Effects of the protein source and content in milk replacers on bone and organ growth in lambs artificially reared from birth until six weeks of age.

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ABSTRACT.

The objective of this study was to investigate if preweaning diets differing in protein source and content altered growth rates as well as bone and organ development in lambs artificially reared from birth until six weeks of age. Lambs removed within the first 48 hours from the dam were randomly allocated to one of the two rearing treatments; MP: milk-protein based milk replacer or WVP: whey and vegetable-protein based milk replacer. Four lambs from each treatment were slaughtered at two weeks of age while the remaining lambs were slaughtered at six weeks of age. Lamb live weight at two weeks of age did not differ ($p > 0.05$) between treatments. There were differences in bone morphology and organ growth, although milk protein-based milk replacer (MP) was associated with greater lamb live weight and average daily gain at six weeks of age ($p < 0.05$). From birth until six weeks of age, the type of milk replacer had no effect on stature measures and bone morphology ($p > 0.05$), meaning that early lamb bone growth is influenced by increases in live weight rather than the type of milk replacer used. Lamb development is driven by live weight gain however, lambs were observed only from birth until six weeks of age, which has limited the observation of live weight gain and thus, changes in bone morphology and organ weight. The differences in the preweaning diets may have altered the future developmental potential growth of bone and organs in lambs but this needs further investigation.

REVIEW OF LITERATURE.

Introduction.

In mammalian species, most growth occurs before puberty and, for many species, the period of most rapid development is the infantile growth phase, which for lambs, represents the period up to weaning (Gibson et al., 2022a, Reis et al., 2001). Early life nutrition in ruminants plays an important role in preweaning growth as well as in the development of bone and organs.

Preweaning growth rates are driven by lamb feeding management and the type of liquid feed used in artificial rearing systems (i.e., restricted milk amounts vs *ad libitum* milk replacer, ewe milk vs milk replacer) (Mialon et al., 2021). While the earliest solid feed introduction combined with restricted amounts of milk replacer is the best to promote rumen development (Danso et al., 2014, Morgan et al., 2007), early lamb performance also relies on the nutritional composition of the milk replacer used (i.e., protein source and content) (McCoard et al., 2021). Lamb milk replacers can be either milk protein-based or plant protein-based, with the main difference between them being the digestion in the pre-ruminants' abomasum. While milk protein-based milk replacer is capable of curding, plant protein-based milk replacers may lead to more health issues due to scouring (Longenbach and Heinrichs, 1998). A high ratio between crude protein and metabolizable energy (CP:ME ratio) in the milk replacer fed ensures greater lamb live weight gain and provides a greater protein amount, which optimises lamb bone growth (Gibson et al., 2022a). In contrast, nutritional deficiencies (i.e., protein deficiency) or malnutrition preweaning can result in reduced body mass, impaired bone growth and increased fracture risk as for first lactation dairy heifers with early osteoporosis leading to spontaneous humeral fractures (Gibson et al., 2022a, Shamay et al., 2005). While some research about protein content in milk replacers and its effects on lamb growth have been conducted (Danso et al., 2018, Herath et al., 2020), there is only limited research examining the effects of protein source in milk replacers preweaning on bone growth and organ development of lambs later in life.

As protein represents 50% of the total bone volume and around one third of bone weight, optimal bone growth relies on sufficient dietary protein intake (Wallace, 2019). To examine bone growth and assess bone health, peripheral quantitative computed tomography (pQCT) scans, a non-invasive, safe imaging modality, are used to measure bone morphology in live animals, as in humans (Weatherholt et al., 2015). To describe bone growth and muscle development in animals and humans, pQCT is generally performed on mid-tibia because it responds to load changes over growth and is easily accessible, compared to other bones (Stagi et al., 2016). An experiment on bone morphology in sheep demonstrates that the mid-tibia gives access to both bone and surrounding muscle without necessarily having to euthanize the animals (Gibson et al., 2022b).

Delayed bone development caused by undernutrition preweaning (i.e., lack of pasture supply in winter) can be compensated for if the rearing management of ruminants provides for all the animals' requirements after the growth check until the end of the rearing period. However, differences in live weight gain and growth rates remain similar and may affect future production of ruminants (i.e., puberty onset, lactation) (Shamay et al., 2005). Therefore, the aims of this thesis were (1) to examine the effects of the content and source of dietary protein on lamb growth rates and bone growth preweaning and (2) to observe the possible effects of preweaning diets on early organ growth in artificially reared lambs. The main objectives of the work presented in this thesis were:

- To determine the effects of the content and source of dietary protein in milk replacers on lamb bone growth preweaning.
- To observe how early organ growth of lambs is influenced by the content and source of dietary protein in milk replacers.

1. Lamb rearing systems in New Zealand.

In New Zealand, the sheep industry has approximately 27.4 million sheep, with lamb international trade contributing $3.8 billion to the New Zealand economy (Beef+LambNZ, 2020, Hall, 2023). Sheep farming can be either extensive on hill or high-country areas or intensive typically in combination with beef farming on highly productive lowlands (i.e., for an area of 4.5 million hectares, it represents around 8.6% sheep on high country against 91.4% sheep on lowlands) (Morris, 2013).

There are two different lamb rearing systems, natural and artificial rearing systems. While in natural rearing systems, lambs are reared by the dam, artificial rearing systems use liquid feed (i.e., whole milk, milk replacer, milk from alternative species) typically supplemented with pellets or meal to meet maintenance and growth requirements of lambs (Jensen et al., 2017). The objective of lamb rearing (i.e., raising lambs to slaughter or dairy replacement stock) and the rearing system used are important factors for farmers to maximize profit (Nieper et al., 2017, Peterson and Prichard, 2015).

While in Europe and America, dairy lambs are mostly artificially reared, in New Zealand, lambs were traditionally reared by the dam, with the exception of specific cases (i.e., orphan or multiple lambs), because lamb artificial rearing was considered uneconomic due to the use of labour, infrastructure and expensive food supplements (David et al., 2014, McKusick et al., 2001, Nieper et al., 2017, Smith and Geenty, 1983). However, the recent development of the New Zealand dairy sheep industry has led to the use of large-scale artificial rearing systems for lambs (Nieper et al., 2017).

In New Zealand dairy (milk harvesting) lamb rearing systems, lambs are typically removed from ewes within two days of birth to maximise saleable milk (Peterson and Prichard, 2015, Nieper et al., 2017). If ewes reared their lambs, suckling lambs would reduce the total harvestable milk yield by 35-40% in the first month of lactation during a 170–180-day ewe lactation (McKusick et al., 1999). Hence, sheep farmers focus on low cost early weaning methods where costs depend on factors such as labour cost, the type of milk replacer used and the occurrence of health issues, whether related to the milk replacer fed or not (Caroprese et al., 2010, McCoard et al., 2021, McMillan et al., 2014).

1.1. Natural rearing.

From birth until weaning, lambs naturally reared live with their dam and have unrestricted access to both their dam and pasture (Gibson et al., 2022b). Lamb suckling frequency is high from birth until two weeks of age, with approximately eight suckling events in a six-hour period (Fletcher, 1971). From two weeks of age, as lambs start eating solid feeds and the rumen develops, lamb suckling frequency decreases and continues to decrease until weaning (Belanche et al., 2019, Fletcher, 1971). Natural rearing is most commonly used in meat production systems (Belanche et al., 2019), where dam's milk is not sold as a product.

1.2. Artificial rearing.

1.2.1. Artificial rearing systems for lambs.

Overview of lamb artificial rearing.

Artificial rearing involves the separation of lambs from their dams and hand-rearing them. Artificial rearing is used when ewes are unable to rear the lamb or have rejected the lamb (Umberger, 2009, Urbano et al., 2017). In artificial rearing systems, lambs are often kept indoors from birth to three weeks of age (Jensen et al., 2017). At four to five weeks of age, a gradual pasture transition can begin, with access to milk replacers and solid feeds (pellets or meal). Lambs are generally milk-weaned between six and eight weeks of age and solid feeds are removed at 10-12 weeks of age to finish transitioning lambs to a complete pasture diet (Khan et al., 2016).

One disadvantage of artificial rearing systems is the occurrence of digestive issues such as abomasal bloat and scouring (Umberger, 2009). Abomasal bloat is a diet-induced health issue that can occur in artificially reared lambs between two and four weeks of age (Bull and Binnie, 2006). If not detected quickly, abomasal bloat can result in serious health issues, or even death, within half an hour of feeding. Feeding lambs milk replacer is also associated with scouring occurrence caused by poor digestibility (Dunière et al., 2023, McCoard et al., 2021), which leads to an increase in labour requirements due to more frequent feeding and milk replacers high cost.

Feeding systems & types of feeds in lamb artificial rearing.

Although the artificial rearing system used is dependent on the number of lambs and farmer preferences (Martin et al., 2010), lambs should be provided with suitable milk replacers and solid feeds to support an efficient and healthy lamb growth (Owen and Davies, 1970). Liquid feed (e.g., milk replacer, whole milk) can be given by bottle feeding, milk/teat bars, multi-nipped containers and automated feeders (Gorrill et al., 1990, Martin et al., 2010).

During the first 18-24 hours of life, colostrum feeding of lambs is crucial and ensures appropriate nutrition and immunity status of lambs (Banchero et al., 2004). If the lamb is rejected by the dam or if the ewe does not produce sufficient colostrum, cow colostrum or colostrum replacers can be fed (Umberger, 2009). Without equalling the effects of ewe colostrum, feeding lambs with a colostrum alternative followed by milk replacer in artificial rearing systems is an adequate option to prevent health issues and results in similar preweaning growth rates and weaning weights with natural rearing (Belanche et al., 2019).

After 24 hours of life, artificially reared lambs are provided with liquid feed (i.e., milk or milk replacer). There is variation in composition between ewe milk and milk replacer due to differences in the source of protein and brand recipes. The ideal milk replacer composition for artificial lamb rearing should be composed of 22-24% of crude protein, 25-35% of crude fat, 0.5-1% of crude fibre, 22-25% of lactose, 5-8% of ash and vitamins (20000, 5000 and 50-100 IU per pound of vitamin A, D and E respectively), which is similar to cow's milk composition (Umberger, 2009). Artificially reared lambs are mostly fed with milk replacers that are based on cow, buffalo, goat or sheep milks (Anjum et al., 2014, Emsen et al., 2004, Kintzel, 2014, Thompson et al., 1993). At the same time, artificially reared lambs are also provided with solid feed meals such as lamb starter to promote early rumen development (Baldwin et al., 2004).

1.2.2. Effects of artificial rearing on preweaning lamb welfare.

Artificial rearing systems affect lamb welfare : negatively related to emotional stress and reduced growth rates for the animals or to improved welfare as lambs may die if rejected by their dam (Napolitano et al., 2008). However, if the lamb was separated too early after birth from its mother (< 12 hours), it probably did not have sufficient time to feed, resulting in a reduced immunity status and thus, lowered growth rates (Dunière et al., 2023).

Adverse effects of artificial rearing systems on lamb welfare can be lowered through social interactions with other lambs or adult sheep (Napolitano et al., 2003) and with human carers (Carbajal and Orihuela, 2001). The presence of adult animals allows the young ones to learn about feeding habits and behaviours (De Paula Vieira et al., 2012). In addition, interactions with human carers lead to easily handled animals and decreased stress rates during management practices involving humans (Pascual-Alonso et al., 2015). Similar observations have been made in artificial calf rearing (Lensink et al., 2000).

1.3. Other factors affecting lamb growth.

Lamb growth rates can be affected by factors related to ewe physiology. Primiparous ewes produce smaller lambs than multiparous ewes due to lamb birth weight being proportional to the maternal live weight (Dwyer and Lawrence, 2000). Moreover, birth rank (single or twin) does not significantly affect lamb birth weight in multiparous ewes but results in lighter weights for twins from a primiparous ewe (Pettigrew et al., 2019).

Nutrition of ewes, either in deficit or in excess, during late gestation can affect foetal development as well as lamb postnatal development (de Sousa et al., 2023). When fed at their requirements or in excess, ewes can maintain or gain body reserves and produce heavier lambs than ewes that needed to mobilise more resources (3.4% heavier when fed high CP:ME ratio) (de Sousa et al., 2023). In addition, ewes using less energy resources lead to more appropriate postpartum behaviours from both ewe and lamb (i.e., standing, suckling, vitality, early maternal behaviour), which leads to greater lamb birth weight (de Sousa et al., 2023, Dwyer, 2003).

The ease of delivery depends on the number of lambs and their presentation at parturition. While lambs deliver more easily when all joints are extended, it is possible that large single lambs or triplet lambs have an incorrect presentation due to a lack of space resulting in birth difficulty for the ewe and reducing postnatal lamb appropriate behaviours (i.e., lack of oxygen due to a difficult birth can lead to a lack of vitality) (Grommers et al., 1985). The occurrence of lamb malpresentations depends on the sheep breed and lamb sex (i.e., Suffolk, male lamb) (Dwyer, 2003). Therefore, neonate lamb status and its future performance are significantly affected by ewe's health conditions and performance, and by birth-related factors (e.g., birth difficulty, birth rank, ewe parity and nutrition).

2. Feeding diets for artificially reared lambs.

2.1. Rumen development in pre-ruminants.

At birth, rumen development lacks due to the effective milk shunting directly to the abomasum by the reflexive closure of the reticular groove (Orskov et al., 1970). Thus, a pre-ruminant solely relies on its own digestive enzymes, found in the abomasum, to digest liquid feeds as its rumen is not developed yet (Baldwin et al., 2004, Longenbach and Heinrichs, 1998). This highlights a physiological need for curding in the abomasum to fully absorb dietary nutrients. The lack of curding can cause a rapid flow of undigested protein into the small intestine (McCoard et al., 2021). The absence of curd formation is considered as a primary cause of diarrhoea in pre-ruminants (Cruywagen et al., 1990). In addition, the rapid emptying of the abomasum can provide a favourable medium for pathogenic bacteria overgrowth (i.e., coliform bacteria) in the duodenum, resulting in diarrhoea (Roy, 1980).

Approximately seven days after birth, pre-ruminants can be introduced to solid feeds (i.e., cereal-based feeds, forage and/or pasture) to stimulate rumen development (Drackley, 2008). Providing pre-ruminants with solid feeds develops their ability to digest and absorb nutrients from feed as well as ensures a smooth transition from liquid to solid feed. Rumen capacity, mostly rumen muscularization and volume, are promoted and increased by forage intake while concentrate intake stimulates the growth of rumen papillae in length and size (Xiao et al., 2018). While the earliest solid feed introduction is the best, the recommended age for the introduction of solid feeds differs among studies, varying from two to ten days after birth (Danso et al., 2014, Liu et al., 2016, Sun et al., 2018). However, late exposure to solid feeds generates delayed growth rates and lower weaning weights (Liu et al., 2016).

By three to four weeks of age, the secretion of digestive enzymes in the abomasum becomes restricted, which limits the digestion of carbohydrate, fat and protein (Cruywagen et al., 1990). This means that nutrient absorption is not dependent on enzymatic digestion because the rumen is sufficiently developed. Thus, curd formation is no longer essential and pre-ruminants may show similar growth rates, regardless of the liquid diet fed until weaning (Longenbach and Heinrichs, 1998).

2.2. Preweaning phase.

There are several milk types to feed artificially reared lambs preweaning such as ewe milk, milk replacers or milk from alternative animal species. The main difference between these feeding options is their chemical and nutritional compositions, which results in different live weight gain and growth rates in lambs (Anjum et al., 2014).

Ewe milk.

Feeding artificially reared lambs with dairy ewe milk, instead of milk replacer, can partially mitigate the negative effects of artificial rearing, although it does not equal the beneficial effects of ewe nursing (Mialon et al., 2021). Lambs reared by the dam or artificially fed with free access to an automatic feeder have about nine meals per day, which can contribute to a greater growth rate than lambs bottle fed milk replacer. In contrast, common feeding practices in artificial rearing systems give lambs about four to six meals per day (McCoard et al., 2020), which might not be adapted to their natural suckling activity and digestive capacities (David et al., 2014).

In terms of growth rates, lambs artificially fed ewe milk do not show any specific advantage compared to lambs artificially fed milk replacer when fed at the same volume, which is probably due to lamb limited abilities to digest high amounts of fat contained in ewe milk during the first few weeks of life (Mialon et al., 2021).

In addition to having higher crude fat and crude protein contents than milk replacers, ewe milk has gut soothing properties due to the presence of β-casomorphin and casein-hydrolysed peptide contents (Blass, 1996). This results in artificially reared lambs fed ewe milk being calmer than lambs artificially reared on milk replacer, with a dirtiness of perianal region similarly reduced as in lambs reared by the dam, meaning that ewe milk is better absorbed than milk replacer by lambs. Due to gut soothing properties of ewe milk, lambs have a higher immune response after vaccination and show a stronger immune system postweaning, compared to lambs fed milk replacer (Gautier and Labussière, 2011, Mialon et al., 2021). This is due to the low rate of antioxidants present in milk replacers, compared to ewe milk (Abuelo et al., 2019).

Milk replacers.

Unlike ewe milk, composition of milk replacers does not fluctuate. Milk replacers generally contain higher levels of lactose and minerals than ewe milk (Azevedo et al., 2023, Glimp, 1972, Mialon et al., 2021). While the composition of ewe milk changes constantly during lactation phase, the constant composition of the milk replacer can increase the risk that lambs are not receiving correct nutrient requirements (Black et al., 1973). Thus, adjustments of milk replacers to meet lamb requirements and maximise growth rates are done through the feeding level given to pre-ruminants and the type of protein the milk replacer is based on (Mialon et al., 2021).

Milk replacers can be based on either milk protein or on plant-derived protein (Huang et al., 2015). Although some plant proteins (i.e., soybean protein or rice protein) are able to induce similar growth rates than whole milk protein, lambs fed milk replacers based on only hydrolysed wheat protein or peanut protein show impaired growth rates (Huang et al., 2015). Milk replacers based on whey and hydrolysed wheat proteins also result in lower average daily gain (ADG) and greater antibiotics use than milk protein-based milk replacers (McCoard et al., 2021). Thus, the choice of protein source in milk replacers is crucial for early diets of pre-ruminants.

The main difference between milk protein- and plant protein-based milk replacers is the digestion in the pre-ruminants' abomasum, as nutrient transfer from the abomasum to the small intestine depends on the characteristics of preweaning liquid diets (e.g., physical, chemical, digestibility) (Longenbach and Heinrichs, 1998). When pre-weaned lambs are fed with milk protein-based products (i.e., whole milk, skim milk), curds are formed then slowly digested and released into the small intestine. Plant protein-based milk replacers are non-curding products because they lack the curd forming casein protein and milk fat (Longenbach and Heinrichs, 1998). Therefore, plant protein-based milk replacers are often associated with decreased growth rates and increased health issues due to impaired nutrient digestion and absorption.

Milk from alternative animal species.

Feeding lambs with cow milk or milk replacer can result in a lower ADG compared to feeding ewe milk (Anjum et al., 2014) or rearing lambs on the dam (Gibson et al., 2022b). In contrast, lambs fed with buffalo milk (at 10% of their liveweight) show similar ADG and cumulative weight gain as lambs fed with ewe milk until 12 weeks of age (Anjum et al., 2014). This difference is probably due to the different compositions of milk alternatives and the digestive abilities of young lambs. Although milk replacers can result in more solid feed intake depending on the feeding level, which leads to a more developed rumen at weaning (Danso et al., 2014), buffalo milk remains a suitable alternative for artificially reared lambs (Anjum et al., 2014).

Solid feed intake.

Artificial rearing systems are commonly linked to an early solid feed intake (i.e., pellets, lamb starter, pasture) in addition to milk to minimize rearing costs. When exposed to solid feeds early (seven days of age), lambs can be weaned at 28 days of age, as rumen microflora developed quickly resulting in improved growth performance (Joyce and Rattray, 1970, Liu et al., 2022). Although pellets supplementation does not significantly affect lamb overall growth (Danso et al., 2014, Jensen et al., 2017), early pellet intake stimulates rumen papillae development, which is where digestion products are absorbed (van Houtert, 1993). This ensures a smooth transition from a poorly developed rumen preweaning to a mature digestive system at weaning, and results in continuous growth rates between preweaning and weaning (Khan et al., 2011).

When lambs are fed a restricted milk intake, they are not provided sufficient nutrition to meet both their maintenance and growth requirements and thus must compensate through increased solid feed intake (Jensen et al., 2017). Although restricted milk intake reduces growth rates in the early preweaning period (Morgan and Owen, 1973), lambs have a higher food conversion efficiency postweaning compared to lambs fed *ad libitum* milk replacer, given that they are provided with high-quality solid feed (Morgan et al., 2007).

2.3. Postweaning phase.

Postweaning growth and lamb finishing weight are influenced by various factors including preweaning growth rates, weaning weight and age, feed quality and quantity, and rumen development (Bhatt et al., 2009, Fraser and Saville, 2000). Postweaning diet is essential to ensure increased lamb growth rates and satisfactory bone and body developments. A preweaning diet that supports rumen development, associated with high-quality pasture being available postweaning are crucial factors to obtain optimum growth rates when rearing lambs (Khan et al., 2016, Morgan et al., 2007).

Pasture characteristics (i.e., herbage quality, quantity and type) should be considered to maximise postweaning performance of lambs. In New Zealand, lambs postweaning are primarily reared on ryegrass-based pasture. However, nutritional properties of pastures are affected by seasonal variation and may not provide sufficient nutrition to lambs postweaning, especially during summer (Beef+LambNZ, 2014). To maintain adequate postweaning growth rates, alternative summer herbage species are a solution (i.e., herb/clover mixed sward rather than perennial ryegrass-based pastures) (Golding et al., 2011).

2.4. Importance of CP:ME ratio in lamb nutrition.

The relationship between protein and energy is quite complex in ruminants due to ruminal metabolism (Lindberg and Jacobsson, 1990, Titgemeyer, 2003). However, greater lamb growth rates are associated with high CP:ME ratio and protein intake preweaning (Gibson et al., 2022a, Herath et al., 2020).

Lamb requirements in terms of CP:ME ratio.

Lamb requirements for crude protein (CP) relative to metabolizable energy (CP:ME ratio) change as it grows. A greater CP:ME ratio is recommended at the initial growth stage (i.e., 14.2 g/MJ at 5 kg of liveweight) to ensure optimal growth rates while during later growth stages, a lower CP:ME ratio meets lamb requirements (i.e., 12.2 g/MJ at 18 kg of liveweight), meaning that protein requirements decrease with growth and age (Danso et al., 2016).

During preweaning, a higher dietary protein amount results in greater lamb growth rates and may affect positively bone growth until peak bone mass at maturity (Gibson et al., 2022a). Later in life, while high protein intake can lead to excessive ammonia levels, which is toxic for ruminants and causes reduced milk production, health issues and even death, protein deficiency results in decreased ruminal activity, weight loss and reduced reproductive performance and milk production (DairyNZ, 2023). In addition, although little is known about lamb requirements of amino acids and their functional roles (McCoard et al., 2016), a deficiency of dietary amino acids, or protein, may be responsible for impaired immune function and increased occurrence of infectious diseases, resulting in decreased growth rates for lambs (Li et al., 2007).

Preweaned lambs generally have a greater proportion of protein deposition than fat in their live weight gain than mature sheep (Searle et al., 1972). This is due to the transition from a liquid to a solid feed diet representing a period of nutritional and digestive changes for lambs (Searle et al., 1972). ME requirements for maintenance are lower for animals that gain more protein than those that gain more fat (Luo et al., 2004). Thus, ME requirements for early growing lambs are lower than those of mature sheep. However, little information is available on ME requirements for lamb growth, which needs further research (Danso et al., 2016).

Effects of CP:ME ratios of milk replacers on early lamb growth.

Solid feed intake is typically higher in lambs fed a milk replacer with a low CP:ME ratio (11.8 g/MJ) than in lambs fed whole milk (i.e., ewe, buffalo or cow) with a CP:ME ratio higher than 13.7 g/MJ (Anjum et al., 2014). During the preweaning growth period, ADG does not differ significantly between lambs only fed high CP:ME ratio milk and lambs fed both milk replacer and pellets (Danso et al., 2014). However, when lambs are only fed same amounts of different milk replacers, the milk replacer with the highest CP:ME ratio results in greatest ADG (Herath et al., 2020). Yet, the ideal CP:ME ratio for ruminants, leading to an optimum growth rates, remains the one where protein levels are adequate (i.e., absence of protein deficit or excess) (Gabler and Heinrichs, 2003).

2.5. Effects of preweaning feeding diets on lamb growth rates from weaning.

Although different preweaning liquid diets do not influence live weight gain until lambs reach 10 kilograms (Hernández-Castellano et al., 2015), the greatest growth rates in pre-ruminants are observed during the preweaning period (Muir et al., 2003). At weaning and postweaning, lamb growth is affected by the type of milk used during preweaning, feeding level (or planes of nutrition) and weaning process. The ideal weaning timing is affected by factors that differ among farming systems, although the most common are live weight and solid feed intake (Greenwood et al., 1997).

The heavier animals are at weaning, the greater growth rates they will achieve postweaning, given that they are sufficiently provided. By improving lamb growth rates preweaning, farmers can choose between sending them directly to slaughter or fattening them postweaning (Schreurs et al., 2010).

2.5.1. Optimal weaning timing.

The optimal preweaning solid feed intake before weaning is crucial for postweaning performance and should not be below 1% of live weight for pre-ruminants (Greenwood et al., 1997, Kertz and Loften, 2013). Weaning calves at six weeks of age is too early according to their solid feed intake as a percentage of live weight, meaning that they will not be able to support their postweaning requirements (only 0.6% instead of 1%) (Greenwood et al., 1997). In contrast, calves weaned at eight weeks have higher solid feed intake preweaning and are able to maintain an appropriate ME intake postweaning, which is possibly due to greater gastrointestinal and metabolic development from higher solid feed intakes during preweaning.

2.5.2. Weaning strategies.

In terms of weaning strategies, farmers can either choose an abrupt weaning or a gradual weaning. For pre-ruminants fed high milk amounts and little solid feed, weaning strategy becomes critical because solid feed intake establishes ruminal fermentation and triggers metabolic and physical development (Baldwin et al., 2004). Although they had higher growth rates preweaning, calves abruptly weaned from high planes of nutrition resulted in reduced solid feed and ME intake postweaning as well as decreased live weight gain due to poor rumen development, compared to calves fed restricted milk amounts preweaning (Steele et al., 2017). In contrast, gradual weaning appears as an ideal solution to prevent any decrease in growth rates. The aim is to “step-down” slowly the milk amount offered with time to achieve optimum solid feed intake before the end of weaning (Khan et al., 2007).

Regardless of weaning strategies used, pre-ruminants are able to adapt their digestive physiology quickly to fit in with changes in nutrient type, as suggested by the minimal differences in ruminal morphology (rumen papillae) between calves abruptly and gradually weaned (Steele et al., 2017). However, calves gradually weaned show larger total rumen mass when full than calves abruptly weaned, suggesting a better adaptation to solid feed diet (7.83 kg vs. 6.02 kg) (Steele et al., 2017). Thus, weaning strategies are crucial to improve pre-ruminant welfare, health and growth postweaning.

3. Lamb performance & growth.

The New Zealand dairy industry is affected by a loss of its replacement heifers due to spontaneous humeral fractures. This condition was first observed in New Zealand in 2008 (Weston, 2008) and has affected approximately 4,620 cows nationally during the 2013/2014 lactation season (Hunnam et al., 2024). First lactation dairy heifers with spontaneous humeral fractures have osteoporosis, which is often associated with periods of inadequate feed quality, leading to decreased bone formation and increased abnormal bone resorption, severely affecting bone quality and strength in affected animals (Craig et al., 2016, Wehrle-Martinez et al., 2023b).

This study aims to examine how preweaning diets affect, in addition to pre-ruminant growth rates, bone development in order to determine whether preweaning nutrition may be the cause of spontaneous humeral fractures in first lactation dairy heifers. As sheep models are frequently used due to similar bone responses to protein deficiency with some mammals (i.e., cattle, sheep, humans) (Cabrera et al., 2018), using lamb models to examine nutrition effects on cattle bone growth, is relevant.

3.1. Bone.

Bone is a unique body component as it can be described both as an organ (macroscopic level) and a connective tissue (microscopic level) (Khan, 2001). In addition, bone protects important organs such as brain and spinal cord and is a site for muscle and tendon attachment (Weiner and Wagner, 1998). Overall, the skeletal system supports mechanical loads, gives a means of locomotion to the body and ensures movement and stabilisation using joints and ligaments (Sinclair et al., 2013). Each bone, especially long bones, is composed of two wider extremities (epiphyses), a tubular shaft in the middle (diaphysis) and an area between them (metaphysis) where bone formation occurs during growth and development (Khan, 2001) (Figure 1). While the diaphysis is designed to resist functional loading patterns of torsion and bending (Sinclair et al., 2013), the metaphysis contains a growth plate of hyaline cartilage called the physis. When an animal becomes mature, growth stops and this cartilage mineralises and is then replaced with an epiphyseal line (Mackie et al., 2008).

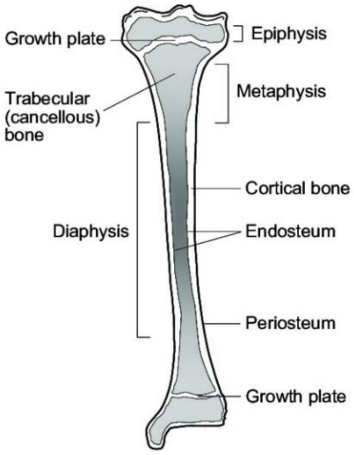

Figure 1. Schematic illustration of a growing long bone (Khan, 2001).

3.1.1. Structure.

Bone is organized into an organic matrix which determines the structure as well as the biochemical and mechanical properties of bone (Rho et al., 1998). 98% of the organic matrix of bone is composed of type I collagen and non-collagenous proteins while the remaining 2% consists of cells including osteoblasts, osteocytes and osteoclasts. These three types of cells are essential for the maintenance of bone homeostasis (Khan, 2001). Osteoblasts produce bone matrix and are responsible for bone formation. Osteocytes are mature bone cells which facilitate cellular communication and relay information about bone loading. Osteoclasts are responsible for bone resorption. Bone formation and resorption are closely related during bone remodelling as bone formation also increases when bone resorption increases (Harris and Heaney, 1969).

There are two types of bone tissues, lamellar and woven bone which are organized into compartments as either trabecular or cortical bone (Rho et al., 1998). Woven bone, also called immature bone, is randomly arranged with collagen, in contrast with the uniform structure of lamellar bone. The skeleton of newborns is fully composed of woven bone but later in life, woven bone is only found at sites of bone remodelling in response to extreme mechanical loads or fracture healing (Forwood and Burr, 1993). Lamellar bone is the bone tissue eventually replacing woven bone when the body grows.

3.1.2. Bone growth & development.

Two different mechanisms are involved in bone formation, intramembranous ossification and endochondral ossification. Intramembranous ossification represents the direct conversion of mesenchymal tissue into bone whereas in endochondral ossification, mesenchymal tissue is first converted into cartilage before getting converted into bone later in life (Bonucci, 1992). However, some bones, especially those associated with locomotion, are formed by both mechanisms, allowing simultaneous longitudinal and circumferential growth.

The main role of the skeletal system is to provide maximum strength with minimal mass to the animal and this is achieved through bone modelling and remodelling (Sinclair et al., 2013). While the animal is growing, bone must proportionally increase in strength and size to preserve bone strain within physiological limits (e.g., mechanostat theorem and Wolffs law) (Frost, 1987). Mechanical strain is the primary cause of bone modelling or remodelling occurrence (Hart et al., 2017). This is defined as a geometric measure of deformation representing the relative displacement between particles in material body. In bone development, mechanical strain occurs due to applied mechanical stresses as gravitational forces, muscle contraction or impact loading. Bone responses to mechanical strain depend on many factors such as the examined site, the animal's age/stage of maturation and the strain characteristics (e.g., environment, rate, magnitude, distribution, frequency, number of loading cycles, rest-recovery periods) (Hart et al., 2017).

3.1.2.1. Bone modelling.

Bone modelling is an organized bone cell process allowing bone growth and which adjusts bone strength through the independent activity of osteoblasts and osteoclasts (Frost, 1990). Modelling improves bone strength by expanding the endocortical and periosteal diameters of bone as well as adding bone mass. Signs of bone modelling include changes in shape with growth and a slow and continuous expansion of the periosteum and endosteum throughout adult life (Cordey et al., 1992). These changes are determined by the mechanical loading of the body and the growth stage (Sinclair et al., 2013).

3.1.2.2. Bone remodelling.

Bone remodelling is a mechanism where damaged bone tissue is removed and replaced by newly formed bone tissue. Remodelling is vital for calcium homeostasis and depends on the sequential work of osteoblasts and osteoclasts (Langdahl et al., 2016). Such a process is needed when microdamage affects bone structure and homeostasis. Microdamage often come from repeating loading cycles and activation of cellular responses responsible for the maintenance and adjustment of the bone matrix in order to preserve resistance to bone strain (Sinclair et al., 2013). During bone remodelling, damaged bone cells are resorbed by recruited osteoclasts before being replaced in equal amounts by new bone cells made up by osteoblasts (Cashman and Ginty, 2003, Langdahl et al., 2016, Frost, 1989). However, although secondary bone remains stronger than the damaged bone matrix replaced, it will never be as strong as primary bone formed during bone modelling (Sinclair et al., 2013).

When growth stops and peak bone mass is achieved, bone remodelling is balanced until later life, when bone loss occurs due to aging. While the body is aging, the ratio between bone resorption and formation becomes unbalanced and insufficient bone is formed to restore bone loss. Bone resorption only takes 10-20 days whereas bone formation takes three to six months (Cashman and Ginty, 2003). This is when bone abnormalities (i.e., osteoporosis) can occur as a result of a net bone loss and can compromise bone strength and mass (Khan, 2001).

Changes due to bone remodelling first occur in the microarchitecture of bone and then alter bone gross morphology, which represents a long process due to bone formation (Firth et al., 2011). Thus, during preweaning, observable changes in bone morphology related to remodelling are restricted or not quantifiable at a peripheral-quantitative-computed-tomography (pQCT) level until later in life, due to the young age of the animals and the slow mineralisation of bone (Gibson et al., 2020, Moallem et al., 2010). In addition, neither the preweaning diet nor the rearing system appear to affect bone growth of pre-ruminants prior to weaning (Gibson et al., 2020, Gibson et al., 2022b). Differences in bone development are more likely to occur from weaning until 600 days of age, when puberty ends and growth stops (Moallem et al., 2010, Shamay et al., 2005).

3.1.2.3. Compensatory growth of bone.

Compensatory growth, partial or complete, can be defined as the increase in growth rate that follows a period of nutritional restriction. The compensatory growth response of animals relies on the age at restriction, the duration and severity of nutritional restriction, and the own response of animals (Ryan, 1990). While the difference in live weight induced by preweaning diets persists over time, the lower measures of wither height, heart girth and hip width of animals undergoing nutritional restriction can be compensated for at different periods up to the first calving/lambing (Shamay et al., 2005).

In lamb artificial rearing, the lack of protein stimulates longitudinal bone growth without concurrent increases in live weight or girth size, compared to ewe reared lambs (Gibson et al., 2022b). This has also been demonstrated in bone growth of cattle, where the growth arrest can lead to a compensatory increase in height after the period of nutritional restriction, suggesting that the lack of protein in early pre-ruminant diets can result in taller animals in later life (Gibson et al., 2022b, Handcock et al., 2021).

Stature measures correspond to all measures related to the growth in size of animals (i.e., wither height, leg height, girth). When pre-ruminants are fed different amounts of liquid feed (i.e., restricted milk replacer (5L per day) vs *ad libitum* fresh milk offered in two 30-min time periods per day), differences in stature measures occur at weaning where calves fed twice a day had greater stature measures. However, if heifers are reared on the same management from weaning to first calving, differences in stature measures (e.g., wither height, heart girth and hip width) between preweaning diets become gradually smaller and are not significant anymore by approximately 600 days of age due to a compensatory mechanism for skeletal growth (Shamay et al., 2005). These results are similar with a study of Moallem et al. (2010) where heifer calves fed similar amounts of either milk replacer or whole milk showed differences in stature measures during the prepubertal period, which become gradually smaller until they faded at first calving. Thus, a delayed bone development due to periods of inadequate feed supply preweaning can be compensated for if the rearing management of ruminants postweaning supplies sufficient nutrition for optimal growth during the rest of rearing period (Shamay et al., 2005).

3.1.3. Bone to muscle relationship.

The development of bone and muscle is highly correlated such that an increase in activity of muscles (i.e., loading) or in muscle mass results in an increase in bone strength (Frost, 1987). Although changes in muscle precede changes in bone and occur approximately three to four time faster (Evans et al., 2012, Ireland et al., 2013), muscle tissue grows relatively more than bone tissue in a constant manner. Equal gains in bone: muscle ratio are related to proportional gains in muscle plus bone weight and thus, the rate at which bone: muscle ratio increases declines as total weight of an animal increases (Young, 1988). This effect may be exaggerated by increasing the contribution of fat or adipose tissue to live weight gain during further development. Local forces in muscle and bone developments also have a crucial role to ensure that both tissues respond to dynamic and static loading, with changes in length and diameter.

3.1.4. Factors affecting bone development.

Nutrition.

Nutrition is the primary factor affecting bone dynamics and development. From an adequate diet, an animal should be provided both sufficient energy and protein. Dietary energy leads to an increase in plasma insulin-like growth factor 1 (IGF-1) concentrations and a decrease in plasma GH (growth hormone) concentrations while dietary protein, in form of collagen, is responsible for 50% of bone volume and one third of bone weight (Wallace, 2019). Through an ideal diet, IGF-1 is released and stimulates an increase in muscle mass (Schüler et al., 2021), which in turn encourages appositional bone growth due to increased strain (Frost, 1987). In addition, early nutrition may restrict bone development but does not alter the proportions of bone or the ratio between bone and muscle, as suggested by bone length not being influenced by preweaning diet (Cake et al., 2006).

While a diet high in protein promotes an increase in protein deposition on the endosteal surface (Gibson et al., 2022a), an inadequate nutrition leads to bone changes and impairs bone growth, modelling or remodelling (Shamay et al., 2005). When an animal is not provided enough dietary energy, its plasma IGF-1 concentrations decrease and in case of severe deficiencies, growth arrest lines are observed (Brameld, 1997). Growth arrest severity is determined through the age of the animal, the phase of skeletal development and the duration the animal is exposed to restriction. While older and mature, animals are usually less impacted by growth arrest, the severity of growth arrest is more significant in younger animals due to their rapid growth rate and bone development, and their needs to meet both maintenance and growth requirements (Craig et al., 2016).

Among bone mass syndromes related to nutritional disorders, osteoporosis is the most common. Osteoporosis is defined as a reduction in bone quantity while bone quality remains intact, leading to weaker bone and increased risks of fractures without trauma. Osteoporosis cannot be detected using radiography or at necropsy unless bone loss is at least 30-50%, making it difficult to treat (Craig et al., 2016). Mechanisms that lead to osteoporosis include failure of bone formation (failure to achieve peak bone mass), excessive bone resorption and/or low bone turnover (Craig et al., 2016, Raisz, 2005).

The two main causes leading to osteoporosis are inadequate feed quality or excessive bone resorption (Seeman, 2003). Osteoporosis can be a consequence of animal starvation due to lack of pasture (i.e., drought) or reduced quality pasture (i.e., overstocking). The main nutritional deficiencies leading to a significant bone mass loss are calcium or copper. This calcium lack comes mainly from diets not meeting animals' requirements. For example, when animals are not provided enough dietary calcium during pregnancy and lactation, additional calcium can be taken from bone to offset this deficit (Reinhardt et al., 1988). However, this leads to a calcium deficit for a balanced bone remodelling and increases the risk of bone fractures because bone strength cannot be maintained (Langdahl et al., 2016). Excessive bone remodelling can also be a cause of bone destabilization, increasing strain of neighbouring bone regions (Langdahl et al., 2016). Thus, nutrition is a key factor for growth and bone development from the earliest age.

Seasonality of feed supply.

Unlike overseas countries, animal production in New Zealand relies on pastoral farming systems which is influenced by seasonality. In winter, when pasture growth is restricted, animals often need feeding supplementation to keep meeting both their growth and production requirements (Rattray et al., 2007). During this period (June to August), a growth check is commonly observed due to nutritional deficit (Handcock et al., 2016). A growth check, shown by the presence of a bone growth arrest line, is the primary consequence of significant and intensive periods of nutritional deficit (Craig et al., 2016).

In New Zealand, where animals are mostly seasonally grown, growth arrest lines are more likely to appear as a result of nutritional restriction than in overseas farming systems where growth rates are more linear, meaning adequate pasture and supplementation are given to animals throughout the year. The two critical periods of reduced growth rates in New Zealand dairy cows are the first winter at approximately nine months of age and the second winter between 22 and 24 months of age (Handcock et al., 2018). Both coincide with low pasture quality and quantity due to winter and are often responsible for the occurrence of growth checks.

Growth trajectories (e.g., linear vs. seasonal) do not affect height or length of 12-month-old heifers however seasonally grown heifers show lower girth circumference than those grown to live weight target (Handcock et al., 2021). By 15 months of age, girth difference is no longer significant but seasonally grown heifers are taller. This height difference is attributed to puberty age achieved 38 days earlier by heifers grown in linear growth rates than by heifers grown in a seasonal pattern (Handcock et al., 2021), as earlier puberty means an earlier end of longitudinal bone growth.

Loading.

Bone needs an adequate development *in utero* and during growth, which depends on mechanical stimulation and is essential for the development of strong weight-bearing bones. Bone changes, or deformations, are named "strain" and can be estimated as the changes in length compared to the original length (Elliott et al., 2016, Sinclair et al., 2013). Loading leads to bone adaptive changes that strengthen skeleton structure (Robling and Turner, 2009). As with muscles, bone mass increases when exercising and atrophies in the absence of motion.

Bone strength increases through greater bone size, thickness and density (Hart et al., 2017). As bone growth is positively affected by live weight gain (Gibson et al., 2020), the initial response to increasing loading on bone is to increase bone size (Firth et al., 2011). In addition, bone density can be affected by age and nutrition (Craig et al., 2016). Animals affected by reduced bone density tend to have a lower bone strength and a higher fracture risk. As cattle and sheep are not cursorial species and rely mainly on pasture grazing, they cannot achieve sufficient strain rates to encourage an increase in bone density in the distal limbs (Arens et al., 2007, Bijen et al., 2020, Logan et al., 2019).

When bone is under normal physiological conditions, a slow turnover occurs and both osteoblasts and osteoclasts are in homeostasis. In contrast, the lack of mechanical stimulation results in a poor development of weight-bearing bones (i.e., limbs), with only 30 to 50% of normal bone mass (Robling and Turner, 2009). Bones lacking mechanical stress (e.g., complete or partial immobilization) result in increased bone resorption and thus into a reduction of bone density and stiffness, making them more susceptible to fractures (Harada and Rodan, 2003). For example, a growing child without mechanical loading stimulation will develop fragile and thin long bones with a lower periosteal circumference whereas mechanical loading on rats forelimbs improved bone strength by 64% (Robling and Turner, 2009).

Puberty.

Puberty is defined as the transition from ovarian inactivity to regular ovulations (Hickson et al., 2011). In animal production, puberty onset relies more on mature live weight achieved than on age, meaning that growth rate is the key factor of puberty onset (McNaughton et al., 2002). Heifers need to reach 48-51% of their mature live weight before puberty onset (Handcock et al., 2021). In contrast, puberty onset of ewe lambs is driven by internal and external cues (Foster et al., 1985) and depends mainly on the animals age (6 to 12 months according to breed and month of birth in seasonal regions), the appropriate weight and metabolic state (when 40-60% of adult bodyweight is achieved, puberty occurs) (Pettigrew et al., 2019) and other factors including breed, nutrition availability and environmental factors (Grazul-Bilska et al., 2014, Junqueira et al., 2019). This difference between species is important as puberty onset means the end of bone growth for the animal (Riggs et al., 2002).

In early puberty, the increase in growth and sex hormones leads to the elongation of long bones, also known as a growth spurt, while in later stages of puberty onset, oestrogen encourages bone resorption and inhibits further appositional growth (Falahati-Nini et al., 2000). Oestrogen also triggers the calcium uptake from the intestinal tract and the retention of calcium in skeletal stores, which increases both bone strength and density (Riggs et al., 2002).

3.1.5. Measuring bone development.

Dual energy X-ray absorptiometry (DEXA).

DEXA is an efficient method to measure both bone mass, mineral content and mineral density. DEXA is a non-invasive and widely used measure due to its low radiation exposure and accuracy for positional and movement variation. However, while DEXA gives accurate bone mass measures, it cannot assess other essential elements of bone strength such as bone architecture or material properties. DEXA provides a 2-dimensional result rather than a 3-dimensional bone model (Gasser, 1995), meaning that it is unable to differentiate between cortical and trabecular bone (Choksi et al., 2018, Khan, 2001).

Three-point bending test.

To quantify the mechanical bone strength, the three-point bending test is a useful method. The three-point bending test is performed by applying force to the centre of a bone with the ends fixed until it achieves its breaking point. Such a method provides necessary data to calculate the elastic modulus, or resistance, of a bone (Walker et al., 2015).

Peripheral quantitative computed tomography (pQCT).

pQCT is a method used to understand bone development in terms of bone volumetric density, geometry and mineral status (Stagi et al., 2016). Bone density obtained via pQCT does not depend on bone size and provides a volumetric measure of bone density, which DEXA cannot do. Unlike DEXA, pQCT provides 3-dimensional results and can differentiate between cortical and trabecular bone (Weatherholt et al., 2015). pQCT is also a useful tool to assess the relationship between bone and muscle by providing information on the muscles surrounding the bone studied. However, pQCT scanners are restricted in the gantry size and so, only peripheral bones of living patients can be assessed. Because pQCT is more sensitive to positional and movement variation than DEXA, animals need to be anaesthetised (Stagi et al., 2016). This could limit the use of pQCT as immobilization is not always practical when working with living animals.

Stress-strain index (SSI) and measures using pQCT.

The stress-strain index, also known as the Young's modulus or elastic modulus, is a measure of the bone stiffness, or bone mineral density, and is defined as the ratio of stress to strain (Ferretti, 1995). The stress-strain index is not associated with live weight and treatment fed to the animals (Gibson et al., 2022b). Stress is determined by the force (i.e., dorso-palmar, lateral or torsional) applied to a bone divided by its cross-sectional area whereas strain is the ratio of the deformation (compression or extension) to the original length of the measured bone. At the same time as it prevents using destructive methods as the three-point bending test, SSI aims at determining bone strength using pQCT, which provides bone mineral density and geometry measurements. In contrast, periosteal circumference measures allow to calculate the total bone area, including bone marrow (Hasegawa et al., 2001).

Bone strength relies on four key elements, size, shape, architecture and composition (Choksi et al., 2018). Bone architecture (or morphology) is crucial as it is primarily affected by many bone disorders such as osteoporosis (Stagi et al., 2016). Therefore, pQCT is considered as an ideal tool to measure bone strength and morphology in heifers impacted by spontaneous humeral fractures and sheep are also studied to understand such a condition.

3.1.6. Radius and tibia development in pre-ruminants.

The radius, combined with the ulna, forms the antebrachium whereas the tibia, combined with the fibula, forms the upper hindlimb of animals. Among the first bones to be developed, both the radius and tibia are primary weight-bearing bones in the fore- and hindlimb respectively (Eurell et al., 2006).

As the radius and tibia are long bones, they develop through endochondral ossification, which occurs prior to birth (Martin, 2015, Succu et al., 2023). Endochondral ossification occurs in two stages, diaphyseal ossification and epiphyseal ossification. Diaphyseal ossification begins between the 7th and 12th weeks of gestation and is responsible for the growth in size on long bones (Martin, 2015). Before birth, cartilage is replaced by woven bone in turn replaced by lamellar bone after birth through bone remodelling. Then, the cartilaginous membrane of long-bone epiphyses persists until birth (Craig et al., 2016). Epiphyseal ossification only begins after birth and proceeds centrifugally in all directions, until the cartilage is replaced by secondary bone. However, an area of cartilage between the epiphysis and the diaphysis will not be replaced by secondary bone to form the metaphysis (Craig et al., 2016). The metaphysis allows bones to grow in length during growth and puberty.

Radius continue to grow after birth until 15 months of age, mostly at the distal growth plate in the radius in cattle (Bartosiewicz, 1984). In contrast, although the postnatal growth of the tibia in sheep appears restricted compared to the proximal limb (Gibson et al., 2022b), the epiphyseal closure of the tibia is complete and occurs at 42-48 months of age (Youssef et al., 2016). The tibia is the longest bone and grows proximally faster than distally with the highest mean values for length, diaphyseal diameter and diaphyseal circumference (Ahmed, 2008). Due to its growth characteristics and particular shape, the tibia can be used as a valid parameter to estimate foetal age by using ultrasound (Succu et al., 2023).

3.1.7. Lamb models to study cattle growth.

Although it has been demonstrated that spontaneous humeral fractures in dairy heifers are related to early osteoporosis resulting from a period of inadequate feed during growth (Wehrle-Martinez et al., 2023a), using cattle to determine the different effects of nutrition on bone growth results in high costs for the animals purchase and to run the trial (Gibson et al., 2022b).

Lamb stature measurements are significantly influenced by live weight (Gibson et al., 2022b), which is in agreement with the study of Gibson et al. (2020) where calves live weight was strongly related to bone measures. Furthermore, spontaneous humeral fractures in dairy heifers are associated with insufficient protein deposition during growth resulting osteoporosis (Dittmer et al., 2016). As results of bone response to protein deficiency are consistent between some mammals (i.e., cattle, sheep, humans), sheep models are also frequently used within human orthopaedic research (Cabrera et al., 2018). Thus, using lamb models to examine nutrition effects on mammals’ growth, especially cattle bone growth, is relevant.

3.2. Organs.

Organ growth is highly correlated with live weight, although organs grow at different rates than the body as a whole (Kirton et al., 1972).

3.2.1. Digestive organs.

As the growth of digestive organs is stimulated by solid feed intake (Danso et al., 2014), the digestive tract is highly responsive to changes in nutrition levels. Variations in form and type of nutrients provided to the gastrointestinal tract can alter the total nutrient use by the gut, cellular proliferation and nutrients available to support growth (Baldwin et al., 2004).

From birth until approximately 20 months of life, all digestive organs grow faster than the body as a whole in ruminants (Bailey, 1986, Kirton et al., 1972). The only exceptions growing more slowly than the body are the small intestine, which is an important absorption site (Dukes, 1947) and the abomasum grown enough at birth to deal with milk digestion (Wallace, 1948). However, organs specifically concerned with ruminant-type digestion (i.e., reticulo-rumen, omasum) grow and increase disproportionately, compared with the remainder of the digestive tract (Bailey, 1986). While the rumen undergoes the greatest growth rates (reaching almost twice that of the body in Romney sheep) (Kirton et al., 1972), the intestinal mass and metabolism change in response to changes in diet, especially changes in ME intake (Freetly et al., 1995).

Rumen development is crucial and ensures that young ruminants transition from relying on liquid diet to solid feed diet (Hamada et al., 1976). During weaning, the rumen increases from 30-70% of the capacity of the digestive tract while the other digestive organs decline as a percentage of live weight as the ruminant becomes mature (Warner et al., 1956). After weaning, diet does not affect the growth of the reticulo-rumen, indicating that nutrition does not regulate the relationship between live weight and reticulo-rumen weight. It is possible that an innate control mechanism ensures that the reticulo-rumen growth is high enough to maintain a high capacity, regardless of the current diet (Bailey, 1986).

3.2.2. Visceral organs.

Visceral organs are the internal organs of the body, specifically those within the thorax (i.e., heart, lungs, spleen) and the abdomen (i.e., gastrointestinal tract, pancreas, liver, kidneys, bladder, fat depots) but it also includes endocrine glands, brain and heart. In sheep growth, most internal organs and endocrine glands (apart from reproductive glands) grow more slowly than the body as a whole (Kirton et al., 1972). In contrast, most reproductive organs grow faster than the sheep body as a whole, which is expected because animals achieve their reproductive maturity at an advanced body size (Kirton et al., 1972).

The thoracic organs mature earlier than the digestive tract (Palsson, 1995), showing that the heart and lungs grow more slowly after birth than the rest of the body (Kirton et al., 1972). Heart weight and final animal body weight are highly correlated, suggesting that the heart might be involved more in body maintenance than in body growth (Hamada et al., 1976). In turn, the brain shows the lowest relative growth after birth and the spinal cord also grows slowly during postnatal phase. This may be because nervous tissue is among the earliest developing and is therefore already well developed at birth (Palsson, 1995).

Liver development is stimulated by solid feed intake and is highly correlated to rumen development (Danso et al., 2016). The liver has a major role as it must keep nutrients available to support animal growth and adapt to patterns of nutrients absorbed linked to rumen ongoing development (Baldwin et al., 2004).

The fat depots show the highest postnatal growth rates and grow much faster than the body with omental fat increasing at more than twice the body increase rate (Kirton et al., 1972). As live weight increases, fat is accumulated in the various depots in the following order: internal fat (mesenteric), intermuscular fat, subcutaneous fat and intramuscular fat (Gotoh et al., 2009). In addition, the pancreas grows at the same rate as the whole body, in contrast to most endocrine glands (Kirton et al., 1972).

3.2.3. Influence of nutrition on lamb organ growth.

As organ weights are not influenced by the CP:ME ratio of milk replacers prior to weaning, the type of milk replacer fed does not affect early organ growth of artificially reared lambs (Herath et al., 2020). However, nutrition of pre-ruminants influences organ development postweaning. Weight of particular organs (i.e., stomach, liver and intestines) differ postweaning according to the preweaning diet type (milk replacer vs. ewe milk; pellets or not) or diet quality (high-protein vs. low-protein) (Danso et al., 2016). In lambs fed both pellets and milk, increased weight of the gastrointestinal organs and liver are probably in response to the metabolic and absorptive requirements related to solid feed intake (Baldwin, 1999). Lambs fed only high-protein milk also show increased kidney and liver weights, compared to lambs fed low-protein milk, proving that visceral organs development is influenced by the diet type and quality preweaning.

4. Summary and implications.

Management of artificially reared animals is the primary key to ensure that high performance and growth rates are associated with reduced labour and rearing costs. While early life nutrition has only minor effects on organ development of lambs, it influences lamb performance and bone development from weaning until later in life. Thus, the choice of preweaning diet is critical.

Protein is one of the most important components of a preweaning diet and should be carefully chosen to increase the development of bone and organ as well as improve lamb growth rates. When not provided sufficient dietary protein, bone cannot reach adequate size and strength, which may result in a decreased bone: muscle ratio and increase the risk of bone disorders such as osteoporosis or spontaneous fractures. One significant example is spontaneous humeral fractures in first-lactation dairy heifers. In addition, the source of protein in milk replacers is another crucial component and need further research, as plant-protein based milk replacers often result in delayed growth rates and increased lamb health issues (i.e., impaired nutrient digestion and absorption, scouring) compared to milk-protein based milk replacers.

Therefore, the main objectives of the work presented in this thesis were:

- To compare the effect of different preweaning diets on lamb performance.

    - Hypothesis: Lambs fed milk protein-based milk replacer show greater live weight gain and growth rates than lambs fed plant protein-based milk replacer.
- To evaluate the effects of preweaning diets on bone development and organ growth from birth until six weeks of age in growing lambs.
    - Hypothesis: From birth until six weeks of age, the type of milk replacer fed does not affect lamb organ growth.
- To examine the effect of different protein sources and contents in milk replacers on lamb performance and bone growth.
    - Hypothesis: The source of protein in milk replacers affects lamb growth rates and early bone development.
    - Hypothesis: Lambs provided protein adequately or in excess preweaning show more developed bones at weaning.

MATERIAL & METHODS.

The experiment was carried out at Massey University, Palmerston North, New Zealand. Lambs were born at the start of September (spring) 2022. The research procedures used were approved by the Massey University Animal Ethics Committee (MUAEC 21/46).

1. Animal management.

1.1. Lamb management.

Twenty-one newborn lambs that were either mismothered or orphaned, were collected from Massey University farms and followed from birth until slaughter. Lambs were housed indoors and fed five times a day (8:00 am; 11:00 am; 2:30 pm; 6:00 pm; 9:00 pm) with a commercial colostrum replacer (Milligans feed Ltd, Oamaru, New Zealand) for approximately the first 24 hours. The nutritional composition of the colostrum replacer was as followed: 15% immunoglobulins (IgG), 52% protein, 12% fat, 26% lactose, 6% minerals and 3% moisture (Excel Plus, Colostrum 15% IgG, Milligans feed Ltd, Oamaru, New Zealand). Within the first 24 hours of birth, lambs were randomly assigned to feed treatments (two different milk replacers) and slaughter groups (either two or six weeks of age) (Table 1).

From approximately two weeks of age, lambs were fed four times a day at 8:00 am, 11:30 am, 3:00 pm and 6:30 pm. Milk volumes were reviewed and increased twice a week (Monday and Thursday) based on live weight measures prior to the first daily feeding. Milk powder was mixed with warm water at a 1:4 ratio. For each feed, milk allocation was weighed individually per lamb, and any refusals recorded post feeding.

1.2. Feed types.

Lambs were assigned to one of two commercial milk replacers and fed at 2.1 times maintenance energy requirements based on their live weight. Lambs assigned to treatment 1 (n = 11) were fed with a milk protein-based milk replacer (MP, Milligans Feed Ltd, Oamaru, New Zealand) and lambs assigned to treatment 2 (n = 10) were fed with a whey and vegetable protein-based milk replacer (WVP, Sprayfo lamb primo, The Netherlands) (Table 2). Lambs were provided with an *ad libitum* access to pellets from one week of age (Milligans Feed Ltd, Oamaru, New Zealand) and free access to fresh water. Individual milk and pen pellets intakes were recorded daily. Lambs were kept in pens of two with a pen size of approximately four meters squared and a bedding composed of rubber matting over concrete. Pens were cleaned every two days.

Table 1. Allocation of lambs in the different treatments (milk protein-based (MP) or whey and vegetable protein-based (WVP) milk replacers) and slaughter groups.

	MP		**WVP**	
	Ram	Ewe	Ram	Ewe
2-week slaughter group	1	3	2	2
6-week slaughter group	5	2	4	2
Total	6	5	6	4

Table 2. Nutrient composition of milk protein-based milk replacer (MP) and whey and vegetable protein-based milk replacer (WVP) fed to lambs.

Chemical composition	**MP**	**WVP**
Dry matter, g/kg	969.8	978.3
Crude protein, g/kg	262.9	213.0
Fat, g/kg	275.0	236.7
Gross energy, MJ/kg	22.7	21.9
Metabolizable energy, MJ/kg	21.8[a]	21.0[a]
Lactose, g/kg	350.4	259.3
CP:ME, g/MJ	12.06[b]	10.13[b]

[a] *calculated as metabolisability = 0.96 (Danso et al., 2016)*

[b] *ratio calculated as crude protein (g/kg) divided by metabolizable energy (MJ/kg)*

1.3. Proximate analysis of samples.

Samples of milk replacer powders were collected and stored at – 20°C until proximate analysis. Samples of each milk replacer were analysed for crude protein content by the Dumas method (method 968.06) (AOAC, 2005). The fat content and milk protein concentrate were determined by the Mojonnier extraction method (method 989.05) (AOAC, 2005) and the gross energy content by bomb calorimetry.

2. Stature and live weight measurements.

Lamb live weights and body measurements were measured twice a week (Monday and Thursday) from birth until slaughter. Body measurements (knee height, leg length, wither height, hock height, tibia length, girth and crown rump length) were taken using a flexible tape measure (Korbond, Lincolnshire, UK). Measures of tibia were not taken in lambs slaughtered at two weeks of age. Hock height was measured from the ground to the *tuber calcis* and the tibia length was measured from the distal end of the lateral malleolus to the proximal edge of the tibial tuberosity. Crown rump length (CRL) was measured from the tip of the scapula to the coccygeal vertebrae.

3. Slaughter.

When lambs reached approximately two weeks of age, four of each treatment group were euthanised. The remaining thirteen lambs were all euthanised at approximately six weeks of age. Lambs were humanely euthanised with a captive bolt before being exsanguinated. The left forelimb (including the scapula) and left hindlimb (including the femur) from each lamb were collected and stored at -20°C until peripheral quantitative computed tomography (pQCT) scanning was carried out. All lambs' visceral and digestive organs were removed and weighed.

4. Peripheral Quantitative Computed Tomography (pQCT).

Limbs were defrosted to allow palpation of the humeroradial and femorotibial joints. Bone length was measured using the same anatomical locations as the body measurements. The radius and tibia were scanned using pQCT (XCT 2000, Stratec Medical, Pforzheim, Germany) to obtain measures of muscle area and bone mass and architecture. The mid-diaphysis of the radius and tibia were scanned at 50% of the total bone length with a voxel size of 0.3 mm^3. After scanning, muscle area and bone parameters were extracted from the pQCT software as an Excel file using contour mode 1 and peel mode 1. The total cross-sectional muscle area at the mid-diaphysis of the radius and tibia was determined in the parameter using a threshold for muscle of 40 mg/cm^3, for bone of 280 mg/cm^3 and cortical bone as > 710 mg/cm^3. The pQCT measurements taken into consideration were total bone area (mm^2), total bone content (mg/mm), total bone density (mg/cm^3), periosteal circumference (mm) and total muscle area (mm^2). Bone to muscle ratio was calculated by dividing each bone measure (i.e., total bone area, total bone content, total bone density and periosteal circumference) by the total muscle area surrounding the bone.

5. Organ proportions.

Lambs' visceral organs were removed and individually weighed by organ on scales (to the nearest 10 g, Wedderburn, New Zealand), except for measures of full rumen, full reticulum and full abomasum in lambs slaughtered at two weeks of age which were not taken. The visceral and digestive organs collected from each lamb were heart, lungs, kidneys, liver, spleen, omental fat, pancreas, gastrointestinal tract, rumen (full and empty), reticulum (full and empty), omasum, abomasum (full and empty), small intestine and caecum together, and large intestine.

6. Statistical analysis & Calculations.

Raw data of lamb live weight was used to draw a scatterplot (Figure 2) using Microsoft Excel 365, an analysis of variance (ANOVA) was then performed to determine differences in lamb live weight and average daily gain (ADG) between treatments (milk replacers fed) and according to slaughter group. Least squares means and standard error for bone and organ parameters were calculated using a general linear model with treatment (milk replacer fed) and slaughter group as fixed effects and live weight as a covariate. Results were considered significant if $p < 0.05$. Statistical analysis was performed using SAS version 9.4 (SAS Institute Inc., Cary, NC, USA).

The efficiency of metabolizable energy utilisation for growth (Table 2) has been calculated and detailed by Danso et al. (2016), and states that for every 100 MJ of gross energy ingested from milk, approximately 96% is retained for growth. The average daily gain was calculated by subtracting live weight at slaughter from birth weight divided by age at slaughter. The ratio between rumen and abomasum was calculated as the empty rumen weight divided by the empty abomasum weight.

RESULTS.

Lamb live weights.

Age and live weight were highly correlated ($R^2 = 0.991$ and $R^2 = 0.997$ for MP and WVP respectively, Figure 2).

There was no significant effect of milk replacer on live weight at two weeks of age (Table 3, $p > 0.05$). At six weeks of age, lambs fed MP were heavier and had a greater ADG than WVP fed lambs ($p < 0.05$) (Table 3).

Bone growth of lambs.

Lambs fed WVP had a greater girth measure than lambs fed MP ($p < 0.05$). Slaughter age group influenced live weight at slaughter, hock height and CRL measurements ($p < 0.05$) (Table 4).

Live weight had a positive relationship with all stature parameters ($p < 0.05$) with the exception of CRL. Girth and height had the greatest increase per kilogram of live weight gain, regardless of the type of milk replacer fed (Table 4).

There was no effect of milk replacer on radius bone measures (Table 5, $p > 0.05$). Live weight had a significant effect on measures of total bone area, total bone content and periosteal circumference ($p < 0.05$). Slaughter age group influenced measures of total bone area and periosteal circumference ($p < 0.05$).

There was no effect of milk replacer and slaughter age group on tibia bone measures (Table 6, $p > 0.05$). Live weight had a significant effect on measures of total muscle area, total bone area, total bone content, periosteal circumference as well as the ratio between total bone density and total muscle area ($p < 0.05$). The ratio between total bone density and total muscle area decreased with increasing live weight (-0.14).

Organ growth of lambs.

There was no effect of milk replacer on lamb organs (Table 7, $p > 0.05$). Measures of heart, kidneys, liver, spleen, pancreas, empty gastrointestinal tract and full reticulum increased as live weight increased ($p < 0.05$). Slaughter age group had a significant effect on measures of empty reticulum, caecum and large intestine, and the ratio between empty rumen and empty abomasum ($p < 0.05$).

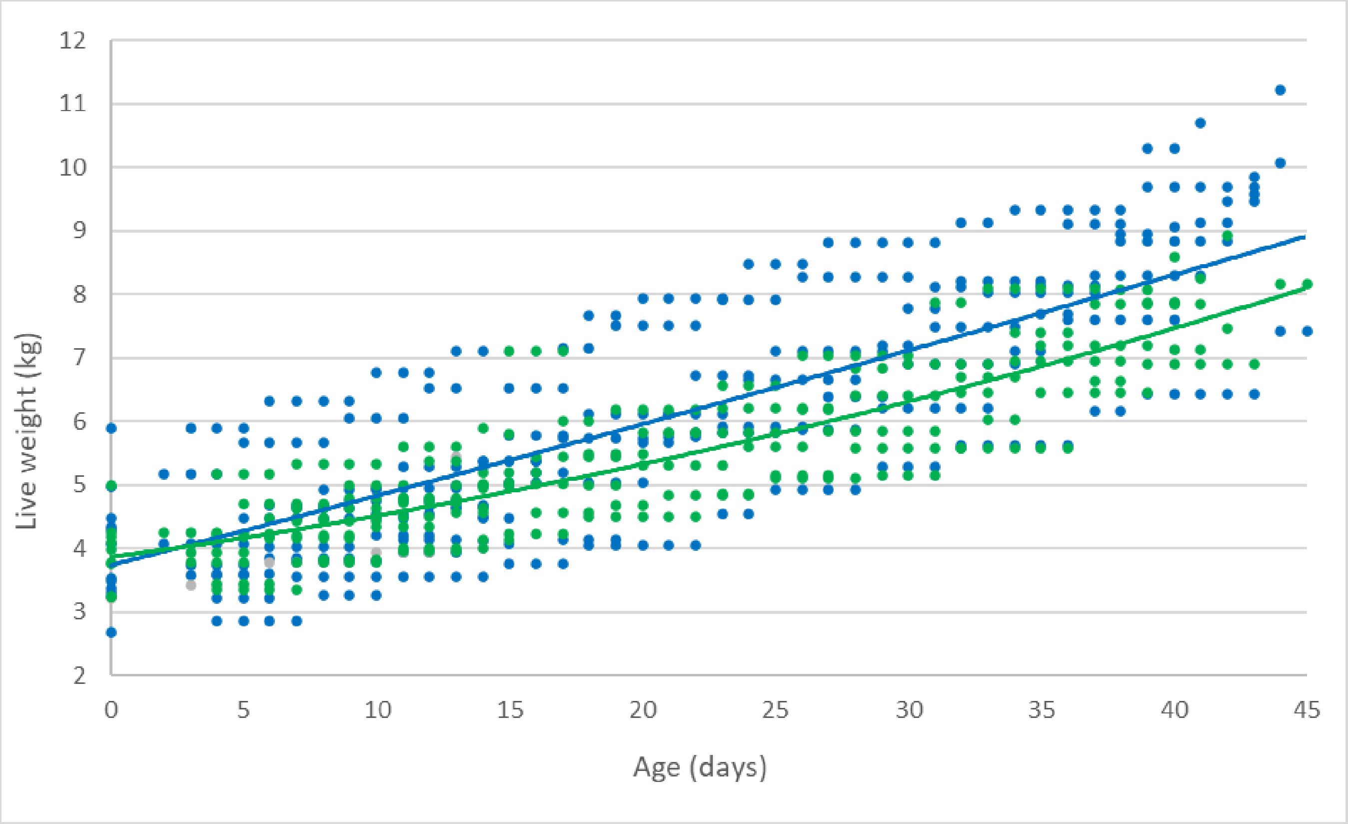

Figure 2. Scatterplot of live weight vs. age for lambs fed milk protein-based milk replacer (MP, blue) and lambs fed whey and vegetable protein-based milk replacer (WVP, green) with trendlines.

Table 3. Least squares means and standard error of live weight measurements (unadjusted for live weight) for lambs fed either milk protein-based milk replacer (MP) or whey and vegetable protein-based milk replacer (WVP) up to 6 weeks of age.

Live weight	**MP**	**WVP**	**P-value Milk replacer**
Birth weight (kg), *n = 21*	4.0 ± 0.2	3.9 ± 0.2	0.623
At 2 weeks of age (kg), *n = 8*	4.4 ± 0.3	4.9 ± 0.3	0.317
ADG at 2 weeks of age (g/day), *n = 8*	96.5 ± 22.6	107.3 ± 22.6	0.748
At 6 weeks of age (kg), *n = 13*	9.8 ± 0.3	8.3 ± 0.3	0.008
ADG at 6 weeks of age (g/day), *n = 13*	120.9 ± 4.4	102.0 ± 4.7	0.014
Total ADG* (g/day), *n = 21*	112.0 ± 8.6	104.1 ± 9.0	0.531

**ADG = average daily gain.*

Table 4. Least squares means and standard error of stature measurements adjusted for live weight at slaughter and slaughter age group for lambs fed either milk protein-based milk replacer (MP) or whey and vegetable protein-based milk replacer (WVP).

STATURE PARAMETERS *(n = 21)*	**MP**	**WVP**	**P-values**			**Live weight coefficient**	**R-square**
			Milk replacer	**Live weight**	**Slaughter age group**		
Live weight at slaughter (kg)	7.2 ± 0.3	6.5 ± 0.3	0.066	-	0.001	-	0.88
Girth (cm)	39.5 ± 0.2	41.0 ± 0.3	0.001	0.001	0.224	2.09	0.77
Height (cm)	40.2 ± 0.2	39.9 ± 0.2	0.395	0.001	0.186	1.70	0.74
Leg length (cm)	24.5 ± 0.2	25.0 ± 0.2	0.056	0.001	0.319	0.78	0.56
Knee (cm)	13.2 ± 0.1	13.4 ± 0.1	0.153	0.001	0.583	0.38	0.38
Hock (cm)	15.8 ± 0.2	16.1 ± 0.2	0.125	0.001	0.001	0.97	0.71
Tibia (cm)	11.6 ± 0.1	11.8 ± 0.1	0.225	0.001	-*	0.40	0.39
CRL (cm)	58.2 ± 0.8	56.2 ± 1.1	0.134	0.592	0.002	0.38	0.89

**Lambs slaughtered at two weeks of age were not measured for tibia so were not included in the analysis.*

Table 5. Least squares means and standard error of radius measurements adjusted for live weight at slaughter and slaughter age group for lambs fed either milk protein-based milk replacer (MP) or whey and vegetable protein-based milk replacer (WVP).

RADIUS ***(n = 21)***	**MP**	**WVP**	**P-values**			**Live weight coefficient**	**R-square**
			Milk replacer	**Live weight**	**Slaughter age group**		
Bone length (mm)	108.0 ± 2.4	108.6 ± 3.1	0.862	0.946	0.644	0.14	0.12
Total muscle area (mm^2)	764.7 ± 90.0	782.8 ± 116.5	0.898	0.550	0.276	-46.67	0.15
Total bone area (mm^2)	84.0 ± 3.8	85.6 ± 4.9	0.796	0.005	0.030	10.44	0.48
Total bone content (mg/mm)	54.7 ± 2.1	54.5 ± 2.7	0.967	0.002	0.051	6.50	0.66
Total bone density (mg/cm^3)	655.8 ± 31.3	641.1 ± 40.5	0.765	0.850	0.498	-5.10	0.11
Periosteal circumference (mm)	32.3 ± 0.7	32.7 ± 1.0	0.743	0.005	0.033	2.00	0.47
Total bone area/Total muscle area	0.2 ± 0.04	0.1 ± 0.05	0.804	0.307	0.405	0.04	0.10
Total bone content/Total muscle area	0.1 ± 0.03	0.1 ± 0.04	0.694	0.333	0.475	0.03	0.11
Total bone density/Total muscle area	1.4 ± 0.4	1.0 ± 0.6	0.650	0.499	0.613	0.26	0.07
Periosteal circumference/Total muscle area	0.1 ± 0.02	0.1 ± 0.02	0.774	0.397	0.479	0.01	0.07

Table 6. Least square means and standard error of tibia measurements adjusted for live weight at slaughter and slaughter age group for lambs fed either milk protein-based milk replacer (MP) or whey and vegetable protein-based milk replacer (WVP).

TIBIA ***(n = 21)***	**MP**	**WVP**	**P-values**			**Live weight coefficient**	**R-square**
			Milk replacer	**Live weight**	**Slaughter age group**		
Bone length (mm)	101.9 ± 2.2	101.8 ± 2.8	0.989	0.064	0.366	3.70	0.40
Total muscle area (mm²)	542.9 ± 19.7	535.3 ± 25.5	0.808	0.018	0.695	43.74	0.71
Total bone area (mm²)	99.3 ± 4.8	102.3 ± 6.2	0.696	0.006	0.072	12.59	0.52
Total bone content (mg/mm)	71.4 ± 3.3	71.1 ± 4.3	0.945	0.012	0.113	7.96	0.50
Total bone density (mg/cm^3)	722.3 ± 22.3	691.6 ± 28.9	0.387	0.411	0.435	-15.97	0.06
Periosteal circumference (mm)	35.1 ± 0.8	35.7 ± 1.1	0.635	0.007	0.072	2.23	0.52
Total bone area/Total muscle area	0.2 ± 0.01	0.2 ± 0.02	0.819	0.501	0.300	0.01	0.10
Total bone content/Total muscle area	0.1 ± 0.01	0.1 ± 0.01	0.901	0.661	0.356	0.003	0.13
Total bone density/Total muscle area	1.4 ± 0.1	1.3 ± 0.1	0.326	0.022	0.586	-0.14	0.65
Periosteal circumference/Total muscle area	0.1 ± 0.004	0.1 ± 0.005	0.973	0.711	0.484	-0.001	0.35

Table 7. Least square means and standard errors of organ measurements adjusted for slaughter age group and live weight at slaughter for lambs fed either milk protein-based milk replacer (MP) or whey and vegetable protein-based milk replacer (WVP).

ORGANS *(n = 21)*		**MP**	**WVP**	**P-values**			**Live weight coefficient**	**R-square**
				Milk replacer	**Live weight**	**Slaughter age group**		
Heart (g)		58.2 ± 2.1	60.8 ± 2.6	0.429	< 0.001	0.063	9.61	0.87
Lungs (g)		158.8 ± 11.2	179.3 ± 14.5	0.260	0.079	0.427	18.38	0.40
Left kidney (g)		21.2 ± 0.8	21.7 ± 1.0	0.686	0.010	0.645	1.95	0.77
Right kidney (g)		21.2 ± 0.8	21.8 ± 1.0	0.663	0.024	0.973	1.75	0.77
Liver (g)		135.1 ± 6.0	150.8 ± 7.7	0.111	0.027	0.400	12.69	0.84
Spleen (g)		14.1 ± 1.1	13.5 ± 1.4	0.730	0.035	0.663	2.25	0.67
Omental fat (g)		10.9 ± 1.2	12.0 ± 1.6	0.565	0.052	0.786	2.22	0.74
Pancreas (g)		13.3 ± 0.7	13.5 ± 0.9	0.816	0.050	0.552	1.30	0.79
GIT empty total (g)		578.5 ± 49.9	564.0 ± 64.4	0.855	0.005	0.115	140.44	0.64
Rumen (g)	*FULL*	405.4 ± 65.5	534.4 ± 81.2	0.301	0.060	-*	118.26	0.35
	EMPTY	72.2 ± 8.0	75.8 ± 10.3	0.772	0.246	0.102	8.38	0.81
Reticulum (g)	*FULL*	25.7 ± 4.0	32.7 ± 5.7	0.402	0.046	-*	8.13	0.45
	EMPTY	13.4 ± 1.1	13.6 ± 1.2	0.810	0.386	0.020	0.67	0.97
Omasum (g)		7.4 ± 0.6	6.6 ± 0.8	0.403	0.668	0.074	0.24	0.75
Abomasum (g)	*FULL*	115.0 ± 17.4	53.5 ± 21.6	0.081	0.234	-*	-18.60	0.30
	EMPTY	49.1 ± 2.7	49.2 ± 3.5	0.974	0.057	0.925	4.82	0.71
Small intestine (g)		376.6 ± 36.1	313.9 ± 46.6	0.283	0.503	0.134	21.61	0.74
Caecum + Large intestine (g)		133.1 ± 6.1	132.5 ± 7.9	0.950	0.439	< 0.001	4.26	0.94
Rumen empty: Abomasum empty		1.3 ± 0.1	1.4 ± 0.2	0.722	0.999	0.017	0.0001	0.78

**Not calculated due to no measurement taken for lambs slaughtered at two weeks of age.*

DISCUSSION.

Lamb live weights.

The slow initial growth rate of lambs in the current study may be a reflection of being removed from dam and having encountered various stresses during the first few days after birth (i.e., rejection by the dam, underfed and/or weaken lambs when they entered the trial). In addition, lamb artificial rearing results in growth lag compared to dam rearing of which the severity of the lag depends on the preweaning diet fed (Gibson et al., 2022a, Gibson et al., 2022b).

Regardless of the type of milk replacer fed, all lambs in the current study had lower ADG (from 108 to 162.9 g/day lower) than artificially reared lambs in previous studies (Gibson et al., 2022a, Gibson et al., 2022b). This could be due to differences in lamb diet management which could have resulted in greater growth lag than those found in previous studies (i.e., ewe colostrum vs. colostrum replacer, milk replacer fed *ad libitum*, access to pasture). The average daily gain in the current study and in the study of Gibson et al. (2022a) were also lower than what has been reported for lambs reared by dam (Gibson et al., 2022b).

The average daily gain for lambs fed MP at six weeks of age was greater than for lambs fed WVP. This is consistent with a study by McCoard et al. (2021) where feeding milk protein-based milk replacer resulted in greater ADG than a whey and vegetable protein-based milk replacer. The differences in ADG were suggested to be due to the ability of milk protein to form curd in the pre-ruminant digestive system and thus to be digested more slowly than plant protein (Longenbach and Heinrichs, 1998). However, differences in ADG between treatments in the study by McCoard et al. (2021) were almost seven times greater than in the current study. It is possible that the low number of lambs in the current study (i.e., 21 lambs vs 206 lambs in the study by McCoard et al. (2021)) resulted in less variation between treatments.

The type of milk replacer affecting slaughter weight at six weeks of age could be due to lambs reaching an age where differences in diet are expressed. In a study by Hernández-Castellano et al. (2015), that examined the difference in feeding lambs commercial milk replacer vs whole powdered cow milk, the preweaning diets fed had no effect on live weight until lambs reached 10 kilograms. However, irrespective of lamb slaughter group, lamb slaughter weight was not influenced by the type of milk replacer. This is in agreement with the findings of McCoard et al. (2021) where differences of ADG between treatments resulted in similar lamb final weights achieved two to three days sooner for milk-protein fed lambs than for plant-protein fed lambs. However, in the current study, lambs were not slaughtered at a specific age (in days) but during the second or sixth week of life. It is thus possible that a difference in slaughter weight existed between treatments, but as precise age at slaughter (in days) was not used in the model, the type of milk replacer did not affect lamb slaughter weight. In addition, despite the differences of average daily gain between lambs fed milk protein- or vegetable protein-based milk replacers in the study of McCoard et al. (2021), lamb live weights reached at the end of the trial were not statistically different, likely reflecting the weekly weighing of lambs, as in the current study.

The type of milk replacer fed influenced lamb girth size. However, lambs were still young and the full effect of preweaning diet on lamb growth may not be expressed until later in life (Moallem et al., 2010). Although girth measure has been found to be highly correlated with live weight (Gibson et al., 2020), the reason for the greater girth size in WVP lambs remains unknown as they had lower live weights than lambs fed MP. The probable cause for this may be the small sample size and variation in lamb breed in the current study.

Bone growth of early growing lambs.

Bone length of the radius and tibia was not affected by the type of milk replacer fed. However, a study by Cake et al. (2006), which examined the effects of nutritional planes on lamb bone growth at the end of an eight-month rearing period, found that nutritional restrictions resulted in impaired bone growth (i.e., shorter and thinner forelimb bones, narrowing of growth plates) and thus altered muscle: bone ratio. Nevertheless, it is possible that, as lambs in the present study were observed only for six weeks and fed at 2.1 times maintenance energy requirements, their diet was sufficient to promote longitudinal bone growth and ensure an adequate bone: muscle ratio preweaning.

Most measures of bone morphology in the radius and tibia had a positive correlation with lamb live weight, which is in agreement with Gibson et al. (2020). Bone strength is highly correlated with live weight; increases in live weight and strain on the bone required greater bone strength which is typically achieved by increasing first bone size and then thickness and/or density (Firth et al., 2011, Hart et al., 2017). In the current study, as lamb live weight increased, bone strength also increased through greater bone size (total bone area/ periosteal circumference). However, total bone density in both the radius and tibia was not affected by lamb live weight, age at slaughter or the type of milk replacer fed. According to the study of Gibson et al. (2020) on dairy calves, it is possible that the lack of increase in bone density with increasing live weight is due to early lamb age. As bone mineralisation is a slow process, it results in minor bone density changes that are not detectable at a pQCT level preweaning (Gibson et al., 2020).

Whilst total bone area and content were positively correlated with live weight, there was a negative correlation of lamb live weight with the ratio between total bone density and total muscle area (-0.14) in the tibia, suggesting that muscle increased proportionally faster than bone density. This is consistent with the findings of Evans et al. (2012), Frost (1987) and Ireland et al. (2013), who found that alterations of muscle and bone are interdependent and positively correlated, although changes in muscle occur approximately three to four times faster and thus precede changes in bone.

The primary driver of early lamb bone growth in the current study was due to increases in live weight rather than the type of milk replacer used. From birth until six weeks of age, the type of milk replacer fed had no effect on stature measures and bone morphology in early growing lambs, with the exception of girth size. As bone remodelling takes approximately two months (Cashman and Ginty, 2003), changes due to bone remodelling first occur in the microarchitecture of bone and then alter bone gross morphology (Firth et al., 2011). As the oldest lambs were slaughtered at six weeks of age, this may have limited the ability to detect differences in bone between treatments at a pQCT level. This is consistent with previous studies, which stated that the effects of preweaning diet or increasing loading on bone growth may not be quantifiable at a pQCT level until later in life (Firth et al., 2011, Gibson et al., 2020). Therefore, if lambs were raised for a longer period, an effect of preweaning diet on lamb bone growth may have been observed.

The absence of effect of the type of milk replacer fed on early lamb bone growth is consistent with Moallem et al. (2010), whereby calves offered milk replacer or reared by the dam had no differences in stature prior to weaning, although calves reared by the dam had greater live weights. While differences in live weight seem constant during the whole rearing period, differences in stature measures may appear from weaning as a result of preweaning nutrition but fade up to become similar between calves fed milk replacer or reared by the dam by 600 days of age (Moallem et al., 2010, Shamay et al., 2005). This lack of stature differences by the end of puberty may indicate the presence of a compensatory mechanism for bone growth (Shamay et al., 2005). However, as lambs in the current study were not observed over a sufficiently long period, it is not possible to determine whether the type of milk replacer used could result in such significant differences in stature measures that a compensatory mechanism of bone growth would occur.

Organ growth of early growing lambs.

Increases in live weight and slaughter age resulted in greater organ size. This is consistent with the findings of Kirton et al. (1972), which demonstrated that organ growth is highly correlated with live weight, although organs grow at different rates than the body as a whole. While digestive organs, poorly developed at birth, must grow rapidly to cope with the diet of a future mature ruminant (Baldwin, 1999), the primary function of visceral organs is to maintain lamb body functions, meaning that as lambs gain live weight, visceral organs must grow proportionally to support lamb body functions (Hamada et al., 1976).

The type of milk replacer fed had no effect on early organ growth of lambs, which is consistent with the findings of Herath et al. (2020), where organ weights were not affected by the CP:ME ratio of milk replacers prior to weaning. This is probably due to the young age of the lambs as well as their limited solid feed intake. Digestive organ growth is stimulated by solid feed intake (Danso et al., 2014). As lambs were still young, solid feed only made up a small proportion of their diet and thus was not included in models of the current study. However, it is possible that at six weeks of age, lambs had increased pellet intake which stimulated the development of some digestive organs.

CP and ME requirements in early lamb nutrition.

In the current study, lambs were fed 2.1 times maintenance requirements and the quantity of milk replacers fed were matched between treatments for metabolizable energy.

However, protein content and source differed between milk replacers, resulting in different CP:ME ratios (12.06 and 10.13 g/MJ for MP and WVP respectively). Therefore, it is not possible to distinguish whether differences in lamb growth were due to protein source or protein content. A similar study by McCoard et al. (2021) determined that the protein source of milk replacers influenced health and growth rates of preweaned lambs. Milk replacers with plant-based protein are non-curding products and cause a rapid emptying of the abomasum (Longenbach and Heinrichs, 1998). Therefore, providing lambs with a plant protein may lead lower growth rates, greater morbidity and animal health costs than milk protein-based milk replacers due to the poor digestibility and nutrient absorption of plant protein, resulting in health issues such as scouring and external infections. However, while lamb morbidity in the current study was not significant between treatments, McCoard et al. (2021) observed a much higher incidence of animal health issues when lambs were fed plant protein-based milk replacer (range from 1.5 to 13 times higher). The difference between studies may be due to the low number of lambs studied in the current study and differences in early nutrition management between the two trials.

As protein contents and sources differed between milk types, the amount and types of amino acids offered in the diet also likely differed (McCoard et al., 2021). This difference in available amino acids may have contributed to the differences in lamb growth in the current study between lambs fed MP and WVP. A deficiency of dietary amino acids or protein is known to impair immune function and increase susceptibility of animals to infectious diseases, resulting in decreased growth rates (Li et al., 2007). Although there is little information on the lamb requirements of amino acids and their functional roles (McCoard et al., 2016), examining the composition of amino acids in milk replacers could determine whether early lamb growth is affected by protein source in addition to protein amount.

Importance of CP:ME ratio in early lamb nutrition.

At two weeks of age, lamb live weight and average daily gain were not affected by treatment, suggesting that the CP:ME ratio of the milk replacers used met the nutritional requirements to support lamb growth (Titgemeyer, 2003). However, at six weeks of age, lambs fed WVP had significantly lower average daily gain and lighter average live weight, which might be due to the small number of lambs studied or to the CP:ME ratio of WVP limiting lamb growth compared to the CP:ME ratio of MP. As MP had a greater protein content and thus a greater CP:ME ratio compared to WVP, it can be concluded that early growth rates are higher when lambs are provided high protein intake. This is in agreement with the study of Gibson et al. (2022a) where lambs fed commercial milk replacer had lower growth rates than lambs provided high protein milk replacer. However, as lambs require a higher CP:ME ratio during early growth than later in growth (13.1 at 5 kg of LW vs 10.9 g/MJ at 18 kg of LW, respectively) (Danso et al., 2016), both milk replacers used in the current study used may have failed to supply adequate CP:ME ratios for early growing lambs. Feeding lambs at 2.1 times maintenance requirements should have partially compensated for the lower CP:ME ratio but might not have been sufficient to meet the minimum protein requirements for lamb early growth (2.74 g CP/ kg $LW^{0.75}$ for maintenance) (Danso et al., 2016).

LIMITATIONS OF THE STUDY.

The source of lambs is one of the limitations of the current study as they were either orphans or rejected by the dam. Lambs used in the current study may have been affected by various stresses during their first few days of life such as undernutrition or a reduced immune status due to low colostrum intake. Before arriving into the trial, it was not always possible to know whether lambs have been able to feed properly and, as a result, they may not have had sufficient colostrum when entering the study. This might have resulted in delayed early growth as well as altered growth rates.

The number of animals assigned to each treatment group was limited to the number of lambs that needed to be removed from the dam and raised, which reduced the power of the current study. A larger sample size improves the robustness of the findings as well as the margins of error are reduced when a greater number of lambs are studied. This also leads to more reliable results while considering variations specific to each lamb to define a growth model and ensuring that observed differences are not due to random variation. However, no retrospective power analysis was performed prior to the trial to define the appropriate number of lambs needed in each feed treatment. As a result, the findings of this trial must be interpreted with caution, as the absence of observed differences could also come from the small sample size, which may have limited the ability to identify significant nutritional effects on lamb growth. Thus, the results of this study could be due either to the low number of lambs studied in the current trial that may have limited the determination of nutritional effects on early lamb growth, or to the nutritional factors studied (i.e., the source and content of protein in milk replacers).

The scope of the current study was limited by the duration of the trial, as the oldest lambs slaughtered were only six weeks of age. Bone remodelling is a process that takes about two months and is driven by lamb live weight gain. Thus, it is likely that the oldest lambs in the trial were only beginning to exhibit changes in stature measures at the time of slaughter, showing that the critical phase of bone growth occurs from weaning. The lack of observable differences in bone morphology and organ weight between treatments may have been due to being raised on the two treatments for an insufficient length of time. The trial duration is significant as inadequate nutrition preweaning could lead to differences in stature measures between lambs, which may only become significant between weaning and first lambing. Thus, the limited duration may not have allowed enough time for the different treatments to apply a measurable impact on lamb bone development. It is possible that if the lambs have been followed for longer that a greater difference in lamb growth would have been observed between treatments.

The aim of this study was to examine the effects of preweaning diet on bone and organ growth in lambs. To do so, the volume of milk replacer fed between treatments was adjusted to match 2.1 times lamb energy maintenance requirements but differed in protein content and source, resulting in two distinct variables that could affect early lamb growth. According to the results of Danso et al. (2016) and Gibson et al. (2022a), protein content in milk replacer influences early lamb growth, which is consistent with the observations of the current study as well. However, the results of the current study, coupled with the paucity of information on the effects of the protein source in milk replacers on early lamb growth, do not allow the differences observed to be significantly attributed to protein content, protein source or both combined. It is therefore not possible to ensure that the results of this study may not be also due to the source of protein in milk replacers used.

In addition, it is possible that the current study might have been limited by the handling of lamb legs for pQCT scanning. In order to pQCT scan-lamb leg, they had to be defrosted and straightened to ensure the bone was straight through the gantry. As the leg was disarticulated, the tension and position of the leg muscles may have been altered, compared to a live animal scan. Although legs were placed in a suitable position for scanning, total muscle area measures may have been slightly different from the lamb muscularity and bone: muscle ratio that can be observed *in vivo*.

FUTURE RESEARCH.

The general aim of this thesis was to examine the effect of preweaning diet on early lamb growth of bones and organs. This thesis investigated the relationship between the protein content of milk replacer used and lamb performance from birth until six weeks of age. However, from these results, new research questions have arisen and require further research on a larger scale.

The number of animals studied in each treatment was low. Conducting this study on a larger scale would reduce the margins of error and provide stronger and more reliable results. Thus, the current study reflects the need for a larger-scale trial to examine the effects of lamb preweaning diet on bone development and organ growth later in life.

The lack of significant differences in bone morphology and organ weight was observed likely due to the lambs being too young at slaughter. Repeating this trial and slaughtering lambs at an older age would therefore make it possible to examine the long-term effects of preweaning diet and the impact of early protein intake on lamb bone and organ growth. Ideally, the study should cover lamb performance from birth to first lambing, enabling the long-term growth and development of bones and organs to be observed.

The treatments were adjusted to have the same amount of energy, resulting in the major differences between the milk replacers used in this trial being the content and source of protein. Previous studies have shown that lambs require a higher CP:ME ratio during early growth than later in life (Danso et al., 2016, Gibson et al., 2022a). However, it is not possible to prove that the results obtained in this trial are due to protein content rather than protein source in the milk replacer, as the protein source used in milk replacers may also alter early lamb growth. To determine the long-term effects of protein source on bone development and organ growth, further research needs to be carried out on lambs, from birth until first lambing, with milk replacers composed of different protein sources but adjusted for CP:ME ratios.

Although previous studies have demonstrated that higher CP:ME ratios in milk replacers are more adequate for early lamb growth than those of the current study (Danso et al., 2016, Gibson et al., 2022a), lambs were fed at 2.1 energy maintenance requirements to meet their protein and energy requirements. The differences in lamb growth rates between the current study and the study of Danso et al. (2016) where CP:ME ratios of milk replacers are adjusted for early lamb growth may not be due only to nutritional effects, as the source of lambs (i.e. breed, sex, orphan or not, birth rank) and lamb management also differed and may partly explain the variations in the results. Repeating this trial by adjusting CP:ME ratios of milk replacers used (i.e., same CP and ME contents) to early lamb requirements based on live weight would allow observation on whether lamb growth is increased when they are provided CP:ME ratios according to their needs and age although the source of protein in milk replacers differs.

CONCLUSION.

Feeding lambs a milk replacer based on milk protein (MP) resulted in greater live weights and average daily gains than feeding them a milk replacer based on whey and vegetable proteins (WVP). Although this difference can be partially attributed to the higher protein content of MP, it is not possible to determine exactly how the protein source in milk replacers affected lamb growth in this trial. Despite restricted changes in bone morphology and organ weight due to the young age at slaughter of the lambs, the differences in the preweaning diets may have altered the developmental potential growth of bone and organs in lambs. Further research should examine the long-term effects of preweaning diet on organ growth and bone development of pre-ruminants until first lambing.

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