data used for this correlation analysis. The pellet intake of lambs varied between treatments, whereby early weaned lambs had greater pellet intake compared to lambs that continued to be offered milk. Further, there was variation in pellet intake of individual lambs within the treatments.

All VFA in the present study, except acetic and n-caproic acids, positively influenced rumen papillae height at the dorsal sampling site. Similar to other studies, which found that both the n-butyric acid content of rumen fluid and the n-butyric acid absorption level impacted rumen papillae development of young goats (Shen et al., 2005). Moreover, butyric acid reduces the rate of apoptosis of cells (Mentschel et al., 2001) leading to increased papillae growth. This likely explains the significant positive correlations observed between papillae height at the dorsal rumen site and both iso- and n- butyric acids content of rumen fluid. Propionic acid has also been found to enhance rumen papillae growth (Baldwin and Connor, 2017, Sander et al., 1959), which is consistent with the positive correlations observed between propionic acid and rumen papillae development in the present study.

Dry matter, ADF, CP, ME and organic matter intakes were positively correlated with the iso-valeric acid content of rumen fluid. All these dietary factors, except CP intake were also positively correlated with iso-butyric content. Iso-valeric and iso-butyric acids are produced as an intermediate product of deamination of proteins by microbes. The dry matter and nutrient intakes from solid feed provide the nutrients required for microbial growth in the rumen. Thus, the observed positive correlations between nutrient intake and

iso-valeric and iso-butyric acid content in the rumen fluid are likely due to the influence of nutrient and feed intake on microbial activity in the rumen.

Papillae height at the ventral sampling site of rumen was positively correlated with dry matter, ME, CP, NDF and organic matter intakes. Further, papillae height at the dorsal sampling site was positively correlated with dry matter, ME, CP and organic matter intakes. While ADF and NDF intakes tended to influence dorsal rumen papillae height. These correlations are likely the result of the positive effect dietary intake has on VFA production by microbes and the positive effects the generated VFA have on rumen papillae development. However, it is unclear whether there is any direct influence of nutrient intake on rumen papillae development. Baldwin and Connor (2017) reported that typically, studies do not provide adequate details on the direct nutrient-gene interactions related to regulatory pathways of rumen development. Thus, future research is needed to better understand the molecular and nutrient direct interactions on the rumen development process.

Empty rumen weight was influenced positively by dry matter, ME, CP, ADF, NDF, hemicellulose and organic matter intakes. Further, papillae height and width at the ventral sampling site and papillae height at the dorsal sampling site had positive correlations with empty rumen weight. The increased VFA production in the rumen with nutrient intake, likely improved rumen papillae development and could result in a heavier empty rumen. This suggests that empty rumen weight is both directly and indirectly affected by the nutrient intake from solid feed. However, the mechanism(s) of the direct effect(s) of nutrient intake on the rumen weight require further investigation. Whilst the effect of rumen weight on the digestive and absorptive capacity of the rumen are not well understood (Baldwin and Connor, 2017), the present study showed that rumen mass was

positively influenced by papillae height. This increase in the nutrient absorptive surface area would impact positively on the nutrient absorption capacity of the rumen. Empty rumen volume was influenced positively by dry matter, ME, CP, ADF and organic matter intakes. An increase in the bulk intake of feed or high fibre content improves rumen musculature and subsequently increases rumen volume (Govil et al., 2017). In the present study, dry matter, ME, CP, ADF and organic matter intake influenced the rumen volume, but not NDF and hemicellulose intake. In summary, empty rumen weight and volume are affected by the nutrient intake from solid feed, suggesting dry matter intake from solid feed and solid feed composition alter the rumen development of lambs during their early life stage.

6.6 Conclusion

In conclusion, rumen fermentation was improved in lambs early weaned at 42 days of age and by feeding low fibre pellets. Early weaning improved rumen dorsal wall thickness compared to milk feeding to 57 days of age. Papillae development was not uniform over the rumen luminal surface and was influenced by pellet fibre content and the age of the lambs' at weaning. Nutrient intake from solid feed influenced volatile fatty acid production and both nutrient intake and volatile fatty acid production impacted rumen physical development.

These results suggest that both lamb diet composition and age at weaning influence rumen function and physical development. Overall, early-weaned lambs fed a low fibre pellets appear to have greater nutrient utilization from solid feed post-weaning. Further research is necessary to confirm if this rearing regimen reduces post-weaning growth check due to rumen immaturity and/or improves post-weaning growth due to improved feed efficiency. Additionally, research is required to examine whether early weaning and diet composition

of lambs reared artificially influence rumen microbial composition and gene expression pathways related to rumen function and development.

Chapter 7 Composition of rumen bacterial community of artificially-reared lambs exposed to three different rearing regimens

7.1 Abstract

This study was performed to investigate the effect of three different rearing regimens on rumen bacterial diversity and abundance in artificially reared lambs and relationships between bacteria abundance, dietary nutrient intake and rumen volatile fatty acids (VFA) content. Lambs were allocated to three dietary and weaning treatments: (i) HFP57 (n=9): commercial milk replacer fed to 57 days of age plus high fibre concentrate pellets; (ii) HFP42 (n=9): commercial milk replacer, high fibre concentrate pellets and weaned from milk replacer at 42 days of age, and offered high fibre pellets until 57 days of age; (iii) LFP42 (n=9): high protein milk replacer from 2-16 days of age followed by commercial milk replacer before being weaned at 42 days and offered low fibre concentrate pellets until 57 days of age. Rumen fluid samples were collected from lambs at slaughter (57 days of age). Bacterial composition was determined by Illumina Miseq platform targeting V3 and V4 regions of the bacterial 16S rRNA gene. Bacteroidetes were the prominent phylum in HFP42 and LFP42 lambs, with Firmicutes being the next most prominent. Phylum Firmicutes was prominent in HFP57 lambs, followed by Proteobacteria. *Prevotella* was the prominent bacteria genus in HFP42 and LFP42 treatments. The most abundant bacteria genus in HFP57 lambs was *Succinovibrio,* followed by *Prevotella*. The abundance of unclassified Bacteroidales was positively correlated ($P<0.05$) with total acid detergent fibre (ADF) and neutral detergent fibre (NDF) intakes. The unclassified Paraprevotellaceae abundance was negatively correlated ($P <0.05$) with total pellet intake, total crude protein (CP) and metabolisable energy (ME) intakes. The abundance of unclassified Clostridiales had a positive correlation ($P<0.05$) with lucerne chaff intake, CP, ME, ADF and NDF intakes from lucerne chaff. The abundance of *Gemmiger*, *Anaerovibrio* and three unclassified Clostridiales were significant ($P>0.05$) predictors of rumen propionate content. The n-butyric acid content was significantly predicted

(P>0.05) by the abundance of *Gemmiger* and *Butyrivibrio*, while iso-butyrate content by *Gemmiger*, Clostridiales, *Anaerovibrio*, unclassified *Ruminococcaceae*, *Bacteroides*, unclassified *Bacteroidales*, *Oscillospira*, *Succinivibrio* and *Desulfovibrio*. Rumen n-valerate content was significantly predicted (P>0.05) by abundance of *Gemmiger* and *Ruminococcus* while iso-valerate content by *Gemmiger*, Clostridiales and unclassified Bacteroidales and *Pseudoramibacter_ Eubacterium*. Overall, this preliminary study suggests that dietary and weaning treatments altered the bacterial composition of artificially reared lambs. Further work is needed to investigate the individual effects of pellet fibre level and concurrent effects of diet, time of weaning and lamb age on rumen bacteria composition to better understand the complex relationships that exist between rumen bacteria abundance, nutrient intake and rumen VFA content and this may help explain differences in lamb growth.

Keywords: 16S rRNA, alpha and beta diversity, Bacteroidetes, Firmicutes, illumina sequencing, neutral detergent fibre, Proteobacteria, volatile fatty acids

7.2 Introduction

Early weaning of lambs can bring economic benefits to farmers by increasing harvestable milk for the sheep milk industry (McKusick et al., 2001) or by reducing the expense of milk replacer and labour for milk feeding in artificial (hand) lamb rearing systems. However, early weaning of lambs onto solid feed could potentially result in impaired post-weaning growth if insufficient rumen development has occurred pre-weaning. The feeding regimen of young ruminants plays an important role in rumen development and maturation. In particular, the nutritional composition of any solid feed offered affects pre-weaning rumen development. An increased fibre level in the diet stimulates rumen musculature development in young ruminants (Tamate et al., 1962, Norouzian et al.,

2011) while low fibre pellets have been reported to improve lamb rumen fermentation, compared to a high fibre pellet (Herath et al., 2021c). Early-weaning of lambs onto a suitable grain-based solid feed does not negatively impact lamb growth (Milis et al., 2019, Herath et al., 2021b). Early weaning of lambs (at 42 days of age) increased pellet intake compared to those still being milk-fed to 57 days age and resulted in a heavier empty rumen and improved rumen fermentation (Herath et al., 2021b, Herath et al., 2021c). Moreover, the interaction effects of weaning age, pellet fibre level and rumen site influence the rumen papillae width, density and rumen wall muscular layer thickness (Herath et al., 2021c). Collectively, these observations indicate that supplementing lambs with a nutrient-balanced, solid-feed diet, which includes adequate fibre, is vital to ensuring optimum lamb growth and rumen metabolic and physical development when applying early weaning strategies (Herath et al., 2021b, Herath et al., 2021c, Milis et al., 2019).

The rumen of a mature ruminant is a continuous fermentation chamber, that is driven by rumen microbes, with a dense population of bacteria (Hobson and Stewart, 1997). Rumen microbial populations in young lambs are less well established (Hobson and Stewart, 1997). Rumen microbes are responsible for rumen fermentation of the diet and the production of volatile fatty acids (VFA), which provides 70-80 % of the energy for mature ruminants (Ahmad et al., 2020, Nagaraja, 2016). The presence of VFA in the rumen also encourage rumen papillae development (Baldwin and Connor, 2017). Additionally, the rumen microbial population is a valuable source of protein for the ruminant animal (Flint, 1997, Rodríguez et al., 2007). Thus, a healthy microbial population in the rumen is essential for optimal animal performance.

The diversity and abundance of rumen bacteria depend on the nutritional composition and physical form of the host animal's diet, the management system and the host's genotype (Beharka et al., 1998, Eadie, 1962, Newbold and Ramos-Morales, 2020, Wang et al., 2017). Mao et al. (2021), Zhang et al. (2019) and Li et al. (2020a) examined the impact of early weaning on the rumen bacterial composition of lambs. They reported early weaning of lambs resulted in a high abundance of certain bacterial genera (*Prevotella*_1, *Succiniclasticum*, and *Prevotellaceae*_UCG-001, *Ruminococcus*) which are involved in carbohydrate fermentation in the rumen. To date, there appear to be no studies that have investigated either the effect of pellet fibre level or the concurrent effect of early weaning and pellet fibre level on lamb's rumen bacterial population.

Therefore, a preliminary study was performed to investigate the effect of pellet fibre level and age at weaning on rumen bacterial diversity and abundance in artificially reared lambs. In addition, relationships between bacteria abundance, and dietary nutrient intake and VFAs were determined. It was hypothesised that the fibre level of pellets and age of weaning would have an impact on rumen bacterial diversity and abundance in artificially reared lambs.

7.3 Materials and method

The experiment was carried out at Massey University, Palmerston North, New Zealand and the research procedures used were approved by the Massey University Animal Ethics Committee (MUAEC 19/64).

7.3.1 Animal management

The experimental feeding regimen for this study have been described elsewhere (Herath et al., 2021b, Herath et al., 2021c) as briefly described below.

Composition of rumen bacterial community of lambs exposed to three rearing regimens

Twin-born, Romney ram lambs (n=27) were allowed to suckle from their dam for the first 24 hours after birth. Then one lamb per twin set was selected for the study and individually penned. Lambs were randomly allocated to one of three dietary and weaning treatments; (i) HFP57 (n=9): commercial milk replacer fed to 57 days of age plus high fibre concentrate pellets; (ii) HFP42 (n=9): commercial milk replacer, high fibre concentrate pellets and weaned from milk replacer at 42 days of age; (iii) LFP42 (n=9): high protein milk replacer from 2-16 days of age followed by commercial milk replacer before being weaned at 42 days and offered low fibre concentrate pellets until slaughter (57 days). All lambs were fed milk replacer at 2.1 times their maintenance energy requirement (ME_m= 0.40 MJ/kg$LW^{0.75}d^{-1}$ (Danso et al., 2016)). Crude protein (CP) and metabolisable energy (ME) content of commercial milk replacer were 262.9 g/kg and 21.8 MJ/kg, respectively (Milligans Feed Ltd, Oamaru, New Zealand). The high protein milk replacer powder was an 80:20 blend of the commercial milk replacer powder with a powdered milk protein concentrate (324.1 g/kg CP and 20.8 MJ/kg ME, Fonterra, Auckland, New Zealand). Daily, one part of milk replacer and high protein milk replacer were mixed with four parts of warm tap water. During the first two weeks of age, lambs were bottle-fed five times daily, then four times daily up to the milk weaning phase at day 38 to 42 for HFP42 and LFP42 lambs and until the end of the experiment for HFP57 lambs (57 days of age).

Lambs had *ad libitum* access to either high fibre concentrate pellet (69.35 g/kg acid detergent fibre (ADF) and 208.56 g/kg neutral detergent fibre (NDF), for HFP57 and HFP42 lambs) or low fibre concentrate pellet (44.40 g/kg ADF and 116.76 g/kg NDF for LFP42 lambs) from 4 to 57 days of age. Lambs were provided water at all times.

Lambs in the HFP42 and LFP42 treatments were offered 50% of their milk allowance for five days during the milk weaning phase (38-42 days of age), while HFP57 lambs were given their full milk allowance until 57 days of age. The HFP42 and LFP42 lambs were bottle-fed twice daily and fully weaned from milk at 42 days. All the lambs were provided with *ad-libitum* access to either high (HFP57 and HFP42 lambs) or low fibre concentrate pellets (LFP42 lambs). Lucerne chaff (114.69 g/kg CP and 7.14 MJ/kg ME; Oaklane Stables Premium Chaff, Hawkes Bay, New Zealand) was offered (40 g/d) for lambs during the 38-57 day period. Pellet, milk replacer and lucerne chaff intakes were recorded daily.

7.3.2 Slaughter and rumen fluid collection

Lambs were slaughtered at 57 days of age (three lambs were excluded due to health issues, 1 and 2 from HFP57 and LFP42 treatments, respectively). The lambs were fasted for 12 hours, slaughtered via captive bolt, exsanguinated, skinned and eviscerated. Rumen fluid samples were collected immediately after opening the rumen into cryovials with phosphate buffer saline (PBS). All rumen fluid samples were immediately snap-frozen and stored in liquid nitrogen, until transfer and storage in a -80 ^{o}C freezer until microbial DNA extraction.

7.3.3 DNA extraction

Total DNA was extracted from rumen fluid samples using a commercial kit (Quick DNA faecal/soil microbe miniprep kit, ZYMO Research, Canada) according to the manufacturer's instructions. Briefly, approximately 150 mg of the rumen fluid sample was mixed with buffer in the kits' bead beating tube and beaten for one minute using a bead beater (BioSpec Products, Inc. Bartlesville, USA) to mechanically disrupt cells. The samples were centrifuged at ≥ 10,000 x *g* for 1 minute. The supernatant (approx. 400µl)

was transferred to a Zymo-Spin™ III-F Filter in a collection tube and centrifuged at 8,000 x *g* for 1 minute. To the filtrate 200 µl of Genomic Lysis Buffer was added, mixed well, and the total volume was transferred to a Zymo-Spin™ IICR Column, centrifuged at 10,000 x *g* for 1 minute and the flow-through is discarded. The column was washed once with DNA Pre-wash Buffer (200 µl), then with the DNA wash buffer (500 µl), both with centrifuge steps of 10,000 x *g* for 1 minute. The DNA was eluted from the column into a clean 1.5 ml microcentrifuge tube with 100 µl DNA Elution Buffer and centrifuged at 10,000 x *g* for 30 seconds.

16S-rRNA amplicon sequencing

The extracted DNA was diluted 1:10 with molecular grade water. Then 20 µl of diluted sample was analysed on a 1% agarose gel along with a high molecular weight marker (#12-352-019, ThermoFisher Scientific, Waltham, MA) to evaluate DNA quality. The DNA concentration of samples was determined by Qubit dsDNA HS assay (#Q32854, ThermoFisher Scientific, Waltham, MA) using Qubit 2.0 fluorometer (Life Technologies, USA) and normalized to 5 ng/µl with Nuclease-Free Water (#4387936, ThermoFisher Scientific, Waltham, MA).

The bacterial composition of rumen fluid was determined by analysis of V3 and V4 regions of the bacterial 16S rRNA gene. The normalised DNA (1 µl) was added to 17 µl of AccuPrime Pfx SuperMix (#12344040, ThermoFisher Scientific, Waltham, MA) and 1 µl each of the barcoded forward (16Sf V3) and reverse (16Sr V4) V3V4 amplicon primers (Table 7.1) for the amplicon PCR, where the barcode was an eight-base sequence unique to each sample.

Table 7.1 Primer Sequences for 16S-rRNA Amplicon Sequencing

Primer	Sequence
16Sf V3	AATGATACGGCGACCACCGAGATCTACACxxxxxxxxTATGGTAATTGG CCTACGGGAGGCAGCAG
16Sr V4	CAAGCAGAAGACGGCATACGAGATxxxxxxxxAGTCAGTCAGCCGGAC TACHVGGGTWTCTAAT

"xxxxxxxx" represents the unique barcode sequence assigned to each sample

The amplicon PCR was performed under the following conditions; 95 °C for 2 minutes, then 30 cycles of 95 °C for 20 seconds, 55 °C for 15 seconds, 72 °C for 5 minutes, followed by a final extension cycle 72 °C for 10 minutes and hold at 4 °C.

The PCR products were cleaned using SequalPrep^{TM} Normalization Plate Kit (# A1051001, ThermoFisher Scientific, Waltham, MA) and library concentration was determined by Qubit HS assay (#Q32854, ThermoFisher Scientific, Waltham, MA). The full library size of ~630 bp was verified on a PerkinElmer LabChip GX Touch HT instrument using the DNA High Sensitivity LabChip Assay. Libraries with equal molarity were pooled, then for sequencing, the pooled library was diluted to 2 nM with 10 mM Tris pH 8.5 with 0.1% Tween 20.

To denature the DNA to a single-strand DNA, 2nM pooled library (10 µl) was mixed with 10µl 0.2N NaOH (pH > 12.5) and incubated at room temperature for 5 minutes. Illumina PhiX Control v3 (#FC-110-3001, Illumina, San Diego, CA) was diluted to 2 nM with 10 mM Tris pH 8.5 with 0.1% Tween 20. Then PhiX Control v3 and 10µl 0.2N NaOH (pH > 12.5) mixed and incubated at room temperature for 5 minutes to denature the DNA. The pooled library was diluted to 8 pM, and PhiX was diluted to 12.5 pM with ice-cold HT-1. The pooled library (800 µl) and PhiX (200 µl) were combined to give a calculated spike of 20 % PhiX. Amplicon sequencing was performed by Illumina MiSeq at the Massey Genome Service, Massey University, Palmerston North, New Zealand. The 600

µl of the mixed sample was loaded into a thawed Illumina MiSeq V2 cartridge for paired-end sequencing with 250 nucleotide read length by Illumina MiSeq platform.

7.3.4 Bioinformatics and data analysis

Sequenced data were processed and analysed by Quantitative Insights Into Microbial Ecology (QIIME2 v.2020.2.) software package (Caporaso et al., 2010, Bolyen et al., 2019) by the Massey Genome Service, Massey University, Palmerston North, New Zealand. Briefly, the data processing included denoising, chimaera checking, pair-joining and clustering through dereplication was performed by DADA2. Sequences were clustered into Amplicon Sequences Variants (ASV) which shared over 97 % similarity in each sample. Then ASVs were filtered to remove any ASVs that appeared in less than ten counts which would likely result in false positives in subsequent steps. All the samples were rarified to the same amount of reads which is a compromise between a number high enough to yield meaningful results but low enough to include as many samples as possible. Alpha diversity of the rumen bacteria within the treatment was analysed using Pielou's evenness index, Faith's PD, observed features (ASVs) and Shannon diversity. Beta diversity of the rumen bacteria between treatments was analysed using the Jaccard index, Bray-Curtis dissimilarity index (Dill‐McFarland et al., 2016), weighted and unweighted UniFrac distance analysis and were visualised with principal coordinate analysis (PCoA). The permutational multivariate analysis of variance (PERMANOVA) was performed to determine differences in bacterial diversity between treatments (Anderson, 2017).

The taxonomic classification of bacteria was obtained using the embedded Naive Bayes fitted classifier, trained on the GreenGenes v 13.8, 99% identity database (accessed May 2020) (DeSantis et al., 2006). Relative abundance of each bacteria phyla was calculated

as total bacteria observed in each phylum divided by the total number of bacteria observed in all phyla of each lamb. Similarly, the relative abundance of bacteria at class, order and genus levels were calculated considering the respective total number of bacteria at class, order and genus levels.

Pearson correlation analysis was performed to determine the relationship between feed or nutrient intake and relative rumen abundance of bacteria at the genus level. The multivariate analysis was carried out by stepwise regression (SAS 9.4, 2013) to determine the relationship between lamb feed and nutrient intakes and abundance of bacteria at phylum, order, class and genus levels. Similarly, multivariate analysis was carried out by stepwise regression (SAS 9.4, 2013) to determine the relationship between rumen volatile fatty acids (VFA) content and abundance of rumen bacteria at phylum, order, class and genus levels. Two lambs from the HFP42 treatment which were not deprived of feed 12 h before slaughter were excluded from the multivariate analysis.

7.4 Results

An average of 31,189 ± 11,187 (SD) bacterial 16S rRNA gene sequences per rumen fluid sample were obtained after processing and filtering by QIIME2 software. The range of bacterial 16S rRNA gene sequences per sample was 14,733 to 57,207, irrespective of treatments. Examination of the rarefaction data showed a plateau at a sequencing depth of approximately 7,000 for all treatments. This indicated that the sequencing depth used for the present study (14,733), was confidently represented the bacterial community in the rumen fluid samples.

7.4.1 Alpha diversity

The alpha diversity was evaluated based on the abundance of ASVs in different samples. Based on Pielou's evenness index, bacteria evenness did not differ (Kruskal-Wallis, P> 0.05) within treatment. Based on Faith's PD index, there were no differences (Kruskal-Wallis, P> 0.05) in the evenness and richness of bacteria of each sample within treatment (Figure 7.1, graph A). The observed ASVs did not differ (Kruskal-Wallis, P> 0.05) in samples within treatment.

Shannon diversity also indicated that there were no differences (Kruskal-Wallis, P> 0.05) in bacterial richness and abundance in each rumen fluid samples within treatment (Figure 7.1, graph B).

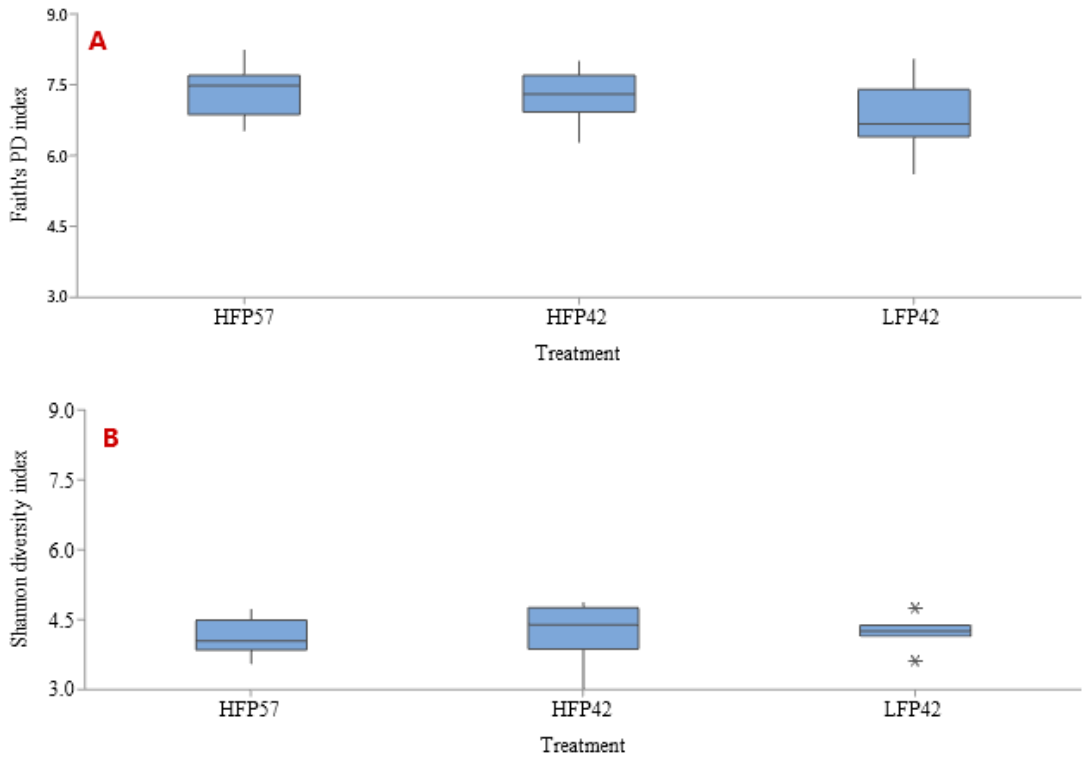


Figure 7.1 Alpha diversity measures; Faith's PD and Shannon diversity indexes of rumen fluid of artificially-reared, early-weaned lambs exposed to three different rearing regimens (HFP57, high fibre concentrate pellets and milk feeding to 57 days of age; HFP42, high fibre concentrate pellets and early weaning; LFP42, low fibre concentrate pellets and early weaning, boxes represent interquartile range (between 25th and 75th percentiles, respectively) for Faith's PD index (A) or Shannon diversity index (B). The horizontal line inside the box represents the median, whiskers

represent the minimum and maximum values and * represents outliers).

7.4.2 Beta diversity

Bacterial community composition differed ($P<0.05$) between treatments based on the Jaccard index (Figure 7.2, graph A). Bacterial abundance in each treatment differed ($P<0.05$) according to the Bray-Curtis dissimilarity analysis (Figure 7.2, graph B). All pairwise comparisons of treatments except the comparison between high fibre pellet and milk feeding to 57 days age (HFP57) and high fibre pellets and early-weaned (HFP42) groups were significantly different (pairwise PERMANOVA, $P<0.05$) in bacterial community abundance. When only considering weaning age, the bacterial community composition of early weaned lambs tended to differ (PERMANOVA, $P=0.052$) from lambs fed milk to 57 days of age.

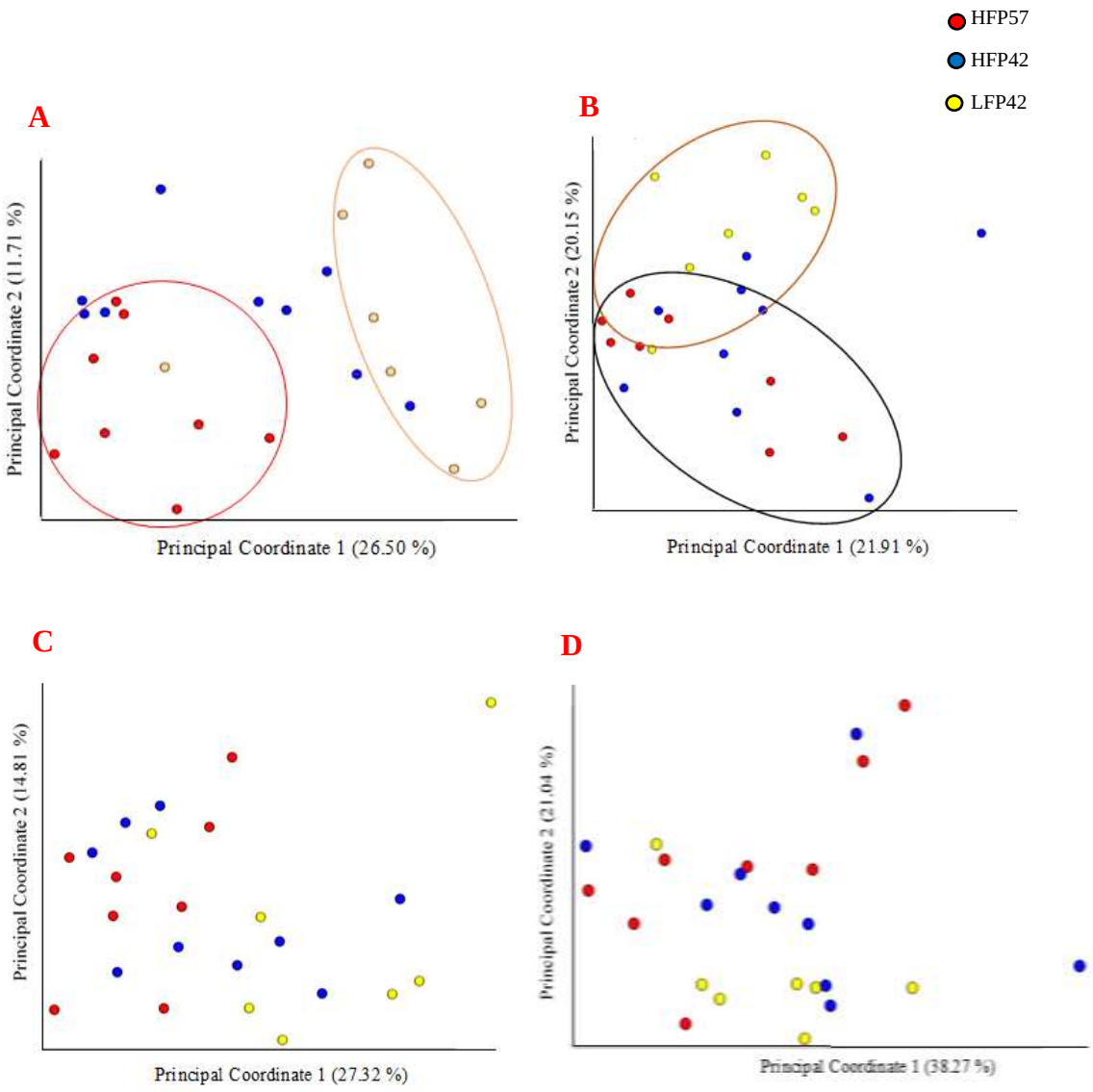


Figure 7.2 Principal Coordinate analysis (PCoA) based on (A) Jaccard index, (B) Bray Curtis index, (C) unweighted UniFrac distance analysis and (D) weighted UniFrac distance analysis on the diversity of the bacterial community of rumen fluid of artificially-reared lambs exposed to three different rearing regimens (HFP57, high fibre concentrate pellets and milk feeding to 57 days of age (●); HFP42, high fibre concentrate pellets and early weaning (●); LFP42, low fibre concentrate pellets and early weaning (●)).

Bacterial community composition based on the unique distance to either treatment in the phylogenic tree differed (P=0.003) as determined by unweighted UniFrac distance analysis (Figure 7.2, graph C). According to the pairwise treatment comparisons, the HFP57 treatment differed from the LFP42 group (pairwise PERMANOVA, P<0.05). The

bacterial composition of early-weaned lambs (HFP42 and LFP42) differed from lambs fed milk to 57 days of age (pairwise PERMANOVA, $P<0.05$). Further, the bacterial composition of high fibre pellets fed lambs differed from low fibre fed lambs (pairwise PERMANOVA, $P<0.05$). The weighted distance in the phylogenetic tree on the abundance of bacterial community did not differ ($P>0.05$) between treatments, as determined by weighted UniFrac distance analysis (Figure 7.2, graph D). Based on all beta diversity analysis parameters, all the metrics except weighted UniFrac distance analysis agreed that treatments groups differed from each other.

7.4.3 Taxa analysis

A total of six phyla, nine classes, 10 orders, 17 families and 37 genera were detected in all of the lamb rumen fluid samples irrespective of treatment. All bacteria were identified at the phylum, class and order levels and only 89.2 and 64.8 % were identified at the family and genus levels, respectively. The six phyla detected in samples were Actinobacteria, Bacteroidetes, Firmicutes, Proteobacteria, Spirochaetes and Synergistetes (Figure 7.3). Bacteroidetes were the prominent phylum in HFP42 and LFP42 lambs (37.3 and 36.9% relative abundance, respectively) and followed by Firmicutes (34.2 vs 33.2% relative abundance, respectively). Phylum Firmicutes was prominent (35.9% relative abundance) in HFP57 lambs and was followed by Proteobacteria (29.7% relative abundance). The relative abundance of Proteobacteria in LFP42 lambs (28.6%) was approximately similar to that in HFP57 lambs, but less abundant in HFP42 lambs (21.3%). The relative abundance of Spirochaetes was greater in HPF57 and HPF42 lambs (6.4 and 6.9 %, respectively) than in LFP42 lambs (1.1%). The relative abundance of Actinobacteria was greater in HPF57 lambs (1.2%) than in both HFP42 and LFP42 lambs (0.07 and 0.08%, respectively). The relative abundance of Synergistetes was less than 0.4% for all the treatments.

Composition of rumen bacterial community of lambs exposed to three rearing regimens

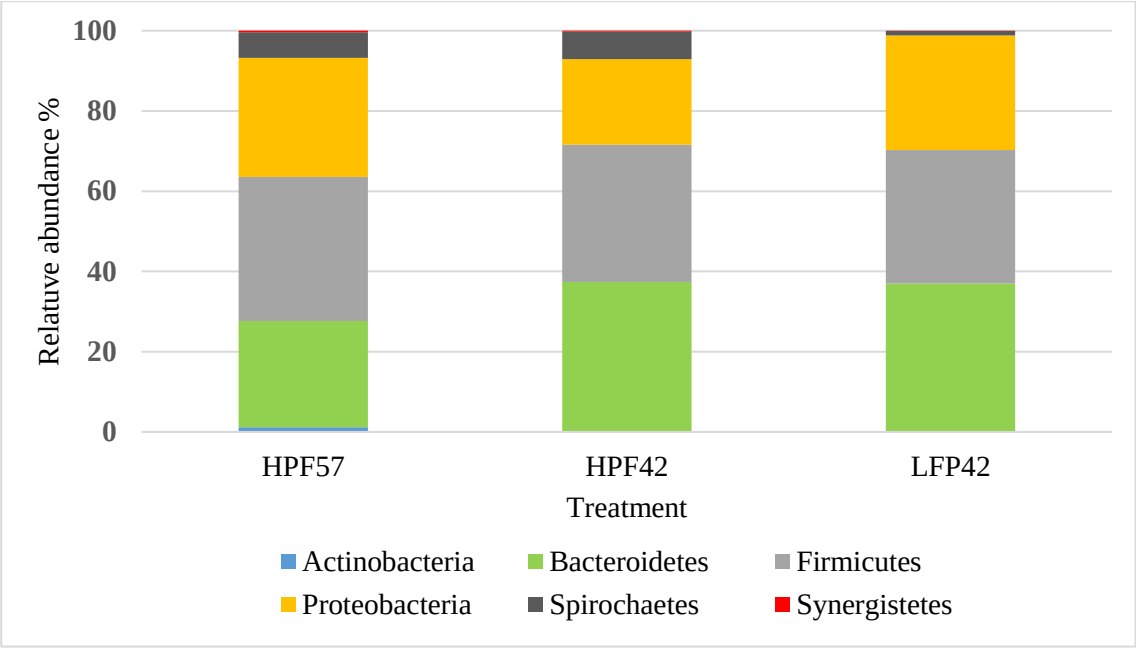


Figure 7.3 The relative abundance of rumen bacteria phyla of artificially-reared lambs exposed to three rearing regimens (HFP57, High fibre concentrate pellets and milk feeding to 57 days of age; HFP42, High fibre concentrate pellets and early weaning; LFP42, Low fibre concentrate pellets and early weaning).

Out of 37 genera identified in the rumen of lambs in all treatments, *Prevotella*, *Succinovibrio*, *Succiniclasticum*, *Butyrivibrio* and *Treponema* were the most abundant rumen genera (Figure 7.4). *Prevotella* was the prominent bacteria genus in HFP42 and LFP42 treatments (31 and 32.5% relative abundance, respectively). The most abundant bacteria genus in HFP57 lambs was *Succinovibrio* (2 %) and was followed by *Prevotella* (20%). The abundance of *Succinovibrio* in LFP42 (28.5%) was similar to HFP57 lambs, while it was 21% for HFP42 lambs.

The relative abundance of *Succiniclasticum* was 8 % for both HFP57 and HFP42 lambs and 5.6% for LFP42 lambs. *Butyrivibrio* relative abundance was similar across treatments and was 4, 7.5 and 7.5% respectively, for HFP42, HFP42 and LFP42 treatments. *Treponema* relative abundance was 6, 7 and 1% for HFP57, HFP42 and LFP42 treatments, respectively.

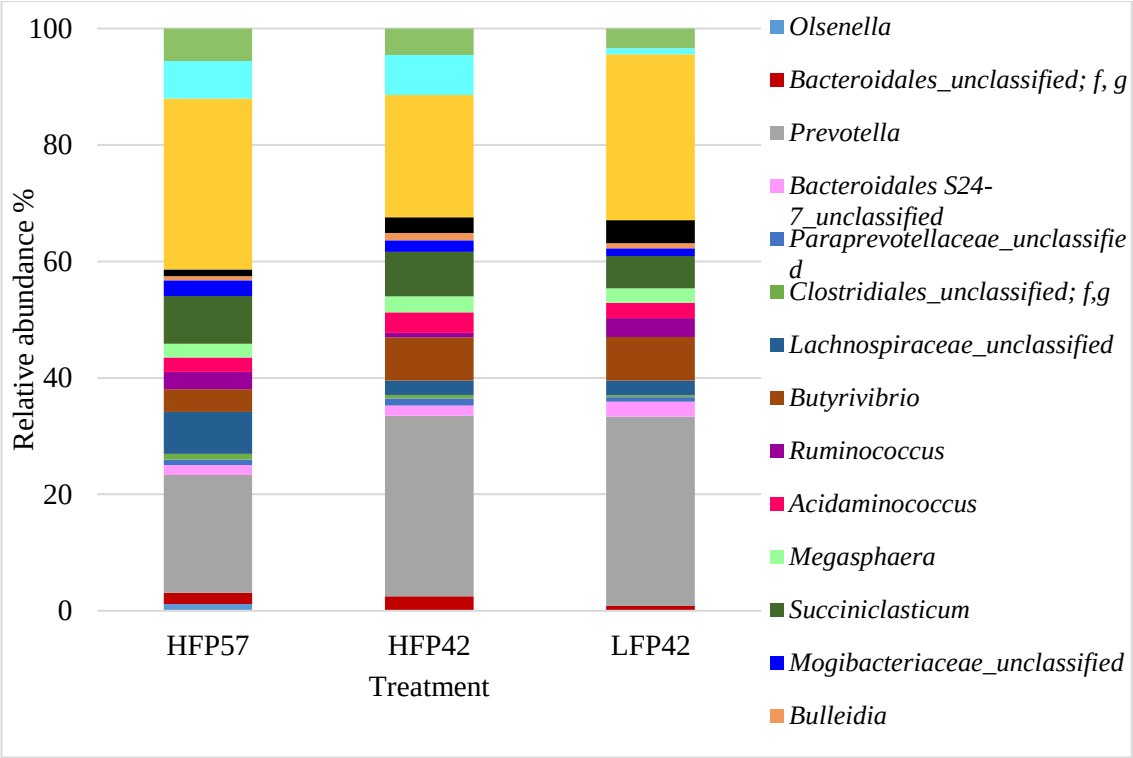


Figure 7.4 The relative abundance of rumen bacteria genera of artificially-reared lambs exposed to three different rearing regimens (HFP57, High fibre concentrate pellets and milk feeding to 57 days of age; HFP42, High fibre concentrate pellets and early weaning; LFP42, Low fibre concentrate pellets and early weaning).

7.4.4 Relationship of bacteria abundance with feed or nutrient intake

Correlation analysis

The results of Pearson correlation analysis on the relationship between feed or nutrient intake and relative abundance of rumen bacteria at the genus level are presented in Table 7.2. The relative abundance of unclassified Bacteroidales was significantly positively ($P < 0.05$) correlated with total acid detergent fibre (ADF) and neutral detergent fibre (NDF) intake from pellets and lucerne chaff. The Paraprevotellaceae_unclassified was significantly negatively ($P < 0.05$) correlated with total pellet intake, crude protein (CP) and meatabolisable energy (ME) intakes from pellets and total CP and ME intakes from both pellets and lucerne chaff. The relative abundance of Clostridiales unclassified at family and genus level had a significant positive ($P < 0.05$) correlation with total lucerne chaff intake, CP, ME, ADF and NDF intakes from lucerne chaff.

Multivariate regression analysis

Based on the multivariate analysis, at the phylum, class and order levels, the relative abundance of any bacteria was not explained (P >0.05) by the feed or nutrient intakes parameters.

Pellets ADF intake and total NDF intake from both pellets and lucerne chaff were the significant (P<0.05) predictors (negatively and positively, respectively) of the relative abundance of unclassified Bacteroidales (Table 7.3). The pellets ADF intake and total NDF intake from both pellets and lucerne chaff explained 34% of the variation in the relative abundance of Bacteroidales_unclassified. There were no improvements in simple correlations of relative abundance of Paraprevotellaceae_unclassified and Clostridiales unclassified with nutrient intake from pellets and lucerne chaff (Table 7.2) by multivariate regression analysis (Table 7.3).

The combined CP to ME intake ratio from both pellets and lucerne chaff was the significant positive predictor of the relative abundance of *Erysipelotrichaceae Eubacterium* unclassified, *Succiniclasticum,* and *Roseburia*, while it was negative for *Mitsuokella* (Table 7.3). The pellet CP and ME intakes were not significant (P>0.05) predictors of the relative abundance of bacteria genus found in this study (data not presented).

7.4.5 Relationship of bacteria relative abundance with VFA production

The variations in rumen acetate content was not explained (P>0.05) by the relative abundance of any of the bacteria genus present in current study (Table 7.4). The genus *Gemmiger, Anaerovibrio* and three unclassified bacteria in order *Clostridiales* were the significant (P<0.05) predictors of the rumen propionate content in lambs' rumen. These bacteria genera explained 73% of the variation in propionic acids in the rumen fluid (Table 7.4).

Table 7.2 Pearson correlation analysis on the relationship between feed or nutrient intake and relative abundance of bacteria in the lambs' rumen

Bacteria genus[1]	Intake														
	Pellet CP	Pellet ME	Lucerne chaff CP	Lucerne chaff ME	Total CP[2]	Total ME[3]	Combined CP:ME[4]	Total pellet	Total Lucerne chaff	Pellet ADF	Pellet NDF	Lucerne chaff ADF	Lucerne chaff NDF	Total ADF[5]	Total NDF[6]
F Paraprevotellaceae unclassified	-0.52	-0.52	-	-	-0.51	-0.51	-	-0.52	-	-	-	-	-	-	-
O Clostridiales unclassified; f,g	-	-	0.53	0.53	-	-	-	-	0.54	-	-	0.54	0.54	-	-
O Bacteroidales _unclassified; f,g	-	-	-	-	-	-	-	-	-	-	-	-	-	0.42	0.43

All significant Pearson correlations at P=0.05 confidence level is presented.

[1] O=Order, F= family, G=genus, unclassified; f,g = unclassified at family and genus level

[2] Total crude protein intake from pellets and lucerne chaff

[3] Total metabolisable energy intake from pellets and lucerne chaff

[4] Combined crude protein to metabolizable energy (CP:ME) intake ratio from pellets and lucerne chaff

[5] Total acid detergent fibre (ADF) intake from pellets and lucerne chaff

[6] Total neutral detergent fibre (NDF) intake from pellets and lucerne chaff

CP, Crude protein; ME, Metabolisable energy

Table 7.3 The multivariate analysis on the relationship between feed or nutrient intake and relative abundance of rumen bacteria at the genus level

Bacteria at genus level[1]	Intercept	Parameter estimate for Intake									
		Pellet	Pellet NDF	Pellet ADF	Combined CP:ME[2]	Lucerne NDF	Lucerne ADF intake	Total NDF[3]	Total ADF[4]	R^2	Model P value
F *Paraprevotellaceae_* Unclassified	1.54	-0.0001	-	-	-	-	-	-	-	0.27	0.0131
O *Clostridiales_* unclassified; f,g	0.007*	-	-	-	-	0.004	-	-	-	0.29	0.0099
O *Bacteroidales;* unclassified f,g	-0.37*	-	-	-0.011	-	-	-	0.0039	-	0.34	0.0185
G *Eubacterium*	-12.27	-	-	-	0.76	-	-	-	-	0.29	0.0103
G *Succiniclasticum*	-207.00	-	-	-	13.13		-	-	-	0.42	0.011
G *Mitsuokella*	15.22	-	-	-	-0.92		-	-	-	0.13	0.0299
G *Roseburia*	-4.84	-	-	-	0.30	-	-	-	-	0.20	0.0359

* The intercept is not significant at P=0.05 confidence level.

[1] O=Order, F= family, G=genus, unclassified; f,g = unclassified at family and genus level

[2] Combined CP:ME intake ratio from pellets and lucerne chaff

[3] Total neutral detergent fibre (NDF) intake from pellets and lucerne chaff

[4] Total acid detergent fibre (ADF) intake from pellets and lucerne chaff

R^2 Coefficient of determination

The n-butyric acid content of rumen was significantly predicted (P<0.05) by the relative abundance of *Gemmiger and Butyrivibrio* and 46% of the variation in n-butyric acid content was explained by the relative abundance of *Gemmiger* and *Butyrivibrio* (Table 7.4). The iso-butyrate content of lamb rumen was significantly predicted (P<0.05) by the relative abundance of *Gemmiger,* order Clostridiales unclassified at family and genus level, *Anaerovibrio*, *Ruminococcaceae_* unclassified, *Bacteroides, Bacteroidales* unclassified at family and genus level, *Oscillospira, Succinivibrio* and *Desulfovibrio.* The relative abundance of *Anaerovibrio, Bacteroides, Oscillospira, Succinivibrio* and *Desulfovibrio* were negatively associated (P<0.05) with the iso-butyric content while other bacterial genera were positively associated (P<0.05) with the iso-butyric content (Table 7.4). These genera explained all most all variation (96%) in the iso-butyric acids content in the rumen.

The n-valerate content of rumen was positively associated with genera *Gemmiger* and *Ruminococcus* and those explained the 44% of the variation in n-valerate content (Table 7.4). The iso-valerate content of rumen fluid was positively associated (P<0.05) with the relative abundance of *Gemmiger,* order Clostridiales and Bacteroidales unclassified at family and genus level and *Pseudoramibacter_Eubacterium.* The iso-valerate content of rumen fluid was negatively associated (P<0.05) with the relative abundance of *Oscillospira* and *Lachnospiraceae* _unclassified (Table 7.4). Ninety-four percent of the variation in iso-valeric acid was explained by the relative abundance of those bacteria genera. There was no bacteria genus that was a significant (P<0.05) predictor of the n-caproic content of rumen fluid. The relative abundance of *Gluconacetobacter* was the significant (P<0.05) predictor of the acetic to propionate ratio of rumen fluid in the present study.

Table 7.4 The multivariate analysis on the relationship between the relative abundance of bacteria and volatile fatty acid production in the rumen

Parameter estimate for VFA[1]	Volatile fatty acids (VFA)							
	Acetate	Propionate	n-Butyrate	Iso-Butyrate	n-Valerate	Iso-Valerate	n-Caproic	Acetate to propionate ratio
Intercept	-	0.35	0.12	0.09	0.05	0.05	-	1.98
G *Gemmiger*	-	0.11	0.06	0.016	0.05	0.04	-	-
O *Clostridiales*_unclassified; f,g	-	0.07	-	-	-	-	-	-
O *Clostridiales*_unclassified2; f,g	-	-0.06	-	-	-	-	-	-
O *Clostridiales* unclassified3; f,g	-	0.06	-	0.014	-	0.03	-	-
G *Anaerovibrio*	-	-0.17	-	-0.05	-	-	-	-
G *Ruminococcus*	-	-	-	-	0.002		-	-
F *Ruminococcaceae*_unclassified	-	-	-	0.11	-	-	-	-
G *Bacteroides*	-	-	-	-0.29	-	-	-	-
O *Bacteroidales*_unclassified; f,g	-	-	-	0.001	-	0.003	-	-
G *Oscillospira*	-	-	-	-0.02	-	-0.05	-	-
G *Succinivibrio*	-	-	-	-0.0006	-	-	-	-
G *Butyrivibrio*	-	-	0.004	-	-	-	-	-
G *Desulfovibrio*	-	-	-	-0.03	-	-	-	-
G *Pseudoramibacter_Eubacterium*	-	-	-	-	-	0.18	-	-
F *Lachnospiraceae*_unclassified	-	-	-	-	-	-0.005	-	-
G *Gluconacetobacter*	-	-	-	-	-	-	-	3.40
Model R^2	-	0.73	0.46	0.96	0.44	0.94	-	0.31
Model P value	-	0.0004	0.0028	<.0001	0.004	<.0001	-	0.0076

All significant relationships at P=0.05 confidence level are presented.

[1] O=Order, F= family, G=genus, unclassified; f,g = unclassified at family and genus level

R^2 Coefficient of determination

7.5 Discussion

This study was a preliminary investigation examining the impact of three different feeding and weaning regimens on rumen bacterial community composition in artificially reared lambs. Relationships between bacteria abundance and both dietary nutrient intake and VFAs were examined.

7.5.1 Bacteria community composition and structure

During the early life of young ruminants, there is a higher abundance of pioneer colonising bacteria (Malmuthuge and Griebel, 2019). These shape the anaerobic rumen conditions for cellulose- and hemicellulose-utilising fungi and bacteria which establish later (Alexander, 1971, Fonty et al., 1987). In support of this, Jami et al. (2013) reported that there was a considerable decline in most aerobic or facultative anaerobic bacterial genera over time, while most strictly anaerobic bacteria genera increased during the first 3 days of age. The succession of the bacterial communities depends on the diet, rearing methods and environment that young animal is exposed to (Palma-Hidalgo et al., 2021, Malmuthuge et al., 2015). The principal coordinate analysis using the Jaccard index suggested that LFP42 lambs had a more diverse bacterial community compared to HFP42 lambs. This is supported by the principal coordinate analysis using the Bray Curtis index, which also showed that LFP42 lambs had a different bacterial composition compared to both HFP57 and HFP42 lambs. This suggests that the fibre level of pellets (LFP vs HFP) influenced the rumen bacterial composition. The polygenetic lineage (unweighted UniFrac distance analysis) of bacteria present in the early-weaned lambs (HFP42 and LFP42) was different from HFP57 lambs, and was also different between low (LFP42) and high fibre pellet fed lambs (HFP57 and HFP42), suggesting an interaction between age at weaning and pellet fibre level on bacteria community diversity and abunadance.

Therefore, further studies on time of weaning and pellet composition on rumen bacteria coomunity would be of interest to better understand the their effects.

Out of six phyla detected in the present study, Firmicutes was prominent in HFP57 lambs, followed by Proteobacteria. Several studies have found that Proteobacteria is the prominent bacteria phylum in young ruminants (Jami et al., 2013, Rey et al., 2014, Jiao et al., 2015a) and that their abundance changes with the later colonisation by other bacterial communities. Further, it is also reported that Proteobacteria is dominated in the small intestine of the neonatal calf and then it transitioned to Firmicutes with calf's age and ingesta (Malmuthuge, 2016). The abundance of Proteobacteria associated with rumen epithelium is reduced, while the abundance of Firmicutes and Bacteroidetes rises with age in goats (Jiao et al., 2015a). Amin et al. (2021) also reported that the abundance of Bacteroidetes increased with age and post-weaning diet changes. Due to continued milk intake, the pellet intake of HFP57 lambs was approximately half that of early-weaned lambs (Herath et al., 2021b). Thus, it might be hypothesised that the bacterial community of HFP57 lambs may still have been in the transitional stage to that of a more mature rumen microbial community. In support of this theory, Bacteroidetes were the prominent phylum in HFP42 and LFP42 lambs, followed by Firmicutes. Bacteroidetes are polysaccharides degrading bacteria and produce propionate (Flint and Duncan, 2014). Early-weaned lambs (HFP42 and LFP42) had a greater pellet intake compared to HFP57 lambs (Herath et al., 2021b) due to the absence of milk feeding, and this could have led to a greater abundance of phylum Bacteroidetes. Contrary to this, the relative abundance of Bacteroidetes phylum and genus *Bacteroides* in the calf rumen was reduced during first five days post-weaning, in which abrupt milk weaning was practised at 30 days of age (Mao et al., 2021), this could be due to weaning stress (Mao et al., 2021). However, in the present study, which practised gradual milk weaning during the period of 38-42

days of age, there was a greater abundance of Bacteroidetes two weeks post-weaning, possibly meaning that lambs were not affected by weaning stress. Although previous studies (Jiao et al., 2015a, Amin et al., 2021, Malmuthuge, 2016) shows that bacteria abundance varies with the age of young ruminants, the present study only investigated the bacterial composition of eight-week-old lambs, therefore, age-related changes in bacteria abundance could not be investigated. Thus, changes in the bacteria composition are resulted due to the different lamb rearing regimens. Future studies on impacts of time of weaning, diet composition and lamb age on bacteria composition are needed.

At the genus level, the findings of Jami et al. (2013) are consistent with the results of the present study as *Prevotella* is the most abundant genus in the Bacteroidetes phylum. The abundance of genus *Prevotella* was greater in early-weaned lambs which had greater pellet intake than HFP57 lambs. *Prevotella* is known to digest starch, xylans and pectin and is engaged in the deamination of protein to produce ammonia (Flint and Duncan, 2014). The abundance of phylum Actinobacteria (genus *Olsenella*) was greater in HPF57 lambs than in both early-weaned lamb treatments (HFP42 and LFP42). The phylum Actinobacteria was more abundant in milk-fed calves, with the abundance reducing as calves aged (Jami et al., 2013). Amin et al. (2021) reported that the relative abundance of the genus *Olsenella* was reduced in early-weaned calves compared to the late-weaned calves, which is consistent with the lower abundance of *Olsenella* in early-weaned lambs in the present study.

7.5.2 Relationship of bacteria abundance with solid feed or nutrient intake

The unclassified bacteria in the Paraprevotellaceae family was correlated with total pellet intake, CP and ME intake from pellets and total CP and ME intakes from both pellets and lucerne chaff. Yang et al. (2018) also found that unclassified genera in Paraprevotellaceae family were positively correlated with CP and NDF intake. Further, they reported that

weaning and associated diet transition affected the relative abundance of the unclassified genera in the family Paraprevotellaceae. The increased solid feed intake in the early-weaned lambs (HFP42 and LFP42 treatments) likely contributed to the significant correlations in the present study.

The relative abundance of unclassified Clostridiales had a significant positive correlation with total lucerne chaff intake, CP, ME, ADF and NDF intakes from lucerne chaff. Most of the Clostridiales found in the present study were assigned to Lachnospiraceae, Ruminococcaceae, Eubacteriaceae, Mogibacteriaceae and Veillaonellaceae families. The members of those families exhibit fibre digestion activities (Dworkin and Falkow, 2006b). Thus, unclassified Clostridiales might also play a role in fibre digestion which could result in significant correlations with nutrient intake from lucerne chaff.

The relative abundance of unclassified Bacteroidales had significant positive correlations with total ADF and NDF intakes from pellets and lucerne chaff. Bacteroidaceae, Prevotellaceae, S24-7 and Paraprevotellaceae families were found in lambs from the present study. The bacteria members of Prevotellaceae and Bacteroidaceae exhibit fibre digestion activities (Flint and Duncan, 2014), and are thus, positively correlated with total ADF and NDF intakes from pellets and lucerne chaff.

7.5.3 Relationship of bacteria relative abundance with VFA production

No bacteria genus was significantly associated with the rumen acetic and n-caproic content. However, differences in rumen propionate content in lambs was explained by the relative abundance of genus *Gemmiger*, *Anaerovibrio* and three unclassified bacteria in the order *Clostridiales.* These bacteria genus belong to the Clostridiales family. Further, many genera in the Clostridiales family produce propionate as a fermentation product,

supporting the association observed in this study. *Anaerovibrio* spp. produce acetate, propionate and butyrate from the fermentation of glycerol and lactate (Hobson and Stewart, 1997), which is consistent with the current study. It is interesting to note that although genus *Gemmiger* has been reported in chicken caeca (Asakura et al., 2021, Paul et al., 2021, Croucher and Barnes, 1983), pigs (Li et al., 2020b) and humans (Kowalska-Duplaga et al., 2019, Chénard et al., 2020), to the author's knowledge there are no studies that report the genus *Gemmiger* in rumen fluid. However, *Gemmiger* spp. produces a variety of acids such as formic and n-butyric, acetic, lactic, succinic, malonic, iso-valeric and pyruvic acids (Gossling and Moore, 1975, Croucher and Barnes, 1983) and some of these are precursors of propionic acid production, which likely contributed to the significant association found between the relative abundance of *Gemmiger* and propionate content in the present study.

The n-butyric acid content of lamb rumen fluid was positively associated with the relative abundance of *Gemmiger* and *Butyrivibrio*. The major fermentation products of *Gemmiger* are formic and n-butyric acids (Gossling and Moore, 1975) and *Butyrivibrio* are a major butyrate-producing bacteria in the rumen (Hobson and Stewart, 1997).

Iso-butyrate is a branch chain fatty acid (BCFA) and a product of protein deamination in the rumen by proteolytic bacteria. In the present study, rumen iso-butyrate content was associated with the relative abundance of members of family Clostridiales (*Gemmiger, Anaerovibrio, Oscillospira,* unclassified *Clostridiales,* unclassified *Ruminococcaceae*), family Bacteroidales (*Bacteroides and* unclassified Bacteroidales) and phylum Proteobacteria (*Succinivibrio* and *Desulfovibrio*) and explained the 96% of the variation in iso-butyrate content. Further, BCFAs are also required for the growth of cellulolytic bacteria (Membrive, 2016) and many genera in Clostridiales are cellulolytic bacteria.

Bacteroides are also reported to require one of BCFA (iso-butyric, iso-valeric, or dl-α-methyl-n-butyric acid) for growth (Bryant and Doetsch, 1955). But little is known about the bacteria that produce or utilise iso-butyric acid in the rumen. As *Desulfovibrio* and *Succinivibrio* are proteobacteria, they may have been involved in the deamination process to produce iso-butyrate, however, negative relationships were found between the relative abundance of those genera and rumen iso-butyrate content in the present study. A dairy cow study reported positive and no correlation between the relative abundance of *Desulfovibrio* and *Succinivibrio*, respectively, with iso-butyrate content in faeces (Mao et al., 2012). Moreover, *Succinivibrio* requires iso-acids (i.e. BCFA) in their growth media (Hespell, 1992). It is difficult to draw clear conclusions on the associations of the bacteria observed in the present study and rumen iso-butyrate content, and further research is warranted.

The n-valerate content of rumen fluid was positively associated with the genera *Gemmiger* and *Ruminococcus*, explaining 44% of the variation in n-valerate content. Valerate can be produced from propionate and ethanol (Oliphant and Allen-Vercoe, 2019). As *Gemmiger* produces the precursors of propionate production, this may explain, in part, the relationship with n-valerate content.

The iso-valerate content of rumen fluid was influenced by the relative abundance of *Gemmiger*, order unclassified Clostridiales and Bacteroidales, *Eubacterium, Oscillospira* and unclassified *Lachnospiraceae*. Iso-valerate is produced by bacteria and then utilised by *Bacteroides ruminicola* and Clostridiales such as *Ruminococcus flavefaciens* for the biosynthesis of cellular leucine in the rumen (Allison et al., 1966). Also, *Gemmiger* produces iso-valeric acid (Croucher and Barnes, 1983). This likely explains the relationship between the relative abundance of bacteria and iso-valerate content.

However, more work on iso-valerate producing and utilising bacteria in the rumen would clarify the complicated associations between VFAs and bacteria abundance.

The acetic to propionate ratio of rumen fluid was positively associated with the relative abundance of *Gluconacetobacter* and explained 31% of the variation in rumen acetic to propionate ratio. *Gluconacetobacter* produces acetic acid from ethanol and oxidates acetate (Dworkin and Falkow, 2006a). Further, some species of *Gluconacetobacter* require acetic acid, glucose and ethanol for growth (Dworkin and Falkow, 2006a). In ruminants, propionate is produced from lactate via two pathways. But it is also reported that *Gluconacetobacter* can convert lactate to carbon dioxide and water (Dworkin and Falkow, 2006a). Consequently, there is a chance that reduced propionic acid production in the rumen is due to oxidation of lactate, rather than propionate production. This would justify the observed relationship between the relative abundance of *Gluconacetobacter* and acetic to propionate ratio of rumen fluid in the present study.

To date, limited research has been carried out to determine the complex relationships between VFA production in the rumen and the composition of the rumen bacterial community. More frequent sampling of rumen fluid over time and across diet transition (milk to solid) to assess the changes in bacterial community composition and VFA production would improve decision making about optimal lamb weaning ages and diets.

Conclusion

The rumen bacterial community composition and structure in artificially reared lambs were affected by variation in three rearing treatments (combinations of pellet fibre level, milk replacer and age at milk weaning). In high fibre and milk-fed lambs to 57 days age, Firmicutes was the predominant phylum followed by Proteobacteria and genus *Succinovibrio* was prominent followed by *Prevotella*. In early-weaned lambs at 42 days

of age, Bacteroidetes were the prominent phylum, irrespective of pellet fibre level and were followed by Firmicutes. *Prevotella* was the prominent bacteria genus in early-weaned lambs at 42 days of age. The abundance of genus *Succinovibrio* in LFP42 was approximately similar to HFP57 lambs while it was less for HFP42 lambs.

Unclassified bacteria in Paraprevotellaceae, Clostridiales, Bacteroidales were associated with some feed and nutrient intake parameters. The relative abundance of some bacteria genera positively or negatively influenced the volatile fatty acids content in the rumen fluid. As the mechanisms that were driven those relationships are unclear, it will be interesting to extend future studies to clarify the complicated relationships between bacterial abundance and rumen VFA content and age-related changes as lambs transition from milk to solid feed.

Chapter 8 Validation of a mechanistic dynamic pre-weaned lamb growth and body composition simulation model

8.1 Abstract

Lamb growth and body composition simulation model can provide valuable insight into the assessment of feeding regimen and rearing systems, to optimise farm profitability without the need for in-vivo trials. The objective of the present study was to evaluate the performance of a published mechanistic dynamic model on the growth and body composition of pre-weaned lambs using independent published and unpublished data sets. Data generated from eight treatments, representing three artificial lamb rearing experiments (n=77 lambs) were used. Initial body composition data were obtained from four lambs at approximately two days of age, with the remaining lambs being provided with defined nutritional treatments until trial end-point. The body composition of lambs at slaughter was determined in six treatments across two experiments. Feed intake and live weight (LW) of lambs were recorded in all treatments. The accuracy of trait predictions by the model was evaluated by mean bias, paired t-test, coefficient of determination (R^2), concordance correlation coefficient (CCC) and relative predictive error (RPE). The overall LW, average daily gain (ADG) and overall ash deposition rates of lambs were accurately simulated by the model (paired t-test, $P>0.05$). The overall empty body weight (EBW), gutfill, protein and fat deposition rates were overestimated, and water deposition rate was underestimated by the model (paired t-test, $P<0.05$). Collectively, these results suggest that the model can be used at the farm level with acceptable accuracy to determine the lamb growth, but not for the determination of protein, fat and water deposition rates, gutfill and EBW. Further improvements in the model are required for protein, fat and water deposition rates, gutfill and EBW to make it more applicable for wider usage.

Keywords: carcass and viscera plus blood composition, feed intake, live weight, maintenance energy requirement, milk replacer, pellets

8.2 Introduction

Lamb growth during the pre-weaning period is an important factor affecting farm profitability (Gascoigne and Lovatt, 2015) and can be altered by diet composition and feeding regimen (Andrews and Ørskov, 1970, Chowdhury and Ørskov, 1997, Danso et al., 2018, Herath et al., 2020). Lamb growth simulation models provide an opportunity to predict the outcome of any given feeding and rearing strategy and could provide valuable information for farm decision making and potentially, the profitability of various lamb rearing options, without the need for *in-vivo* experimentation. Dynamic and mechanistic models exist which simulate the growth, intake and body composition of lambs from birth to maturity or in the post-weaning period (Sainz and Wolff, 1990, Finlayson et al., 1995, Johnson et al., 2012, Graham et al., 1976, Osorio et al., 2015). However, it appears there is only one model available for the growth and body composition of lambs in the pre-weaning period (Anim-Jnr et al., 2020).

The model of Anim-Jnr et al. (2020) is a mechanistic, dynamic, model aiming to predict pre-weaning lamb growth and body composition. The simulation model of Anim-Jnr et al. (2020) predicts daily body protein, fat, water and ash depositions based on milk replacer and pellet intakes and their composition. Based on those predictions empty body weight (EBW) and finally, lamb live weight (LW) is determined. To date, this model has not been validated utilising an independent data set. Recently, three artificial lamb rearing experiments were undertaken in which the lamb live weight and daily feed intake were recorded (Herath et al., 2020, Herath et al., 2021a, Herath et al., 2021b) and the body composition of lambs at slaughter was determined (Herath et al., 2020, Herath et al., 2021b). Therefore, the objective of this study was to evaluate the model of Anim-Jnr et al. (2020) using data generated from the aforementioned three lamb rearing experiments.

8.3 Materials and methods

8.3.1 Model description and validation

The pre-weaned lamb growth and body composition simulation model (Anim-Jnr et al., 2020) predicts daily body protein, fat, water and ash depositions based on the milk replacer and pellet intakes, their crude protein (CP), metabolisable energy (ME) contents and pellet neutral detergent fibre (NDF) content. Empty body weight (EBW) is determined based on daily body protein, fat, water and ash depositions and finally, live weight (LW) of lambs is determined as a function of EBW and NDF intake of the pellets. In this study, validation of the Anim-Jnr et al. (2020) model was tested using data collected from studies of Herath et al. (2020) (Chapter 3), Herath et al. (2021) (Chapter 4) and Herath et al. (2021b) (Chapter 5) utilising a total of 77 lambs. Four lambs of the 77 were slaughtered at approximately two days of age to obtain initial body composition data. The remaining 73 lambs were provided with defined milk replacers and pellet treatments across three studies, with eight different milk replacer and pellet feeding and age at weaning combinations being tested. A summary of these milk replacer, pellet and age of weaning treatment combinations is presented in Table 8.1.

The validation of the Anim-Jnr et al. (2020) model for LW and average daily live weight gain (ADG) was carried out using data obtained from all eight treatment groups (Table 8.2) while the validation of body composition was only based on six treatment groups (NMNP'17, HPMNP'17, MBNP'17, NMHF57'19, NMHF42'19 and HMLFE'19), due to the study of Herath et al. (2021a) not determining lamb body composition. A summary of data used in the validation of the growth and body composition simulation model is presented in Table 8.3.

8.3.2 Calculations

The actual lamb ADG was calculated as LW gain divided by the number of days in the individual experiment. Actual protein, fat, water and ash depositions per day in the carcass and viscera plus blood of the individual lamb were calculated as differences in protein, fat, water and ash depositions at the start of the experiment and at slaughter, divided by the number of days in the experiment. Simulated ADG and simulated daily protein, fat, water and ash depositions rates in the empty body weight were calculated as differences in simalated live weight, protein, fat, water and ash depositions at the start of the experiment and at slaughter, divided by the number of days in the experiment.

The model of Anim-Jnr et al. (2020) simulates the protein, fat, water and ash depositions in the empty body weight excluding blood, while the data of Herath et al. (2020) and Herath et al. (2021b) estimated protein, fat, water and ash depositions in the carcass and viscera, plus blood (Table 8.2). Therefore, corrections for actual protein, fat, water and ash deposition rates of carcass and viscera plus blood for the data from the studies of Herath et al. (2020) and Herath et al. (2021b) were undertaken by adding head, skin and feet composition deposition rates based on Danso et al. (2018). Protein, fat, water and ash depositions were further adjusted for deposition rates of blood composition by subtracting calculated protein, fat, water and ash depositions of each lamb based on fresh blood composition reported in Feedipedia (2012) and estimated or measured weight of blood at the start of the experiment and at slaughter. The initial blood weight of lambs in Herath et al. (2021b) was calculated as 0. 055 times the initial live weight which was estimated based on the baseline slaughter group (n=4, at 2 days old). The gutfill of lambs was determined as the difference between the predicted LW and the predicted EBW (De Lange et al., 2003)

Table 8.1 Summary of treatments (milk replacer, pellet and age at weaning combinations) of each of the three experiments used for the validation of the Anim-Jnr et al. (2020) lamb growth and body composition simulation model

Year	Treatment[4]	n[5]	Milk replacer composition			Pellet composition				Lucerne chaff composition				AP[6] (d)	AW[7] (d)	AE[8] (d)	Reference
			ME MJ/kg	CP g/kg	CP:ME g/MJ	ME MJ/kg	CP g/kg	CP:ME g/MJ	NDF g/kg	ME MJ/kg	CP g/kg	CP:ME g/MJ	NDF g/kg				
2017[1]	NMNP	10	21.9	240.0	10.96	11.5	180.0	15.6	118	-	-	-	-	14	-	70.2	Herath et al., 2020
2017[1]	MBNP	8	21.8	259.8	11.88	11.5	180.0	15.6	118	-	-	-	-	14	-	63.4	
2017[1]	HPMNP	9	21.3	338.7	15.89	11.5	180.0	15.6	118	-	-	-	-	14	-	65.4	
2018[2]	NMNP	10	21.2	260.8	12.28	10.3	184.4	17.8	199.7	7.4	207.5	28.2	294.7	8	-	57.8	Herath et al., 2021a
2018[2]	MBNP	12	20.8	305.7	14.73	10.3	184.4	17.8	199.7	7.4	207.5	28.2	294.7	8	-	58.2	
2019[3]	NMHF57	8	21.8	262.9	12.05	10.9	185.6	17.1	208.6	7.1	114.7	16.1	594.3	4	-	56.8	Herath et al., 2021b
2019[3]	NMHF42	9	21.8	262.9	12.05	10.9	185.6	17.1	208.6	7.1	114.7	16.1	594.3	4	42	55.3	
2019[3]	HPLF42	7	21.6	279.5	12.96	10.7	180.0	16.9	114.7	7.1	114.7	16.1	594.3	4	42	56.9	

[1] Chapter 3 - Herath et al. (2020) study, [2] Chapter 4 - Herath et al. (2021a) study, [3] Chapter 5 - Herath et al. (2021b) study

[4]NMNP'17- commercial milk replacer and normal pellets, 2017 experiment; MBNP'17 - milk blend and normal pellets, 2017 experiment; HPMNP'17 - high protein milk and normal pellets, 2017 experiment; NMNP'18 - commercial milk replacer and normal pellets, 2018 experiment; MBNP'18 - milk blend and normal pellets, 2018 experiment; NMHF57'19 - commercial milk replacer and high fibre pellet feeding to 57 days of age, 2019 experiment; NMHF42'19 - commercial milk replacer, high fibre pellet and early weaning, 2019 experiment and HPLF42'19 - high protein milk replacer for two weeks followed by commercial milk replacer, low fibre pellet and early weaning, 2019 experiment.

[5] Number of lambs in each treatment, [6]Age at pellet provision (d), [7]Age at weaning (d) of lambs, [8]Age at the end of the experiment for Herath et al., (2020) and Herath et al., (2021b) experiments and age at the end of the pre-weaning period for Herath et al., (2021a) experiment.

Table 8.2 Summary of data collected and analysed chemical composition of body components of each of the three experiments and their treatments that were utilised for the validation of the Anim-Jnr et al. (2020) lamb growth and body composition simulation model

Year	Treatment[4]	Data collected and analysed chemical composition of body components						
		Feed intake[5]	LW[6]	Carcass	Viscera[7]	Blood[7]	Head	Skin and feet
2017[1]	NMNP	✓	✓	✓	✓	✓	X	X
2017[1]	MBNP	✓	✓	✓	✓	✓	X	X
2017[1]	HPMNP	✓	✓	✓	✓	✓	X	X
2018[2]	NMNP	✓	✓	X	X	X	X	X
2018[2]	MBNP	✓	✓	X	X	X	X	X
2019[3]	NMHF57	✓	✓	✓	✓	✓	X	X
2019[3]	NMHF42	✓	✓	✓	✓	✓	X	X
2019[3]	HPLF42	✓	✓	✓	✓	✓	X	X
Model (Anim-Jnr et al., 2020)	All treatments	✓	✓	✓	✓	X	✓	✓

[1] Herath et al. (2020) study ,[2] Herath et al. (2021a) study, [3] Herath et al. (2021b) study

[4]NMNP'17- commercial milk replacer and normal pellets, 2017 experiment; MBNP'17 - milk blend and normal pellets, 2017 experiment; HPMNP'17 - high protein milk and normal pellets, 2017 experiment; NMNP'18 - commercial milk replacer and normal pellets, 2018 experiment; MBNP'18 - milk blend and normal pellets, 2018 experiment; NMHF57'19 - commercial milk replacer and high fibre pellet feeding to 57 days of age, 2019 experiment; NMHF42'19 - commercial milk replacer, high fibre pellet and early weaning, 2019 experiment and HPLF42'19 - high protein milk replacer for two weeks followed by commercial milk replacer, low fibre pellet and early weaning, 2019 experiment.

[5] Milk replacer, pellet and lucerne chaff (if applicable) intakes, [6] Live weight, [7]The chemical composition of viscera and blood was determined after mixing both components in 2017 and 2019 experiments.

Table 8.3 Summary data of each treatment of the three experiments that were utilised for the validation of the Anim-Jnr et al. (2020) lamb growth and body composition simulation model.

Treatment[1]	n[2]	Feed intake on an as fed basis				Nutrient per kg dry matter intake[3]			Mean lamb variables		
		Milk replacer (kg)	Pellet (kg)	Lucerne chaff (g)	Total dry matter (kg)	CP (g/kg)	ME (MJ/kg)	NDF (g/kg)	Initial LW (kg)	Final LW (kg)	ADG (g/d)
NMNP'17	10	17.2	8.4	0.0	24.1	234.4	19.7	40.8	5.9	22.3	235.7
MBNP'17	8	15.7	9.6	0.0	23.7	244.8	19.1	47.8	5.9	22.7	266.8
HPMNP'17	9	16.3	7.6	0.0	22.5	306.9	19.4	39.2	6.0	23.0	260.8
NMNP'18	10	12.7	9.7	187.8	21.0	272.1	17.4	82.2	5.3	22.2	304.3
MBNP'18	12	12.6	9.8	183.1	21.0	244.6	17.7	82.5	5.2	22.1	302.5
NMHF57'19	8	13.6	5.9	347.9	18.7	253.1	19.8	71.7	5.0	17.1	220.8
NMHF42'19	9	7.8	10.5	250.6	17.0	238.7	17.2	132.3	4.9	14.7	184.1
HPLF42'19	7	7.8	9.6	397.7	16.4	239.2	16.9	80.8	4.9	14.3	171.3
Mean		13.1	8.9	163.1	20.7	255.1	18.4	72.4	5.4	20.1	248.5
SD		3.6	2.9	194.9	3.8	23.8	1.3	32.5	1.0	3.9	56.4

[1] NMNP'17- commercial milk replacer and normal pellets, 2017 experiment; MBNP'17 - milk blend and normal pellets, 2017 experiment; HPMNP'17 - high protein milk and normal pellets, 2017 experiment; NMNP'18 - commercial milk replacer and normal pellets, 2018 experiment; MBNP'18 - milk blend and normal pellets, 2018 experiment; NMHF57'19 - commercial milk replacer and high fibre pellet feeding to 57 days of age, 2019 experiment; NMHF42'19 - commercial milk replacer, high fibre pellet and early weaning, 2019 experiment and HPLF42'19 - high protein milk replacer for two weeks followed by commercial milk replacer, low fibre pellet and early weaning, 2019 experiment.

[2] Number of lambs in each treatment.

[3]The combined intake of milk replacer, pellets and lucerne chaff (CP = crude protein; ME = metabolisable energy; NDF = neutral detergent fibre).

LW, Live weight; ADG, Average daily live weight gain

8.3.3 Statistical analysis

The overall differences in actual and simulated values for data from all lambs, irrespective of treatments were determined by a paired t-test (SAS 9.4, 2013). Similarly, the differences in actual and simulated values of each experimental treatment were determined by a paired t-test (SAS 9.4, 2013). The coefficient of determination (R^2) was calculated by linear regression between simulated and actual values to measure the precision of the model prediction. The mean bias of each parameter was calculated as actual value minus simulated value (Anim-Jnr et al., 2020). The concordance correlation coefficients (CCC) were computed as described by (Lin, 1989) to determine model reproducibility. A CCC value between 0.81-1.00 is considered as almost a perfect prediction, 0.61-0.80 is a substantial prediction, 0.41-0.60 is a moderate prediction and 0.21-0.40 is a fair prediction (Fuentes-Pila et al., 1996, Visser et al., 2012).

The relative prediction error (RPE) was calculated as follows to evaluate the prediction accuracy of the model (Fuentes-Pila et al., 2003, Visser et al., 2012).

$$Relative\ prediction\ error\ (RPE)\ =\ (MPE/\bar{A})\ \times\ 100$$

$$Mean\ Prediction\ Error\ (MPE) = \sqrt{\frac{1}{n}\sum_{i=1}^{n}(Ai - Pi)^2}$$

Where A*i* is the *i*th observed actual value and P*i* is the *i*th simulated value by model, and $\bar{A}$ is the mean of the actual value and n is the number of observations.

A RPE value less than 10% is considered a satisfactory prediction, between 10-20% is an acceptable prediction and greater than 20% is an unsatisfactory prediction (Fuentes-Pila et al., 1996, Fuentes-Pila et al., 2003, Anim-Jnr et al., 2020).

8.4 Results

8.4.1 Overall lamb body composition

Simulated values of protein, fat and water deposition rates for each lamb irrespective of treatment differed from their actual values ($P<0.05$, Table 8.4, Figure 8.1; graph A, B, C). Overall, the relationship between actual and simulated values demonstrated higher R^2 for daily fat and protein deposition rates while daily water deposition rates had less R^2. The model reproducibility of daily protein deposition was substantial, while daily fat and water deposition prediction only had moderate reproducibility as demonstrated by the CCC values. Based on the RPE values, protein and water deposition predictions were of an acceptable level, while fat deposition predictions were unsatisfactory. The model accurately simulated the overall ash deposition rates of lambs (Table 8.4, Figure 8.1; graph D) as simulated values did not differ ($P>0.05$) from actual values. The R^2 of the relationship between actual and simulated daily ash deposition values were low ($R^2=0.15$) and had a fair reproducibility based on CCC with a higher RPE.

8.4.2 Body composition of lambs in each experimental treatment

Protein deposition rates of lambs in NMNP'17, MBNP'17 and HPMNP'17 treatments were accurately simulated by the model ($P>0.05$, Table 8.5). Protein deposition rates of the NMHF57'19, NMHF42'19 and HPLF42'19 treatments were overestimated and simulated values differed ($P<0.05$) from actual protein deposition rates. The R^2 of the relationship between actual and simulated protein deposition rate of HPMNP'17 and MBNP'17 treatments were less than 0.50, while R^2 was higher for all other treatments ($R^2 > 0.50$). Overall, the model of Anim-Jnr et al. (2020) simulated daily protein deposition accurately for some pre-weaned lamb treatments but with varying precision.

Table 8.4 Statistical indicators of Anim-Jnr et al. (2020) overall model performance for the body composition of lambs, which had different milk replacer and feeding and age at weaning combinations from studies Herath et al. (2020), Herath et al. (2021a) and Herath et al. (2021b)

Trait	n[1]	Actual ± SD	Simulated ± SD	Mean Bias[2]	P value[3]	R square[4]	Concordance correlation coefficient[5]	Relative prediction error[6] %
Protein deposition, g/d	51	34.9 ± 5.8	39.2 ± 3.5	-4.3	<.0001	0.45	0.42	17.3
Fat deposition, g/d	51	19.8 ± 8.2	38.6 ± 16.7	-18.8	<.0001	0.65	0.31	109.0
Water deposition, g/d	51	123.0 ± 20.5	117.1 ± 8.3	5.9	0.0239	0.22	0.30	15.4
Ash deposition, g/d	51	5.6 ± 1.4	5.5 ± 0.4	0.1	0.67	0.15	0.21	23.5

[1] Total number of lambs in all treatments.
[2] Calculated as actual mean value minus simulated mean value.
[3] P>0.05 indicates the model simulated value for each trait did not differ from the actual value.
[4] Relationship between actual and model-simulated values of experimental treatments.
[5] A concordance correlation coefficient (CCC) value between 0.81-1.00 indicates an almost perfect prediction, 0.61-0.80 a substantial prediction, 0.41-0.60 a moderate prediction and 0.21-0.40 a fair prediction (Fuentes-Pila et al., 1996, Visser et al., 2012).
[6] A Relative prediction error (RPE) value <10% indicates a satisfactory prediction, between 10-20% an acceptable prediction and >20% an unsatisfactory prediction (Fuentes-Pila et al., 1996, Fuentes-Pila et al., 2003, Anim-Jnr et al., 2020).

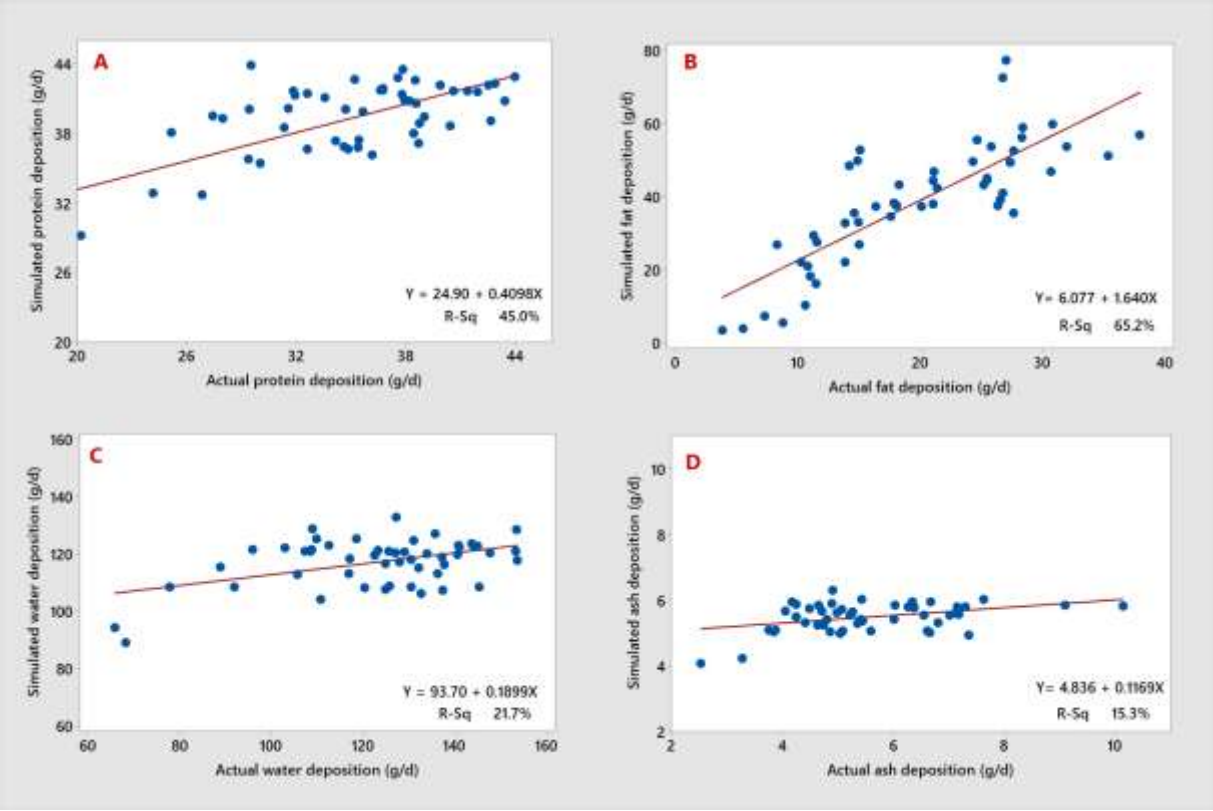


Figure 8.1 Relationship between the actual and Anim-Jnr et al. (2020) model simulated protein (Graph A), fat (Graph B), water (Graph C) and ash (Graph D) deposition rates of individual lambs in six experimental treatments, which had different milk replacer and pellet feeding and age at weaning combinations. Data sourced from Herath et al. (2020) and Herath et al. (2021b).

Table 8.5 Statistical indicators of the Anim-Jnr et al. (2020) model performance for the prediction of daily protein and fat depositions in the body of lambs in each treatment of two experiments, which had different milk replacer and pellet feeding and age at weaning combinations.

Trait/Treatment[1]	n[2]	Actual ± SD	Simulated ± SD	Mean Bias[3]	P value[4]	R square[5]
Protein deposition rate g/d						
NMNP'17	10	36.5 ±2.5	37.3 ±0.9	-0.8	0.20	0.51
MBNP'17	8	39.4 ±3.2	40.9 ±0.8	-1.5	0.21	0.11
HPMNP'17	9	39.4 ±2.3	41.8 ±1.3	-2.3	0.0577	0.29
NMHF57'19	8	35.1 ±5.2	39.8 ±3.5	-4.7	0.0053	0.61
NMHF42'19	9	29.9 ±6.4	36.6 ±5.7	-6.8	<.0001	0.91
HPLF42'19	7	27.9 ±2.7	39.2 ± 3.4	-11.3	<.0001	0.66
Fat deposition rate, g/d						
NMNP'17	10	26.4 ± 2.8	45.7 ±7.5	-19.3	<.0001	0.12
MBNP'17	8	30.2 ±4.6	51.0 ±6.6	-20.8	<.0001	0.21
HPMNP'17	9	20.7 ±3.8	42.8 ±7.5	-22.1	<.0001	0.67
NMHF57'19	8	17.9 ±5.9	47.1 ±19.4	-29.3	0.0007	0.82
NMHF42'19	9	11.4 ±4.7	26.7 ±15.4	-15.3	0.0044	0.72
HPLF42'19	7	10.5 ±2.1	14.4 ±6.1	-3.9	0.10	0.59

[1] NMNP'17- commercial milk replacer and normal pellets, 2017 experiment; MBNP'17 - milk blend and normal pellets, 2017 experiment; HPMNP'17 - high protein milk and normal pellets, 2017 experiment; NMHF57'19 - commercial milk replacer and high fibre pellet feeding to 57 days of age, 2019 experiment; NMHF42'19 - commercial milk replacer, high fibre pellet and early weaning, 2019 experiment and HPLF42'19 - high protein milk replacer for two weeks followed by commercial milk replacer, low fibre pellet and early weaning, 2019 experiment.
[2] Number of lambs in each treatment.
[3] Calculated as actual mean value minus simulated mean value.
[4] P>0.05 indicates the model simulated value for each trait did not differ from the actual value.
[5] Relationship between actual and model-simulated values of experimental treatments.

Table 8.6 Statistical indicators of the Anim-Jnr et al. (2020) model performance for the prediction of daily water and ash depositions in the body of lambs in each treatment of two experiments, which had different milk replacer and pellet feeding and age at weaning combinations.

Trait/Treatment[1]	n[2]	Actual ± SD	Simulated ± SD	Mean Bias[3]	P value[4]	R square[5]
Water deposition rate, g/d						
HPMNP'17	9	135.4 ±10.1	120.9 ±1.73	14.5	0.0018	0.20
MBNP'17	8	139.8 ±8.5	119.0 ±2.6	20.8	0.0002	0.05
NMNP'17	10	127.9 ±10.4	108.5 ±2.8	19.4	0.0002	0.03
HPLF42'19	7	100.3 ±13.9	120.9 ±6.9	-20.6	0.0005	0.85
NMHF57'19	8	125.2 ±19.7	121.8 ±7.7	3.4	0.55	0.52
NMHF42'19	9	105.7 ±23.9	113.7 ±13.0	-7.9	0.11	0.84
Ash deposition rate, g/d						
HPMNP'17	9	7.0 ±1.7	5.8 ±0.1	1.2	0.0559	0.07
MBNP'17	8	6.2 ±1.1	5.6 ±0.2	0.6	0.18	0.15
NMNP'17	10	5.6 ±1.3	5.1 ±0.2	0.5	0.30	0.004
HPLF42'19	7	4.7 ±0.4	5.6 ±0.3	-0.9	0.0017	0.04
NMHF57'19	8	5.3 ±1.2	5.7 ±0.4	-0.4	0.33	0.28
NMHF42'19	9	4.5 ±1.1	5.2 ±0.7	-0.7	0.0055	0.73

[1] NMNP'17- commercial milk replacer and normal pellets, 2017 experiment; MBNP'17 - milk blend and normal pellets, 2017 experiment; HPMNP'17 - high protein milk and normal pellets, 2017 experiment; NMHF57'19 - commercial milk replacer and high fibre pellet feeding to 57 days of age, 2019 experiment; NMHF42'19 - commercial milk replacer, high fibre pellet and early weaning, 2019 experiment and HPLF42'19 - high protein milk replacer for two weeks followed by commercial milk replacer, low fibre pellet and early weaning, 2019 experiment.
[3] Calculated as actual mean value minus simulated mean value.
[4] P>0.05 indicates the model simulated value for each trait did not differ from the actual value.
[5] Relationship between actual and model-simulated values of experimental treatments.

The model did not simulate the fat deposition rate accurately as simulated fat deposition rates differed from actual values ($P<0.05$), except for the HPLF42'19 treatment (Table 8.5). Fat deposition rate was overestimated by 21.3 g/d on average for all treatments, except HPLF42'19. The R^2 of the relationship between actual and simulated fat deposition rates were higher for four treatments, but less for the NMNP'17 and MBNP'17 treatments. In summary, the model of Anim-Jnr et al. (2020) did not simulate the daily fat deposition rate of pre-weaned lambs particularly well.

Daily water deposition of NMHF57'19 and NMHF42'19 lambs were simulated accurately ($P>0.05$), but it was poorly predicted in all other treatments (Table 8.6). Daily water depositions of early-weaned lambs treatments (HPLF42'19 and NMHF42'19) were underestimated. The R^2 values for the relationship between actual and simulated daily water depositions were greater than 0.80, showing there was a precise prediction by the model. All the other treatments had R^2 values less than 0.20 indicating less precision, except for the NMHF57'19 treatment ($R^2=0.52$). In summary, the prediction of daily water deposition by the model of Anim-Jnr et al. (2020) was not at a satisfactory level for most pre-weaning lamb treatments.

The ash deposition rates did not differ ($P>0.05$) between actual and simulated values in the four treatments (Table 8.6). The R^2 for the relationship between actual and simulated daily ash deposition was less than 0.30 for all the treatments, indicating less precision except for the HPLF42'19 treatment which had a greater R^2 value (0.73). In summary, the model of Anim-Jnr et al. (2020) simulated daily ash deposition of pre-weaned lamb treatments accurately but, with less precision.

The concordance correlation coefficient (CCC) and relative prediction error (RPE) values for protein, fat, water and ash deposition rates of each treatment varied between

treatments and are presented in Appendix 3. The RPE and CC values vary between treatments for each parameter and had no clear pattern of change.

8.4.3 Lamb growth

8.4.3.1 Overall lamb live weight and average daily gain

Live weight of individual lambs, irrespective of treatment, were accurately simulated by the model (P>0.05). The R^2 for the relationship between actual and simulated values was greater confirming a satisfactory model prediction (Table 8.7, Figure 8.2). The CCC values indicated that LW prediction was almost perfect and based on RPE, it was a satisfactory model prediction.

The ADG of each lamb, irrespective of treatment, was simulated accurately (P>0.05, Table 8.7, Figure 8.3) with a higher R^2 for the relationship between simulated and actual values. The CCC indicates the ADG prediction was almost a perfect prediction and based on RPE, it was an acceptable model prediction.

8.4.3.2 Lamb live weight and average daily gain (ADG) in each experimental treatment

The LW of lambs in HPMNP'17, MBNP'17 and NMNP'17 treatments were accurately predicted by the model (P>0.05, Table 8.8). However, lamb LW in MBNP'18 and NMNP'18 treatments were not predicted accurately (P>0.05). Lamb LWs in HPLF42'19, NMHF57'19 and NMHF42'19 treatments were overestimated (P>0.05). The R^2 for the relationship between simulated and actual LW was greater in MBNP'18, HPLF42'19, NMHF57'19 and NMHF42'19 treatments ($R^2 > 0.85$). All the other treatments had R^2 values less than 0.35 indicating a less precise model prediction, except for the NMHF57'19 treatment (R^2=0.55). In summary, the model of Anim-Jnr *et al.* (2020) simulated LW accurately for some pre-weaned lamb treatments with less precision.

Table 8.7 Statistical indicators of Anim-Jnr et al. (2020) overall model performance for the growth of lambs, which had different milk replacer and pellet feeding and age at weaning combinations from studies Herath et al. 2020, Herath et al. 2021 and Herath et al. 2021b.

Trait	n[1]	Actual ± SD	Simulated ± SD	Mean Bias[2]	P value[3]	R square[4]	Concordance correlation coefficient[5]	Relative prediction error[6] %
Final live weight, kg	73	20.1 ± 3.9	20.0 ± 2.8	0.1	0.67	0.88	0.89	8.0
Average daily gain, g/d	73	248.6 ± 56.4	247.4 ± 34.1	1.1	0.75	0.83	0.81	11.6
Empty body weight, kg	51	16.2 ± 3.7	17.0 ± 2.7	-0.8	<.0001	0.94	0.89	9.3
Gutfill, kg	51	1.7 ± 0.5	2.8 ± 0.6	-1.1	<.0001	0.28	0.17	70.4

[1] Total number of lambs in all treatments.
[2] Calculated as actual mean value minus simulated mean value.
[3] P>0.05 indicates the model simulated value for each trait did not differ from the actual value.
[4] Relationship between actual and model-simulated values of experimental treatments.
[5] A concordance correlation coefficient (CCC) value between 0.81-1.00 indicates an almost perfect prediction, 0.61-0.80 a substantial prediction, 0.41-0.60 a moderate prediction and 0.21-0.40 a fair prediction (Fuentes-Pila et al., 1996, Visser et al., 2012).
[6] A Relative prediction error (RPE) value <10% indicates a satisfactory prediction, between 10-20% an acceptable prediction and >20% an unsatisfactory prediction (Fuentes-Pila et al., 1996, Fuentes-Pila et al., 2003, Anim-Jnr et al., 2020).

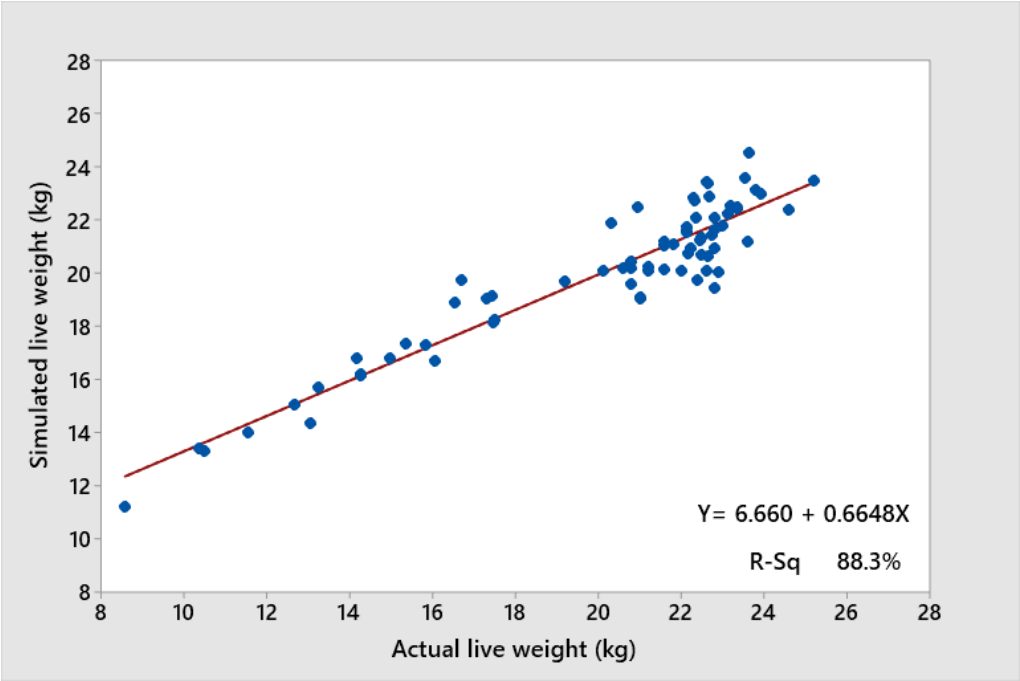


Figure 8.2 Relationship between actual and Anim-Jnr et al. (2020) model simulated live weight for eight experimental treatments of lambs, which had different milk replacer and pellet feeding and age at weaning combinations. Data sourced from Herath et al. (2020), Herath et al. (2021a) and Herath et al. (2021b).

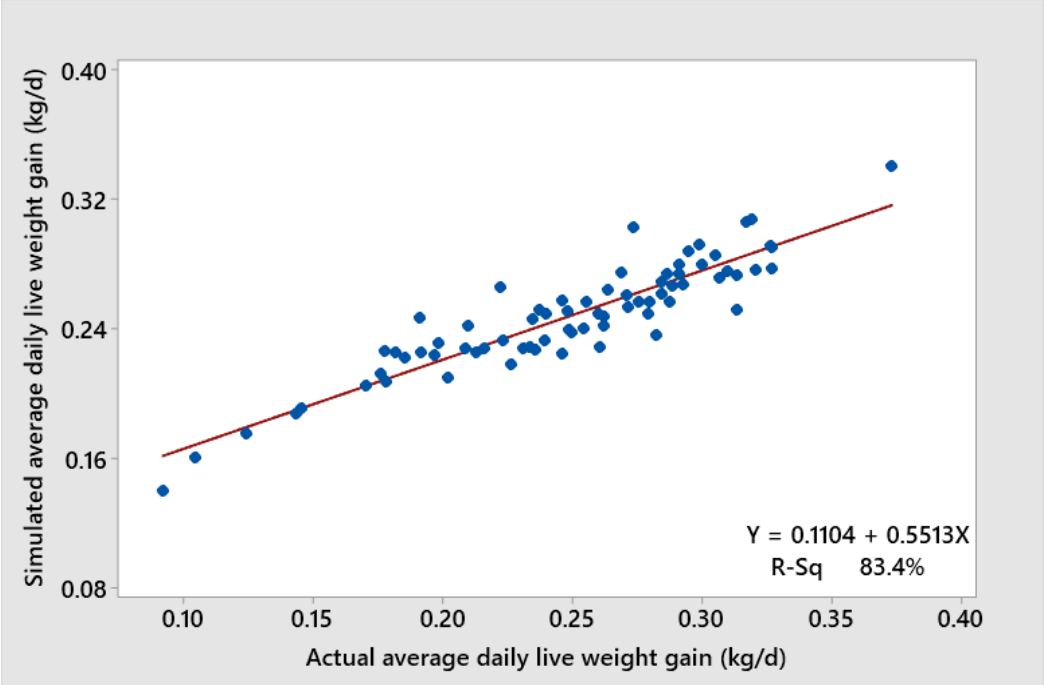


Figure 8.3 Relationship between actual and Anim-Jnr et al. (2020) model simulated average daily live weight gain for eight experimental treatments of lambs, which had different milk replacer and pellet feeding and age at weaning combinations. Data sourced from Herath et al. (2020), Herath et al. (2021a) and Herath et al. (2021b).

The ADG of lambs in the HPMNP'17, MBNP'17 and NMNP'17 treatments were accurately simulated by the model (P>0.05, Table 8.8). The ADG was underestimated in the MBNP'18 and NMNP'18 treatments and the simulated values differed from actual values (P<0.05). Lamb ADG was overestimated in the HPLF42'19, NMHF57'19 and NMHF42'19 treatments (P<0.05). The precision of ADG prediction, as indicated by the R^2 value, was greater in the MBNP'18, HPLF42'19, NMHF57'19 and NMHF42'19 treatments (R^2 > 0.82) but was between 0.50 and 0.65 for all the other treatments except the HPMNP'17 treatment. The model of Anim-Jnr *et al.* (2020) simulated ADG accurately for some pre-weaning lamb treatments.

The concordance correlation coefficient (CCC) and relative prediction error (RPE) values for LW and ADG of each treatment varied between treatments and had no clear pattern of change (Appendix 3).

Table 8.8 Statistical indicators of the Anim-Jnr et al. (2020) model performance for the prediction of live weight (LW) and average daily live weight gain (ADG) of lambs in each treatment of three experiments, which had different milk replacer and pellet feeding and age at weaning combinations.

Trait/Treatment[1]	n[2]	Actual ± SD	Simulated ± SD	Mean Bias[3]	P value[4]	R square[5]
Live weight, kg						
HPMNP'17	9	23.0 ± 0.4	22.2 ± 1.2	0.8	0.06	0.17
MBNP'17	8	22.7 ± 0.7	22.1 ± 1.3	0.6	0.10	0.55
NMNP'17	10	22.3 ± 0.6	22.1 ± 0.8	0.3	0.37	0.0003
NMNP'18	10	22.1 ± 0.9	20.4 ± 1.1	1.7	0.0003	0.34
MBNP'18	12	22.2 ± 1.6	21.0 ± 1.2	1.3	<.0001	0.85
HPLF42'19	7	14.3 ± 2.1	16.4 ± 1.9	-2.1	<.0001	0.99
NMHF57'19	8	17.1 ± 3.3	18.5 ± 2.6	-1.5	0.0071	0.92
NMHF42'19	9	14.7 ± 3.8	16.4 ± 3.1	-1.7	0.0017	0.94
Average daily live weight gain, g/d						
HPMNP'17	9	260.7 ± 16.3	247.7 ± 9.6	13.1	0.06	0.01
MBNP'17	8	266.8 ± 21.0	255.7 ± 14.6	11.1	0.07	0.52
NMNP'17	10	235.7 ± 21.7	230.5 ± 12.6	5.2	0.25	0.67
NMNP'18	10	302.5 ± 29.9	271.3 ± 27.3	31.2	0.0004	0.64
MBNP'18	12	304.3 ± 26.0	281.5 ± 21.4	22.8	<.0001	0.83
HPLF42'19	7	171.3 ± 20.2	209.7 ± 22.7	-38.4	<.0001	0.99
NMHF57'19	8	220.8 ± 48.8	247.4 ± 36.6	-26.6	0.0069	0.87
NMHF42'19	9	184.1 ± 56.0	215.8 ± 41.4	-31.7	0.0015	0.92

[1] NMNP'17- commercial milk replacer and normal pellets, 2017 experiment; MBNP'17 - milk blend and normal pellets, 2017 experiment; HPMNP'17 - high protein milk and normal pellets, 2017 experiment; NMNP'18 - commercial milk replacer and normal pellets, 2018 experiment; MBNP'18 - milk blend and normal pellets, 2018 experiment; NMHF57'19 - commercial milk replacer and high fibre pellet feeding to 57 days of age, 2019 experiment; NMHF42'19 - commercial milk replacer, high fibre pellet and early weaning, 2019 experiment and HPLF42'19 - high protein milk replacer for two weeks followed by commercial milk replacer, low fibre pellet and early weaning, 2019 experiment.

[2] Number of lambs in each treatment.

[3] Calculated as actual mean value minus simulated mean value.

[4] P>0.05 indicates the model simulated value for each trait did not differ from the actual value.

[5] Relationship between actual and model-simulated values of experimental treatments.

8.4.3.3 Overall lamb empty body weight and gutfill

The EBW of each lamb irrespective of treatment was not accurately predicted by the model (P<0.05, Table 8.7, Figure 8.4). However, the simulated EBW value had almost perfect prediction according to the CCC and RPE values.

Gutfill was poorly predicted by the model (P<0.05, Table 8.7, Figure 8.5). The R^2 of gutfill simulated and actual value was poor and based on both CCC and RPE, the model prediction was unsatisfactory.

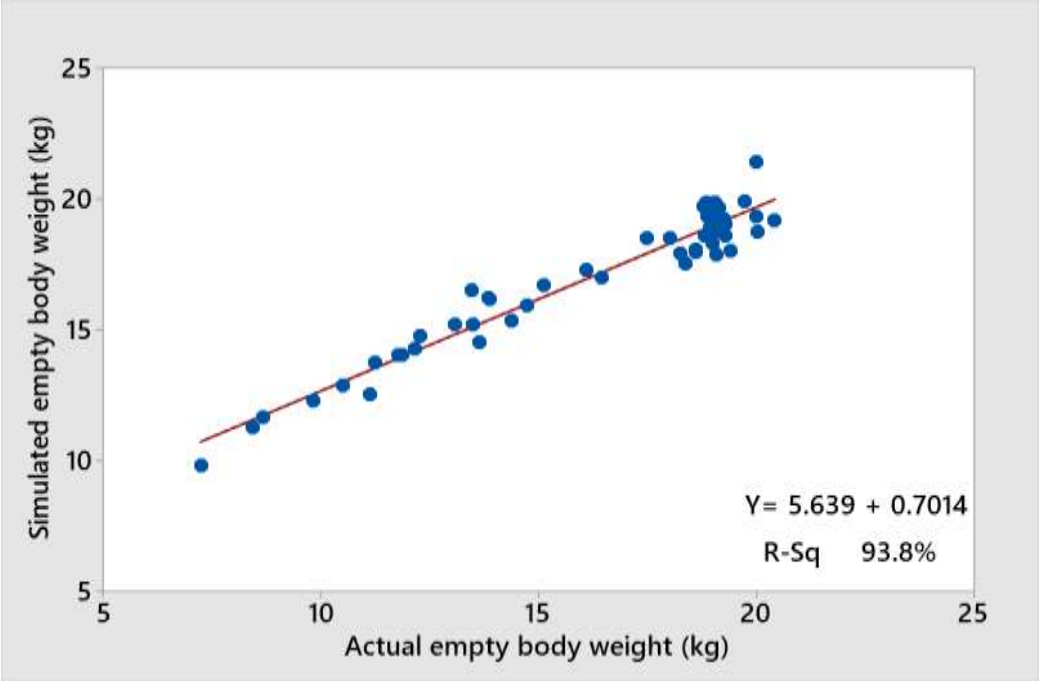


Figure 8.4 Relationship between actual and Anim-Jnr et al. (2020) model simulated empty body weight for six experimental treatments of lambs, which had different milk replacer and pellet feeding and age at weaning combinations. Data sourced from Herath et al. (2020) and Herath et al. (2021b).

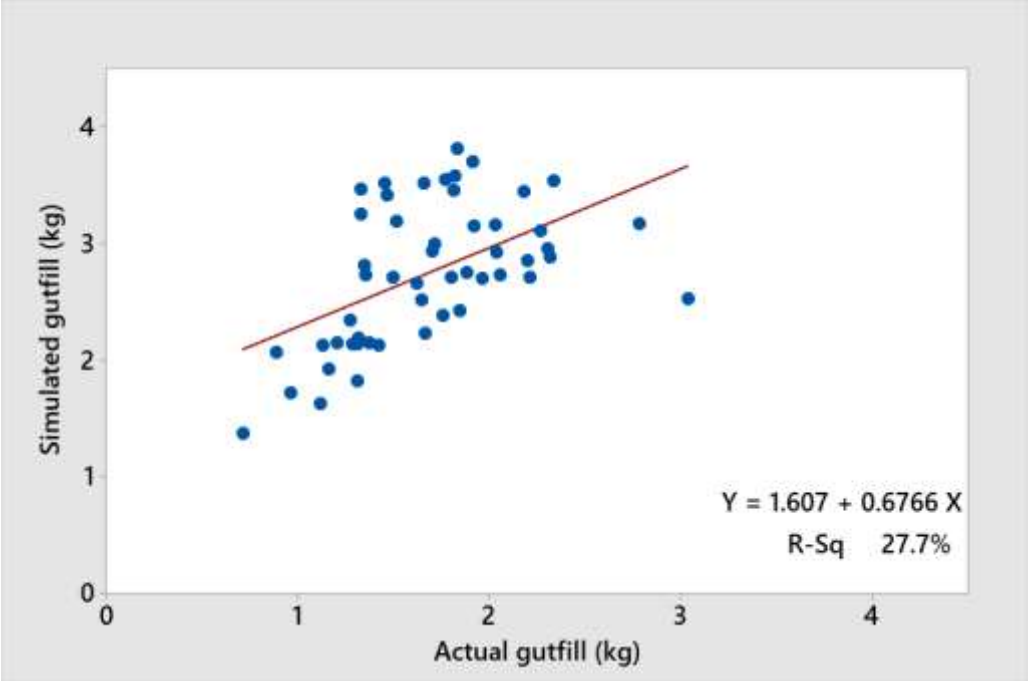


Figure 8.5 Relationship between actual and Anim-Jnr et al. (2020) model simulated gutfill for six experimental treatments of lambs, which had different milk replacer and pellet feeding and age at weaning combinations. Data sourced from Herath et al., (2020) and Herath et al. (2021b).

8.4.3.4 Empty body weight (EBW) and gutfill in each experimental treatment

The EBW of lambs in the HPMNP'17, MBNP'17 and NMNP'17 treatments were accurately simulated (P>0.05, Table 8.9). The EBW of lambs in the HPLF42'19, NMHF57'19 and NMHF42'19 treatments were overestimated (P<0.05, Table 8.9). The R^2 for the relationship between EBW predicted and actual values was greatest in the HPLF42'19, NMHF57'19 and NMHF42'19 treatments (R^2 > 0.88) and it was higher for MBNP'17 treatment (R^2 > 0.55) than all the remaining treatments, which had R^2 values less than 0.13. The model of Anim-Jnr *et al.* (2020) simulated EBW accurately for some pre-weaning lamb treatments with varying precision.

The model did not simulate the gutfill accurately in all the treatments (P<0.05, Table 8.9), with gutfill being overestimated in all treatments. The R^2 for the relationship between actual and simulated gutfill values was greater in the HPLF42'19 and NMHF42'19

treatments (R^2 =0.79 and 0.74, respectively) while all the other treatments had reduced prediction precision (R^2 < 0.32). The model of Anim-Jnr *et al.* (2020) did not predict gutfill accurately and most treatments had low model precision.

Table 8.9 Statistical indicators of the Anim-Jnr et al. (2020) model performance for the prediction of empty body weight (EBW) and gutfill of lambs in each treatment of two experiments, which had different milk replacer and pellet feeding and age at weaning combinations.

Trait/Treatment[1]	n[2]	Actual ± SD	Simulated ± SD	Mean Bias[3]	P value[4]	R square[5]
Empty Body Weight, kg						
HPMNP'17	9	19.1 ± 0.5	19.1 ± 0.9	0.1	0.82	0.13
MBNP'17	8	19.3 ± 0.6	18.9 ± 1.2	0.4	0.20	0.55
NMNP'17	10	19.1 ± 0.5	19.0 ± 0.6	0.1	0.79	0.03
HPLF42'19	7	11.5 ± 1.7	13.7 ± 2.8	-2.3	<.0001	0.98
NMHF57'19	8	14.5 ± 2.8	16.1 ± 2.2	-1.6	0.0015	0.88
NMHF42'19	9	12.2 ± 3.1	14.0 ± 2.5	-1.8	0.0001	0.95
Gutfill, kg						
HPMNP'17	9	1.9 ± 0.6	3.0 ± 0.4	-1.1	0.0018	0.10
MBNP'17	8	1.8 ± 0.3	3.1 ± 0.4	-1.3	<.0001	0.05
NMNP'17	10	2.0 ± 0.4	3.2 ± 0.4	-1.6	0.0001	0.06
HPLF42'19	7	1.2 ± 0.5	1.9 ± 0.4	-0.7	0.0002	0.79
NMHF57'19	8	1.6 ± 0.4	2.6 ± 0.6	-1.1	0.0005	0.35
NMHF42'19	9	1.6 ± 0.4	2.4 ± 0.4	-0.8	<.0001	0.74

[1] NMNP'17- commercial milk replacer and normal pellets, 2017 experiment; MBNP'17 - milk blend and normal pellets, 2017 experiment; HPMNP'17 - high protein milk and normal pellets, 2017 experiment; NMHF57'19 - commercial milk replacer and high fibre pellet feeding to 57 days of age, 2019 experiment; NMHF42'19 - commercial milk replacer, high fibre pellet and early weaning, 2019 experiment and HPLF42'19 - high protein milk replacer for two weeks followed by commercial milk replacer, low fibre pellet and early weaning, 2019 experiment.
[2] Number of lambs in each treatment, [3] Calculated as actual mean value minus simulated mean value.
[4] P>0.05 indicates the model simulated value for each trait did not differ from the actual value.
[5] Relationship between actual and model-simulated values of experimental treatments

8.5 Discussion

The objective of this study was to validate the mechanistic dynamic model of Anim-Jnr *et al.*, (2020) which was developed to predict pre-weaning lamb growth and body composition using data generated from three lamb rearing experiments.

8.5.1 Body composition

The model overestimated overall protein deposition rate in the lamb empty body weight. This could be due to changes in the gastrointestinal tract (GIT) development and differences in the feed digestibility *in-vivo,* compared to what was assumed in the model. The average pellet intake of lambs in the present study was twice that in all the treatments used for the development of Anim-Jnr et al. (2020) model. Therefore, average NDF intake of lambs was almost double. Greater energy expenditure for ruminal and intestinal growth and less retained energy in tissues have previously been reported in growing lambs and heifers fed a higher proportion of a fibre containing diet (Reynolds et al., 1991, McLeod and Baldwin, 2000).

Further, the total weight of the empty stomach, intestine and liver relative to the final LW of lambs in the studies of Herath et al. (2020) and Herath et al. (2021b), was approximately 10% while that in Anim-Jnr et al. (2020) model was 8%. The growth of liver and GIT is closely related to the maintenance energy requirement (ME_m) of the animal, indicating that growth changes in those organs can considerably alter the total animal energy expenditure and gain of body chemical composition (Ferrell, 1988). Thus, due to greater pellet and NDF intakes and GIT development, ME_m would likely have increased over time in the pre-weaning *in-vivo* experiments, compared to the fixed ME_m value (0.45 MJ/kg$LW^{0.75}$) utilised in the model of Anim-Jnr et al. (2020). The use of fixed and likely underestimated ME_m value in the model simulation could result in the overestimation of available energy for deposition compared to the actual energy

availability of *in-vivo* experiment. This could lead to a greater protein deposition in the model simulation than actual. Supporting this, the model did not show energy limitation for protein deposition when lambs had greater pellet intake, though available energy for protein deposition theoretically could reduce with greater pellet intake due to possible improved GIT development.

The protein deposition rate of lambs in HPMNP'17, MBNP'17 and NMNP'17 treatments was accurately simulated, while it was overestimated in HPLF42'19, NMHF57'19 and NMHF42'19 treatments. Solid feed intake improves the rumen development of lambs transitioning them from non-ruminant to ruminant stage. This alters the digestibility of feed stuff in the rumen. The efficiency of ME utilization for maintenance decreases as feed digestibility decreases (Nicol and Brookes, 2007), resulting in a higher ME_m requirement. The increased fibre level in the diet reduces the digestibility (Porter et al., 2007). The lambs in HPLF42'19, NMHF57'19 and NMHF42'19 treatments had greater fibre intake due to the greater fibre content in pellets and /or greater pellet intake which would have likely increased ME_m. The fixed ME_m in the model of Anim-Jnr et al. (2020) would have resulted in an overestimation of protein deposition, which explains why these three studies were poorly predicted.

The model did not simulate overall fat deposition at a satisfactory level, and in fact, consistently overestimated it in all treatments. This is likely caused by a mismatch in energy and protein partitioning in the model, compared to the actual biological process. Under scenarios of excess energy and protein, after utilisation for maintenance, the model deposited the protein, depending on the energy available for growth and daily maximum protein deposition and finally fat deposition (Anim-Jnr et al., 2020). The possible underestimation of ME_m and use of a fixed ME_m over time in the lamb growth likely

results in excess energy for a greater fat deposition in the model, than in *in vivo* experiments. Moreover, Anim-Jnr et al. (2020) reported that the model underestimated the fat deposition rate of lambs fed only milk replacer and restricted pellet-fed lambs. In *in vivo* experiments, those milk fed lambs might have less ME_m requirement compared to the model prediction, thus, they could deposit more fat *in-vivo* than the model does, leading to underestimation of fat deposition. Thus, both situations suggest that the use of a fixed ME_m in the model prediction could over or underestimate the fat deposition, depending on the ME_m requirement of lambs in *in-vivo* experimental conditions. The changes in MEm requirement of lambs with their GIT development could be determined by a series of *in-vivo* experiments, which would be designed considering age, live weight and fibre intake of lambs, and then would derive an equation to simulate the ME_m requirement in the model.

Overall and for each treatment, water deposition rates of empty body weight were poorly simulated, except for lambs fed high fibre pellets and commercial milk replacer (NMHF57'19 and NMHF42'19). Water deposition was simulated based on protein deposition, but water deposition did not show the same trend as protein deposition. The water to protein ratio of lambs varies with empty body weight and the dietary protein content (Ørskov et al., 1976). Further in pigs, it has been found to change with body tissue type and genotype (De Lange et al., 2003). Some of the treatments in the present study were different combinations of dietary protein to energy levels which could change the ratio between water and protein deposition in each treatment. The equations used to simulate water deposition could be adjusted further with large data set to increase the prediction accurately. However, the actual cause is unknown and needs exploration.

Overall and each treatment ash deposition rates were accurately simulated except HPLF42'19 and NMHF42'19, those lambs were weaned early and had a pellet-only diet for two weeks. The ash deposition rate of lambs was predicted based on the protein deposition in the body. Ørskov et al. (1976) suggested that the ratio between protein to ash in empty body weight and the carcass is constant at equal live weights. The lambs in HPLF42'19 and NMHF42'19 had the lowest live weight at pre-weaning than all the other treatments. Thus, the ratio between protein to ash could be changed in the i*n-vivo* experiment, but, the model did not account for it. However, the model can be used to simulate the overall daily ash deposition of pre-weaned lambs.

8.5.2 Lamb growth, empty body weight (EBW) and gutfill

The model was found to satisfactorily simulate the overall lamb growth (LW and ADG) and overestimated the overall empty body weight (EBW). The LW, ADG and EBW of lambs in HPMNP'17, MBNP'17 and NMNP'17 treatments were accurately simulated and overestimated in HPLF42'19, NMHF57'19 and NMHF42'19. The model underestimated LW and ADG in MBNP'18 and NMNP'18 treatments. The overestimation of protein and fat deposition of HPLF42'19, NMHF57'19 and NMHF42'19 lambs are likely cause for the overestimation of EBW.

The model of Anim-Jnr et al. (2020) consistently overestimated the gutfill of lambs in all the treatments. Gutfill was estimated based on a derived equation using NDF intake of lambs in the studies of Danso et al. (2016) and Danso et al. (2018). The poor simulation of gutfill is likely due to greater NDF intake of lambs in three of the treatments (HPLF42'19, NMHF57'19 and NMHF42'19) used in the present study. Considering the lambs in Herath et al. (2020) and Herath et al. (2021b) experiments used in this validation study, gutfill accounted for approximately 9.7% of the total live weight compared to 8.6%

in Danso et al. (2016) and Danso et al. (2018). The model determined the lamb LW as a function of EBW and NDF content of pellets as the gutfill. Thus, overestimation of EBW and higher NDF intake of the HPLF42'19, NMHF57'19 and NMHF42'19 treatments are the probable explanation for the overestimation of LW of those lambs. However, the small overestimation of gutfill in HPMNP'17, MBNP'17 and NMNP'17 lambs did not make a considerable change in their final LWs. Compared these data indicate gutfill estimation in the model needs to be improved, to account for varying NDF intakes.

8.5.3 Improvements required for model prediction

The model used a fixed ME_m value, which was determined by ME_m values at lamb weights of 9, 16 and 17-18 kg (Danso et al., 2016, Danso et al., 2018). Thus, the use of corresponding actual ME_m values for each lamb live weight rather than an average value for the entire pre-weaning period would improve the model prediction of body composition during pre-weaning. Therefore, the metabolisable energy requirement for maintenance needs to be experimentally re-evaluated. This would require defined feeding schedules to represent the changes in ME_m requirements for maintenance along with gastrointestinal tract development associated with the level of feed intake, LW and age of lamb. Cannas et al. (2010) also reported that uncertainty of the ME_m requirement for organ development is a limitation of small ruminant nutritional requirement prediction. It would be worthwhile to further investigate the energy requirement of lambs for maintenance during the transition from liquid to solid feed also. Alternatively, a predicted ME_m value based on the physiological development (the ratio between LW and mature LW) could be used which would more accurately represent the ME_m requirement of growing lambs compared to using chronological age, breed or LW (Freetly et al., 2002). But this would require testing. Because ME_m varies with the weights of metabolically active organs (Koong et al., 1985), an alternative model could be developed considering visceral organ and carcass

growth separately and then combining them to determine the final lamb LW. This would improve the model by reducing possible errors of energy partitioning for organ growth and carcass depositions.

Body compositions of two lamb treatments (HPLF42'19 and NMHF42'19) were determined two weeks post-weaning when they were fed only a pellet diet. However, the model of Anim-Jnr et al. (2020) was developed for pre-weaned lambs only. Thus, it may not be surprising that data from early-weaned lamb treatment (HPLF42'19 and NMHF42'19) was not accurately predicted for growth and body composition. Further studies are required to provide additional data to better enable the model to predict lambs in the early post-weaning period.

The model of Anim-Jnr et al. (2020) uses the composition of only milk replacer and pellets as input parameters. But lambs in the treatments of MBNP'18, NMNP'18, HPLF42'19, NMHF57'19 and NMHF42'19 had also been fed lucerne chaff, although lucerne chaff intake was less than 1% of total dry matter intake and was unlikely to have much effect on lamb growth and body composition. To cater for such additional feedstuffs, the inclusion of additional input parameters would likely improve prediction accuracy. Further inclusion of input parameters into the model of Anim-Jnr et al. (2020) to help calculate the cost-effectiveness of each feeding strategy would also ensure a wider application in the evaluation of commercial pre-weaning lamb feeding systems.

8.6 Conclusion

Overall, the mechanistic dynamic model developed to predict pre-weaning lamb growth and body composition (Anim-Jnr et al., 2020) could be used to predict live weight and average daily live weight gain of lambs given different feeding management with satisfactory model prediction and precision. However, simulation of lamb body

composition needs to be improved to achieve higher accuracy. The model of Anim-Jnr et al. (2020) could be improved by accounting for the changing metabolisable energy requirement for lamb maintenance to account for changes in energy utilisation due to gastrointestinal tract development during the pre-weaning period. The inclusion of additional model parameters to determine the cost-effectiveness of the feeding regimen would enable better decision-making by farmers.

Chapter 9 General Discussion

9.1 Introduction

Artificial rearing is the separation of lambs from ewes and the hand-rearing of them to help ensure optimum and healthy lamb growth (Owen and Davies, 1970). It is practised in the dairy sheep industry and in orphan rearing meat production systems. Although artificial lamb rearing provides several advantages to farmers, there has not been the need to develop systems on a large scale in New Zealand, until the recent growth of the dairy sheep industry; thus, little farmer-useful information is available (Nieper, 2017). Therefore, there is a need to devise optimum artificial rearing strategies for new-born lambs, to optimise outcomes for lambs and profits for farmers in both milking and non-milking lamb-rearing systems.

Feeding management during artificial rearing is the major driver of growth and development in lambs in that period. Recently, a series of lamb nutrition studies was conducted in New Zealand examining artificial lamb rearing systems and one of those studies suggested that lambs required a higher protein to energy (CP:ME) ratio at lighter live weights (13.1 g/MJ at 5 kg live weight) and a lower ratio at heavier weights (10.9 g/MJ at 18 kg (Danso et al., 2016)). This suggests that in many artificial lamb rearing feeding systems, where the CP:ME ratio in milk replacer is kept constant, lamb growth can be limited due to low protein intake per unit of energy intake in early lactation, while protein intake per unit energy is in excess in later life (approximately after six weeks of age), when solid feed intake becomes the major nutrient source (Danso *et al*., 2016). Combined, these results suggest it may be possible to improve lamb growth and reduce nutrient wastage by feeding lambs with a milk replacer and/or pellets adjusted to better match the changes in CP:ME requirements for growth as lambs age. However, this has not been tested. Further, early milk weaning of young ruminants has been reported to reduce the cost of milk feeding (Jones and Heinrichs, 2007), but it is currently unknown

if early milk weaning combined with varying pellet fibre levels improved lamb growth and rumen development during artificial rearing. This also warrants investigation.

In addition, prediction of lamb growth and body composition without the need for *in-vivo* experiments would bring many advantages to farmers and research development (Pettigrew, 2018). However, there appears to be only one published stimulation prediction model for pre-weaned, artificially-reared lamb growth and body composition (Anim-Jnr et al., 2020). To date, this model has not been validated against an independent dataset.

Thus, to address the above gaps in knowledge, the following objectives were investigated in this thesis.

1. The effect of dietary protein to energy ratio (CP:ME) in milk replacer (to 22 kg live weight) on growth and body composition of pre-weaned lambs reared artificially (Chapter 3)
2. The effect of dietary CP:ME ratio (to 22 kg live weight) on growth performance of pre-and post-weaned lambs (Chapter 4)
3. The influence of pellet fibre level, in addition to milk replacer composition and time of weaning (42 vs 57 days age), on the growth and body composition of lambs reared artificially (Chapter 5)
4. The impact of varying pellet fibre levels and milk replacer composition on rumen development in artificially-reared, early-weaned lambs (Chapter 6)
5. The effect of varying pellet fibre levels on the composition of the rumen bacteria community in artificially-reared, early-weaned lambs (Chapter 7)

6. Validation of a mechanistic dynamic pre-weaned lamb growth and body composition simulation model (Anim-Jnr et al., 2020) based on data from Chapters 3, 4, and 5 (Chapter 8)

The general outcomes of the experiments, limitations, implications and recommendations for further studies are discussed in this section (Chapter 9). The summaries of the results found are outlined in the following section based on research themes rather than on a chapter by chapter basis.

9.2 Summary of research findings

9.2.1 Lamb growth, body composition and organ development

9.2.1.1 Lamb growth

Lamb growth during the pre-weaning period varied with the milk replacer CP:ME ratio. A greater average daily live weight gain (ADG) was observed in lambs fed milk replacers with a high CP:ME ratio (15.89 g/MJ) and with a milk replacer that had the CP:ME ratio adjusted to match the lambs changing requirements (Chapter 3) than the traditional commercial milk replacer with low and static CP:ME ratio (10.96 g/ MJ). Consequently, based on the findings of this study and previous research (Danso et al., 2018), the CP:ME ratio of commercial milk replacers in New Zealand has been increased by the industry to 12.28 g/MJ. Chapter 4 suggested that feeding commercial milk replacer with an improved CP:ME ratio (12.28 g/MJ), in addition to *ad libitum* access to pellets (CP:ME 17.93 g/MJ) pre-weaning resulted in similar ADG to those of lambs fed CP:ME adjusted milk replacers to meet their theoretical nutrient requirements. Moreover, post-weaning ADG was similar for both improved and adjusted CP:ME milk replacer fed lambs reared to 45 kg live weight. Chapter 4 also demonstrated that fulfilment of CP:ME requirements at an early stage of life (approximately 5 to 22 kg live weight) depended on both milk replacer and pellet intake. If pellet intake is substantial, it may be possible to early milk-

wean lambs, without compromising their growth, due to improved rumen development. Chapter 5 was designed to examine how fibre level in pellets, a milk replacer with CP:ME 12.28 g/MJ (except for an initial two weeks of low fibre pellets fed lambs) and time of weaning, affected lamb growth and body composition. It showed that milk-weaning at 42 days of age does not impair the overall growth rate to 57 days of age in artificially reared lambs. Further, the fibre level of pellets also did not affect the overall lamb growth rate.

Across the three lamb rearing experiments, the ADG of lambs was highest for lambs in Chapter 4 (reaching 22 kg LW at 57 days of age). Those lambs were fed either improved milk replacer (12.28 g/MJ CP:ME) or CP:ME ratio adjusted milk replacer, as the lambs aged, in addition to *ad libitum* access to pellets. The early weaned lambs in Chapter 5 had the lowest ADG of all experiments. Lambs fed an increased CP:ME ratio in Chapters 3 and 4 had higher pre-weaning ADG. Considering the pre-weaning period of lambs in Chapters 3 and 4, the combined CP:ME intake between 13.5 to 14.5 g/MJ from both milk replacer and pellets seem to be optimal for lamb growth, resulting in ADG of 300 g/d or greater. However, adjustments of the CP:ME ratio in milk replacer to meet the lamb's changing nutritional requirement as it ages and grows, would not be effective in improving lamb growth, if lambs also have considerable pellet intake, as the combined milk plus pellet intake will modify the overall intake CP:ME ratio. That might lead to reduced growth as there is an energy cost associated with excretion of excess nitrogen (Reed et al., 2017, Kozloski et al., 2001).

Based on the data generated in Chapters 3, 4 and 5, overall ADG and live weight of pre-weaned lambs can be accurately predicted by the mechanistic, dynamic pre-weaning lamb growth model of Anim-Jnr et al. (2020) (Chapter 8).

9.2.1.2 Body composition

Chapter 3 showed fat deposition rate in the whole lamb body was higher in lambs fed an adjusted CP:ME ratio milk replacer to meet the changing requirements in pre-weaning. Feeding milk replacer with a high CP:ME ratio (15.89 g/MJ) resulted in a reduced fat deposition rate in the whole body (Chapter 3), this could be due to the use of energy for excess nitrogen excretion, leaving less energy for deposition. Lamb whole body protein deposition rate was not affected by the CP:ME ratio of milk replacers chosen for the Chapter 3 experiment.

Early weaning reduced daily fat deposition rates in the lamb whole body compared to those fed milk to 57 days age (Chapter 5). Early weaned lambs could have greater energy expenditure for gastrointestinal tract (GIT) development than lambs fed milk to slaughter. In addition, they could have used stored body fat for maintenance, if they did not have adequate energy for maintenance due to lower nutrient utilisation efficiency from pellets. However, pellet fibre level and early weaning did not affect the protein deposition rates in the whole lamb body (Chapter 5).

Considering both Chapters 3 and 5, the protein deposition rate in whole body increased with increased CP intake and ME intake and reached a plateau. The protein deposition rate depends on the availability of both protein and energy (Andrews and Ørskov, 1970). The plateau reached with further increases in either CP or ME intakes was most likely due to the limitation of ME or CP, respectively. The fat deposition rate in the whole body increased with increasing ME intake. The fat deposition rate increased with increasing CP intake and plateaued, possibly due to insufficient ME for further deposition or energy cost associated with excess nitrogen excretion.

Based on Chapter 8, the Anim-Jnr et al. (2020) model did not simulate lamb protein, fat and water deposition rates in the empty body weight accurately, but ash deposition rate was accurately simulated. This could be due to increased ME requirement for maintenance of lambs in the present study due to greater rumen development *in-vivo.* However, the model used a fixed value for ME requirement for maintenance throughout lamb growth. Thus, the altered ME requirement for maintenance could not be accounted for by the model. Consequently, the model could have greater energy allocation for depositions than the actual availability of *in-vivo* experiments, resulting in poor body composition simulation by the model of Anim-Jnr et al. (2020).

9.2.1.3 Organ development

Empty small intestine weight was greater in lambs fed low CP:ME (10.96 g/MJ) milk replacer than those fed high CP:ME (Chapter 3). It could be due to inefficient nitrogen absorption in the proximal small intestine in low CP:ME fed lambs resulting in increased surface area of the small intestine. Early weaned lambs fed either high or low fibre pellets had lighter empty small intestine compared to lambs fed milk to 57 days of age (Chapter 5). A recent study reported only duodenal villi width was greater in early weaned lambs compared to late weaned lambs (six weeks of age) and found no changes in other measures of tissue morphological development (Carballo et al., 2019). However, it is not well established that any impact of empty small intestine weight changes on its morphology and nutrient absorption capasity.

Omental fat content was lower in high CP:ME milk replacer fed lambs than both CP:ME low and adjusted milk replacer fed lambs. This could be due to the use of energy for excess nitrogen excretion in lambs that had greater protein intake. Further, early weaning also resulted in reduced omental fat content (Chapter 5) as those lambs could have less

energy for deposition due to poorer efficiency of nutrient utilisation from solid feed compared to the milk replacers (Danso et al., 2016). This could slightly reduce the fat content in the viscera, as the contribution of omental fat to total visceral fat is low. The weights of total empty viscera and specific organs (liver, kidney, empty stomach, empty large intestine, heart, lungs and testis), blood, head, feet and tail and whole skin of lambs was not affected by the CP:ME ratio of milk replacer (Chapter 3) or early weaning and pellet fibre level (Chapter 5).

9.2.2 Lamb rumen development

Chapters 6, and 7 examined how pellet fibre level in addition to milk replacer composition and time of weaning affected rumen development (physical, metabolic, and bacterial composition) of artificially reared lambs. Chapter 6 suggested that rumen n-butyric, which influences rumen papillae development, increased with early lamb weaning (42 days of age). The greater solid feed intake of early-weaned lambs could have resulted in greater rumen n-butyric content. The inclusion of lactose in the pellets improves rumen n-butyric content (Chamberlain et al., 1993). Hence, the low fibre pellet fed lambs benefited by both greater pellet intake and inclusion of skim milk powder in the pellets as a lactose source. Although a positive relationship was found between the n-butyric acid content and height of papillae at the dorsal site of the rumen (Chapter 6), there was no difference in the papillae height between treatments. This could be due to a small sample size in each treatment as variable papillae height was not use in power analysis to determine the sample size of the experiment. In addition, greater variation in the papillae development of lambs within the treatments could also lead to a lack of difference between treatments.

Feeding low fibre pellets resulted in higher n-valeric content compared to high fibre pellet fed lambs, irrespective of weaning age (Chapter 6). It is also found that n-valeric content of the rumen fluid positively influenced the papillae height of the rumen dorsal site (Chapter 6). Thus, papillae height of the lambs fed low fibre pellets could have been impacted by both early weaning and the low fibre content of the diet. However, the absence of a difference in the papillae height of the lambs between treatments could be due to an insufficient number of lambs or the variations in the papillae development of lambs within the treatments.

The absence of a significant difference in dorsal rumen wall thickness between treatment at 36 days suggests that prior to early weaning rumen development of all the lambs was similar. But ultrasound examination of the rumen at 49 days of age suggested that early-weaned lambs had a thicker rumen dorsal wall compared to milk feeding to slaughter at 57 days of age (Chapter 6). The greater pellet intake and n-butyric acid content of those lambs could result in a thicker rumen dorsal wall. In addition, Chapter 6 also found, the rumen dorsal wall thickness measured via ultrasound examination was positively correlated with the height of rumen papillae at the rumen dorsal site measured via histology examination, suggesting ultrasound examination of rumen would be a tool to monitor rumen development of young ruminants.

The combined effect of pellet fibre level and weaning age and the site of rumen tissue sampling affected the rumen papillae width, density and rumen muscular layer thickness (Chapter 6). Lambs fed milk replacer to 57 days of age had narrow papillae but higher papillae density at the ventral rumen site compared to other combinations of treatments and the rumen tissue sampling sites. Papillae height, percentage of longer papillae and ratio between the length of papillae outer boundary and respective straight length of

rumen tissue were greater in the ventral than the dorsal site of the rumen (Chapter 6). These results highlight the importance of examinating all rumen sites for papillae development in future studies to provide an informative outcome as papillae development varies with rumen sites.

Chapter 6 also found that nutrient intake from solid feed (ME, ADF, dry matter and organic matter intake) positively influenced the rumen iso-valeric and iso-butyric fatty acid content, which are growth factors of cellulolytic bacteria. Although only iso-valeric content was positively affected by the dietary protein intake (Chapter 6), both iso-valeric and iso-butyric fatty acids are produced from deamination of proteins by the rumen bacteria (Membrive, 2016). However, in Chapter 7, it was found that differences in rumen iso-valeric and iso-butyric contents were explained positively or negatively by the relative abundance of many rumen bacteria genera. This suggests nutrient intake may affect the growth of bacteria and those bacteria genera may be engaged in the production or utilisation of those branched-chain fatty acids.

The CP:ME ratio of milk replacer did not affect the rumen empty weight (Chapter 3). This could be due to the presence of oesophageal groove in bottle-fed lambs, which directed milk to the abomasum and bypassing the rumen (Ørskov et al., 1970) resulting in a negligible effect on the rumen development.

Early weaning of lambs resulted in heavier empty rumen compared to those fed milk to 57 days age (Chapter 5). Further, those early-weaned lambs had a greater proportion of rumen relative to their total empty stomach weight (Chapter 5). Although the effects of rumen weight on the digestive and absorptive capacity of the rumen are not well understood (Baldwin and Connor, 2017), Chapter 6 showed that rumen mass was positively influenced by papillae height at both the dorsal and ventral rumen sites.

Moreover, papillae height at the dorsal rumen site is positively associated with rumen propionic**,** iso-butyric, n-butyric, iso-valeric and n-valeric contents (Chapter 6). Hence, the improved VFA production in the rumen with solid feed intake and bulkiness of diet due to the dietary fibre level, likely improved rumen papillae development and could result in a heavier empty rumen. This could increase the nutrient absorptive surface area and would impact positively on the nutrient absorption capacity of the rumen.

The feeding of lambs with milk replacer to 57 days of age resulted in a prominent abomasum relative to their total empty stomach weight to digest milk rather than rumen development to utilise nutrients from solid feed. Hence, an extended period of milk feeding, or late milk weaning, would have negative impacts on the rumen development and thereby, transition and post-weaning lamb growth.

Chapter 7 demonstrated that Bacteroidetes, which are polysaccharides degrading bacteria that produce propionate (Flint and Duncan, 2014), were the prominent phylum in early-weaned (42 days of age) lambs, irrespective of pellet fibre level. This was followed by Firmicutes. The lambs fed high fibre pellets and milk to 57 days of age were found to have Firmicutes as the prominent phylum followed by Proteobacteria. Proteobacteria is reported to be the prominent bacteria phylum at the early life stage of young ruminants (Jami et al., 2013, Rey et al., 2014, Jiao et al., 2015a) and the abundance of rumen epithelium associated Proteobacteria was reduced, while the abundance of Firmicutes and Bacteroidetes rose with goats' age (Jiao et al., 2015a). Therefore, early weaned lambs would have developed rumen bacterial population to digest the solid feed, while rumen bacterial population of milk-fed lambs could still transition from milk-based diet to solid feed to improve rumen digestion.

This is also supported by the analysis of relative abundance of bacteria at the genus level. *Prevotella* was the prominent genus in early-weaned lambs; they are involved in the digestion of starch, xylans and pectin and deamination of protein to produce ammonia. The most abundant bacteria genus in milk-fed lambs to 57 days of age was *Succinovibrio,* which contribute to ruminal starch digestion, *Prevotella* were the next most abundant.

Overall, results of Chapters 6 and 7 suggest that both lamb diet composition and time of weaning influence rumen physical development, metabolic development and the rumen bacterial community.

9.2.3 Cost analysis

The feeding of a high CP:ME ratio or adjusted milk replacer was more expensive than feeding a traditional low CP:ME ratio milk replacer to lambs during the pre-weaning period. The lowest feed cost per kilogram live weight gain of lambs was achieved by the improved CP:ME (12.28 g/MJ) commercial milk replacer, followed by the CP:ME adjusted milk replacer (Chapter 4). The results indicated that feeding either a high or low CP:ME milk replacer would not be financially beneficial, due to either the high cost of milk replacer or low ADG, respectively, and thus should be avoided by farmers. Although the feed cost per lamb was lowest in early-weaned lamb groups (Chapter 5) compared to lambs in Chapter 4, the feed cost per kilogram of live weight gain was higher (Chapter 5). This is because the lambs needed more time to achieve a set weaning or market weight, requiring additional feed.

9.3 Limitations

Statistical power analyses were carried out to determine the minimum number of animals to be used to detect the differences in growth of lambs, but with high statistic power (>0.8). However, power analyses were not undertaken for every trait investigated in the

various experiments. For some traits, such as gut measurements, for which a power analysis was not possible, having a large number of animals in each treatment group would have reduced the variation, potentially enabling the discovery of important differences. In addition, all the experiments were carried out with male Romney sheep. As there are differences in growth among sheep breeds and sexes, it would be prudent to evaluate the performance of other breeds and sexes.

High CP:ME ratio milk replacer reduced the fat deposition rate in the whole lamb body (Chapter 3), and this could be due to the use of energy to eliminate excess nitrogen. But no information on the energy cost of excreting excess nitrogen in lambs was collected. The energy required could be determined by measuring the nitrogen excretion through faeces and urine, in lambs consuming varying protein levels of milk replacer. Measuring the nitrogen excretion would also have helped to better understand the nitrogen balance of high protein fed lambs.

Ideally, the pasture intake of lambs should have been measured during the transition and post-weaning period in Chapter 4. This would have enabled the assessment of any difference in pasture intake of lambs in each treatment, and any consequent impact on live weight gain during those respective periods. The experiment was designed to provide lambs ryegrass and white clover-based pasture at *ad libitum* pasture level during the transition and post-weaning periods. To ensure lambs were provided *al libitum* access to pasture, pre-and post-grazing pasture measurements were taken, and all measurements were above 1200 kg DM/ha (Morris and Kenyon, 2004). If this experiment was to be repeated, the pasture intake of lambs should be assessed by pre-and post-grazing pasture measurement by plate meter, with pasture growth during the grazing period being measured using random quadrat pasture cuts at pre-and post-grazing. Further, pasture

grab would collect to determine the nutritional composition and calculated the nutrient intake of lambs.

Many studies have reported that pellet intake of lambs is negligible when lambs are younger than 21 days, particularly when milk is provided simultaneously. Therefore, the effect of pellet intake on combined CP:ME intake was not considered in the experimental design. However, the lambs in Chapter 5 had pellet intakes of approximately 100 g/d during 21-30 days and greater pellet intake thereafter. Thus, lambs fed milk replacer with adjusted CP:ME to meet their theoretical growth requirement likely had greater combined milk replacer plus pellet CP:ME intake than their requirements, due to considerable pellet intake. Being able to account for the effect of CP:ME intake from pellets in the experimental design would have avoided excessive intake. Therefore, in the future, CP:ME intake from pellets needs to be considered in experimental designing and individual pellet intake could either be measured manually by weighing pellets offered and refused or using automatic feeders.

The digestibility of the individual feedstuffs (i.e. milk replacer, pellets and lucerne chaff) were not measured in Chapter 5. Ideally, these could have been determined for different ages or live weights of lambs before the start of the experiments. This would have helped optimise feed formulations, and to ensure high digestible feed at the initial stages of lamb growth and to improve nutrient utilisation.

Another consideration is that the composition of blood and viscera was analysed after they were mixed in Chapters 3 and 5. The composition of blood and viscera could have been analysed separately to better understand the alterations in the visceral composition. This knowledge would have provided information on blood and visceral composition of lambs.

The rumen study was limited to the examination of papillae development in the ventral and dorsal rumen sites. Chapter 6 demonstrated that papillae growth at the dorsal and ventral sites of the rumen was different. Thus, to investigate the effect of dietary or lamb rearing system on rumen development, the collection of representative rumen tissue samples from all rumen sites would have provided informative and accurate results on the entire rumen development in future studies.

The experiment (Chapter 5) did not measure the volume of the rumen content at slaughter, but, knowing the rumen content volume and correlating it with the calculated rumen volume in Chapter 6 would have provided an assessment of accuracy of the calculated volume. Rumen volume of sheep varies even when animals are fed at the same level of feed intake, which suggests there are considerable differences in rumen environment affecting the rumen fermentation (Purser and Moir, 1966). Rumen volume is determined directly by manual emptying of the rumen content in fistulated animals or evacuation by a cannula and indirectly by using digesta flow markers (France et al., 1991, Moloney et al., 1993). As there are negative impacts of direct rumen volume measurement methods on the rumen fermentation after the return of digesta, it would have benefited if rumen volume was measured in slaughtered animals.

In Chapter 6, the volatile fatty acid (VFA) content of rumen fluid was measured from lambs approximately 12 hrs after the last feeding, which could alter the actual VFA content resulting from diet and weaning interventions. This is because some of rumen VFAs content increased markedly after feeding, while that of others reduced depending on the VFA production and absorption (Fenner et al., 1967). Thus, rumen fluid samples for VFA analysis could have been collected after either two to four hours (Pless et al., 2018) or three hours after feeding (Fenner et al., 1967) to assess the direct effect of diet,

and to avoid any impact of fasting time on VFA content. Collection of rumen fluid for VFA analysis after two to four hours of feeding could allow the determination of the maximum VFA production (Pless et al., 2018) related to diet and weaning treatments. As the primary objective of the experiment in Chapters 5 was to determine the effect of diet and time of weaning on the body composition of lambs at slaughter, which is important for meat industry, this approach was not undertaken. However, lamb rumen fluid samples were collected at slaughter for the study in Chapter 6. Further, fasting of lambs before slaughter might have affected the rumen bacteria composition presented in Chapter 7. Rumen fluid could have been collected via a stomach tube after two to four hours from the last feed for the determination of VFA and bacterial composition of rumen fluid.

Lambs in Chapter 7 were penned in-doors separately and they shared the same outdoor area for approximately five hours a week during the cleaning of pens until the end of the experiment. Lambs were provided a common water source when they were in the shared outdoor area. The dam, feed, partners and housing and environment are sources of bacteria in neonatal ruminants (Diao et al., 2019). It was also found that bacteria from ambient air and pen floors are dominat in the guts of bottle-fed lambs in addition to microbes from the mother's vagina (Bi et al., 2018). In addition, drinking water provided to calves immediately after birth was found to have an impact on gut microbial composition (Wickramasinghe et al., 2019). Thus, in the present study, there were possibilities for microbial cross-contamination during milk bottle cleaning, milk preparation and pellet weighing and feeding and cleaning of pens. Therefore, the actual effects of pellet fibre levels and early weaning on the composition of rumen bacteria may be masked. Therefore, if this experiment was to be repeated, these possible routes of cross-contaminations should be controlled.

The experiments reported in Chapters 5, 6, and 7, did not include the fourth treatment of a low fibre diet and milk feeding to 57 days of lamb age, due to insufficient space to accommodate the required lambs in the indoor lamb rearing facility. The inclusion of that treatment would have provided more insight into how a low fibre pellet diet with milk replacer influenced lamb growth during pre-weaning. However, the three treatments provided adequate comparisons to examine the main objective of the study: the effect of pellet fibre level and time of weaning on growth and rumen development of lambs.

The body composition of early-weaned lambs at slaughter in the Chapter 5 experiment was determined two weeks post-weaning. Data generated from those lambs were used for the validation of the body composition simulation model of Anim-Jnr et al. (2020) (Chapter 8). However, the model of Anim-Jnr et al. (2020) was developed for pre-weaned lambs, and exclusion of data from those early-weaned lambs from model validation would have enabled a more accurate evaluation of model performances. Therefore, an alternative approach could have been the analysis of body composition of a few, representative, early-weaned lambs in each treatment at the end of the pre-weaning. These could then also have been used for validation of body composition prediction by the model of Anim-Jnr et al. (2020). But validation of the model of Anim-Jnr et al. (2020) was not the main objective of the experiments in Chapters 5, 6, and 7 and therefore this approach was not undertaken.

9.4 Further Studies

Across and within the lamb rearing experiments and treatments presented in this thesis, there was significant variation in pellet intake. Pellet intake had a positive impact on lamb growth and rumen development. However, what drives pellet intake is not well understood. Further research that focuses on factors that drive individual lamb pellet

intake of artificially reared lambs, during the pre-weaning growth stage would be useful. These factors could potentially include live weight, breed, restricted milk feeding, nutrient requirement fulfilment, age, sex, breed, behavioural changes due to absence of the dam in artificial rearing, time of pellet feeding and/or composition of milk replacer and pellets. Research would require a series of experiments controlling each of the possible factors while monitoring lamb pellet intake and growth.

Early solid feed intake improved rumen development and provided adequate protein to energy for lambs. Thus, approaches to increase solid feed (pellets and/or pasture) intake at the early stage of pre-weaning of lambs needs to be evaluated. Possible approaches would be the examination of the effects of time of solid feed introduction, composition and digestibility of pellets, rearing of lambs with adult animals, rearing system, age at weaning on the pellet intake of lambs.

Although a greater growth rate pre-weaning was observed with increased CP:ME intake (Chapters 3 and 4), and this would bring benefits to farmers in terms of early lamb growth, however, increased CP:ME intake on longer-term impacts in replacement stocks are unknown. Therefore, it would be useful to explore the long-term impacts of greater pre-weaning growth rates and early weaning on subsequent lamb growth, milk production and reproductive performance of replacement ewe lambs. This would require ewe lambs to be reared and then monitored over a long period and their performance recorded. The present studies were focused on male lambs only.

Lambs' small intestinal development was affected by the CP:ME ratio of milk replacer (Chapter 3) and early milk weaning (Chapter 5). However, it is unclear which mechanism/s drive this development and what the effects of changed empty weights of the small intestine are on anatomy and the nutrient absorptive capacity of lambs. Thus,

further work on intestinal development with different dietary treatments and weaning age is warranted. This would be determined by histology examination of villi development and nutrient absorptive surface area in the small intestine of lambs given the specific dietary or management treatment.

Early weaned lambs (Chapter 5) may have had higher energy expenditure for GIT development and protein synthesis and consequently, resulted in lower fat deposition. The energy used by lambs to excrete excess nitrogen intake could be a possible reason as to why lambs fed a milk replacer with a high protein to energy ratio have less fat. Ammonia produced in the rumen needs to be transported to the liver and converted to urea and excreted (Owens and Basalan, 2016). Therefore, gene expression related to rumen ammonia transporters in the rumen and urea cycle in the liver could have been altered, which would warrant the exploration of any changes in gene expression rumen and liver tissues.

Rumen fermentation and papillae morphology were altered by pellet fibre level and early weaning (Chapters 6 and 7). It is also well established that there are positive influences of VFA on papillae development. Thus, gene expression of rumen tissues responsible for fatty acid metabolism, VFA transportation, cell differentiation and epithelial cell development and cell apoptosis of lambs might have changed with respect to pellet fibre level and weaning age. Consequently, genes involved in gluconeogenesis in the liver may have been altered, but this has not been evaluated to date. Therefore, the effects of pellet fibre level and early weaning of lambs on gene expression of rumen tissue and liver need to be investigated. In addition, there appears to be no information on direct nutrient-gene interactions related to regulatory pathways of rumen development. So, future

research is needed to better understand the molecular and nutrient direct interactions on the rumen development process.

Chapter 6 found a positive correlation between empty rumen mass and rumen papillae height. The greater papillae development improved nutrient absorption in the rumen. However, the effects of rumen weight on the digestive and absorptive capacity of the rumen are not well understood (Baldwin and Connor, 2017). Thus, further work on the effect of rumen mass changes on nutrient absorption in the rumen and the use of empty rumen mass as an indicator of rumen papillae development would be of interest. Chapter 6 also found that there was a correlation between dietary nutrient intake and rumen mass, but mechanisms were not obvious, therefore, this also warrants further investigation.

Ultrasonographic examination of lamb rumen development demonstrated a positive correlation between rumen wall thickness measured via ultrasound and papillae height measured by histological study. Ultrasonographic scanning is a real-time, non-invasive method (Crilly et al., 2017), which potentially could be used to investigate rumen development. Thus, future studies should utilize ultrasonographic examination to determine morphological changes in the rumen, such as size and wall thickness in different sacs of the rumen, at different ages in the same animal, in adapted feeding programs, early weaning strategies and rearing systems to ensure wider application.

In Chapter 7, the rumen microbiota of the dam was not characterized, but it is known that the rumen microbial community of neonatal ruminants is affected by microbial sources provided by the dam (Yeoman et al., 2018). Therefore, further studies on rumen microbial establishment in the new-born ruminant and alterations with diet interventions should require the analysis of the microbiota (e.g. faecal, oral, vaginal) of the dam, especially if the offspring was naturally born and/or nursed by its dam during the first few days of life.

Moreover, there have been limited recent studies to clarify the growth substrates of the rumen bacteria, their rumen fermentation products and complex associations of the bacteria community and ruminal VFA content, warrant future studies. The effects of pellet fibre level on rumen branched-chain fatty acids content, and the relationship of branched-chain fatty acids with rumen bacterial composition and rumen function is also deserving of further exploration. Gaining insight into the complex associations in the rumen would improve feeding management and lamb performance.

It appears that metabolisable energy requirements for maintenance changed with lamb rumen development (Chapter 8). Therefore, it will be worthwhile to experimentally re-evaluate the pre-weaned lamb's metabolisable energy requirement for maintenance using defined feeding schedules to track the changes in metabolisable energy with gastrointestinal tract development. The model of Anim Jnr et al. (2020) could then be improved by adjusting the metabolisable energy requirements of lambs with rumen development which would improve body composition prediction by the model.

9.5 Implications of the results found in the current studies

Feeding of milk replacer with a higher protein to energy ratio and protein to energy ratio adjusted milk replacer to meet the requirement of lambs improve the lamb growth rate pre-weaning. Lamb growth rate is also increased by feeding protein to energy ratio improved milk replacer (12.28 g/MJ) when given in addition to pellets containing a high CP:ME ratio, which allows lambs to reach optimum CP:ME requirement by both milk replacer and pellet intakes. However, pellet intake is less at the initial lamb growth stage and varies between lambs. So, farmers should implement strategies to either encourage and ensure adequate pellet intake at the early stage of lamb growth or provide milk replacer with a higher protein to energy ratio until lambs boost their pellet intake.

Feeding of either high or low fibre pellets introduced at early as four days of lamb age and milk weaning of lambs at 42 days of age does not impair the growth rate of lambs reared to 57 days of age and result in a leaner carcass. Further, the inclusion of a source of lactose (e.g. skim milk powder) in pellet improves butyric acid production, which enhances the rumen papillae development. This would assure a smooth transition of lambs from a milk-based diet to a solely solid diet, due to improved pellet intake and rumen development.

Considering the feed cost associated with rearing lambs, feeding of a higher protein to energy milk replacer and pellets benefits the farmers due to the greater pre-weaning lamb growth, which reduces the number of days to reach the target weight or heavier weight at a fixed age and therefore should reduce overall costs. Although feed cost was lowest in early-weaned lambs, they would need to be fed a solid feed for a longer period to reach the target live weights, which would increase costs associated with lamb management and labour. Therefore, overall, these results suggest farmers are better to feed the lambs with protein to energy improved milk replacer with *ad libitum* pellets if they wish to ensure fast lamb growth rates and lower overall rearing costs.

Validation of the model of Anim-Jnr et al. (2020) suggests that the model can be used to predict the overall pre-weaning lamb growth accurately based on the feed composition and intake. However, the model of Anim-Jnr et al. (2020) lacks accuracy for the pre-weaned body composition. Therefore, if farmers use this model to predict the lamb's growth, they are required to have feed intake and feed composition data during the pre-weaning period.

9.5.1 List of recommendations based on the current studies

The following guidelines are suggested for the farmers to achieve cost-effective artificial lamb rearing.

- Lambs need to be provided ewe's colostrum for the first two days postnatal.
- In artificial lamb rearing, it is recommended to:
    - feed lambs with CP:ME ratio improved milk replacer (12.28 g/MJ) at 2.1 times their maintenance requirement from two days of age lambs until milk weaning,
    - provide *ad libitum* access to pellets (NDF 117 g/kg) and free access to water from the start of artificial rearing,
    - provide lucerne chaff when lambs have considerable pellet intake (approximately 5 weeks of age),
    - practise gradual milk weaning of lambs starting at 38 days of age and fully milk weaned by 42 days of age, and
    - provide *ad libitum* pellets, lucerne chaff and free access to water post-weaning.

9.6 Conclusions

The lamb rearing experiments undertaken in this thesis allowed the following conclusions to be drawn.

- Adjusting the crude protein and metabolisable energy content of milk replacer to match the lamb's requirements and high protein to energy milk replacer feeding increased pre-weaning lamb growth rates.

- Feeding of lambs with improved protein to energy milk replacer (12.28 g/MJ) with *ad libitum* access to pellets, with a high protein to energy ratio, resulted in similar pre- and post-weaned lamb growth rates as protein to energy-adjusted milk replacer feeding.
- Body fat content was reduced by feeding milk replacers with a high protein to energy ratio.
- Early weaning of lambs at 42 days of age did not impair growth, but reduced body fat deposition.
- Early weaning and pellet fibre levels altered rumen fermentation, papillae development and bacterial community composition.
- Feed cost per kilogram of live weight gain was lowest in lambs fed milk replacers with improved protein to energy ratio and *ad libitum* pellets
- The lamb growth simulation model can be applied to estimate the pre-weaned lamb growth performances with acceptable accuracy, but not for body composition.

Collectively, the findings of the thesis advance the knowledge on how the composition of milk replacer and pellets and age of milk weaning impact the lamb growth, body composition and rumen development in artificial rearing. Apart from its direct value in providing information to optimise newborn lamb rearing strategies in artificial rearing systems, the research in this thesis provided a basis for future research.

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Appendices

Appendix 1 Pearson correlation coefficients of dietary factors and rumen metabolic development parameters of artificially reared lambs given three rearing treatments (DMI, dry matter intake; ME, metabolisable energy; CP, crude protein; ADF, acid detergent fibre; NDF, neutral detergent fibre)

Parameter	Acetic, mg/g	Propionic, mg/g	n-Butyric, mg/g	n-Valeric, mg/g	n-Caproic, mg/g	Acetic:Propionic	$PC1_{(md)}$	$PC2_{(md)}$
Total DMI from pellets, kg	0.04	0.23	0.33	0.40	0.29	-0.38	0.21	-0.03
Total DMI from lucerne chaff, kg	0.23	0.30	-0.01	0.29	-0.04	-0.04	0.27	0.10
Total DMI, kg	0.05	0.24	0.32	0.40	0.28	-0.37	0.22	-0.02
Total ME intake, MJ	0.04	0.23	0.32	0.39	0.28	-0.38	0.21	-0.03
Total CP intake, g	0.04	0.23	0.32	0.39	0.27	-0.38	0.21	-0.03
Combined CP:ME intake, g/ MJ	-0.22	-0.19	-0.30	-0.41	-0.32	-0.14	-0.27	-0.18
Organic matter intake from pellets and lucerne chaff[1], kg	0.05	0.24	0.33	0.41	0.29	-0.37	0.23	-0.02
Total ADF intake, g	0.06	0.23	0.22	0.31	0.15	-0.33	0.20	-0.01
Total NDF intake, g	-0.01	0.16	0.19	0.23	0.12	-0.35	0.12	-0.06
Total hemicellulose intake[2], g	-0.04	0.12	0.17	0.18	0.10	-0.35	0.08	-0.08

[1] Calculated as total dry matter intake from pellet and lucerne chaff minus ash intake of respective feedstuff.
[2] Calculated as total NDF intake minus total ADF intake.

Appendix 2 Pearson correlation coefficients of rumen metabolic and physical development parameters of artificially reared lambs given three rearing treatments

Variables	Empty rumen weight, g	Rumen volume, ml	Papillae height Dorsal, μm	Papillae height Ventral, μm	Papillae width Dorsal, μm	Papillae width Ventral, μm	Muscle layer thickness Dorsal, μm	Muscle layer thickness Ventral, μm
Acetic, mg/g	-0.04	0.20	0.33	0.11	-0.01	0.09	-0.09	0.22
Propionic, mg/g	0.19	0.31	0.49*	0.23	-0.13	0.28	-0.16	0.19
Iso-Butyric, mg/g	0.35	0.30	0.48*	0.14	-0.04	0.43*	-0.14	-0.28
n-Butyric, mg/g	0.22	0.17	0.61*	0.23	-0.26	0.17	-0.26	0.10
Iso-Valeric, mg/g	0.31	0.24	0.42*	0.10	-0.05	0.36	-0.11	-0.33
n-Valeric, mg/g	0.28	0.35	0.48*	0.21	-0.17	0.25	-0.21	0.08
n-Caproic, mg/g	0.07	0.001	0.22	0.33	-0.18	-0.14	-0.05	0.09
Acetic: Propionic	-0.48	-0.12	-0.16	-0.22	0.18	-0.39	0.09	0.20

* Significant at confidence level of 0.05.

Appendix 3 The concordance correlation coefficient (CCC) and relative prediction error (RPE) values of the Anim-Jnr et al. (2020) model performance for the protein, fat, water and ash deposition predictions in the body of lambs in each treatment of three experiments, which had different milk replacer and pellet feeding and age at weaning combinations.

Treatment[1]	n[2]	Protein deposition, g/d			Fat deposition, g/d			Water deposition, g/d			Ash deposition, g/d		
		Paired *t*-test[3]	CCC[4]	RPE[5] %	Paired *t*-test[3]	CCC[4]	RPE[5] %	Paired *t*-test[3]	CCC[4]	RPE[5] %	Paired *t*-test[3]	CCC[4]	RPE[5] %
NMNP'17	10	-1.39	0.41	5.56	-8.66	0.03	73.70	4.60	0.05	12.53	2.23	0.03	28.44
MBNP'17	8	-1.36	0.13	8.16	-9.80	0.05	71.51	7.10	0.02	15.88	1.49	-0.12	19.92
HPMNP'17	9	-2.21	-0.24	9.60	-13.69	0.08	108.76	5.95	0.02	16.45	1.10	0.02	21.72
NMHF57'19	8	-3.99	0.45	15.98	-5.82	0.15	180.18	-6.79	0.24	21.84	-5.38	0.04	21.55
NMHF42'19	9	-10.31	0.56	23.44	-3.92	0.24	165.57	0.63	0.48	11.59	-1.05	0.28	20.53
HPLF42'19	7	-15.08	0.09	41.10	-1.94	0.32	60.60	-1.82	0.70	13.90	-3.77	0.55	20.68

[1] NMNP'17- commercial milk replacer and normal pellets, 2017 experiment; MBNP'17 - milk blend and normal pellets, 2017 experiment; HPMNP'17 - high protein milk and normal pellets, 2017 experiment; NMHF57'19 - commercial milk replacer and high fibre pellet feeding to 57 days of age, 2019 experiment; NMHF42'19 - commercial milk replacer, high fibre pellet and early weaning, 2019 experiment and HPLF42'19 - high protein milk replacer for two weeks followed by commercial milk replacer, low fibre pellet and early weaning, 2019 experiment.

[2] Number of lambs in each treatment.

[3] Paired *t*-test value for analysis of significant differences between the model simulated and actual value of each parameter.

[4]A concordance correlation coefficient (CCC) value between 0.81-1.00 indicates an almost perfect prediction, 0.61-0.80 a substantial prediction, 0.41-0.60 a moderate prediction and 0.21-0.40 a fair prediction (Fuentes-Pila et al., 1996, Visser et al., 2012).

[5] A Relative prediction error (RPE) value <10% indicates a satisfactory prediction, between 10-20% an acceptable prediction and >20% an unsatisfactory prediction (Fuentes-Pila et al., 1996, Fuentes-Pila et al., 2003, Anim-Jnr et al., 2020).

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Animal Feed Science and Technology

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- Describe the contribution that the candidate has made to the manuscript/published work:
Gayani collected all the data, completed all the analyses and prepared the manuscript.

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