Impact of casein- versus whey-based lamb milk replacers on growth and health of artificially reared lambs

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Abstract

High cost of milk proteins has driven development of cheaper whey-based milk replacer (WBMR) as an alternative to skim-milk (casein)-based milk-replacer (CBMR) for artificial-rearing of young ruminants. This study compared growth, antibiotic/anti-inflammatory use and mortality in lambs reared on either WBMR (n=138) or CBMR (n=151) in the first three weeks of rearing at commercial scale. There was a diet x rank interaction (P=0.001) whereby CBMR-fed single/twin and triplet/quad lambs had the highest average daily gain (ADG) independent of birth-rank (362 *vs.* 358 g/d respectively, SED=16.7) compared to WBMR-fed lambs where higher ADG was observed in triplet/quad compared to single/twin lambs (275 *vs.* 213 g/d respectively, SED=16.7). Males tended to have higher ADG than females (311 *vs.* 294, SED=9.7 g/d, P=0.064). Overall, there was a tendency for greater mortality (11% *vs.* 4%; P=0.055), and overall incidence rate (*N*/100 animal weeks) for use of antibiotics (4.43 *vs.* 1.27; P=0.073) and anti-inflammatories (4.59 *vs.* 1.59; P=0.099) in WBMR- than CBMR-fed lambs. These results highlight that CBMR under an *ad-libitum* milk-feeding regime supports greater ADG independent of birth-rank and reduces the incidence of disease in the first three weeks of life compared to WBMR. Furthermore, WBMR under an *ad-libitum* milk-feeding regime results in lower ADG of single/twin-born lambs compared to their triplet/quad counterparts.

Keywords: lambs; artificial rearing; milk replacer; composition; growth; health; mortality

Introduction

In nature, lambs are naturally suckled by their dams and receive numerous small feeds of whole milk. One of the most important nutrients in milk replacers is protein. Therefore, choosing an appropriate protein source influences feed quality and animal production costs (Erickson et al. 1989). Milk replacers generally contain protein from two sources: milk protein and non-milk protein. Milk protein has balanced amino acid constituents, is highly digestible and is, therefore, considered an excellent protein source. However, the high cost of milk proteins in the US and Europe has resulted in skim-based milk replacers being uneconomic for calf-rearing systems (Davis & Drackley 1998). As a result, non-curding whey milk replacers have been developed and tested under United States and European conditions and are being used in artificial-rearing systems for ruminants including lambs and calves.

Recent studies indicate lower weight gain in artificially reared lambs (McCoard et al. 2021) and calves (Thomson et al. 2018) fed whey-based versus casein-based milk replacers while disease and mortality were similar. Artificial rearing of lambs is an essential part of some dairy sheep systems for generating replacements, and for for rearing surplus progeny and orphan/multiple-born lambs on traditional meat- and wool-producing sheep farms. As with calf-rearing systems, the cost of lamb rearing is a key consideration for producers. While the impact of whey-based milk replacers in calves has received attention in the literature (Huang et al. 2015; Thomson et al. 2018), the production performance of artificially reared lambs on casein- or whey-based milk replacers has not been described.

The objective was to evaluate the effect of a casein-based versus whey-based lamb milk replacer on the growth, health and survival of artificially reared East Friesian cross-bred lambs in the first three weeks of rearing using an *ad-libitum* milk-feeding system at commercial scale.

Materials and methods

All procedures in this study were approved by the AgResearch Grasslands Animal Ethics Committee (AE13960).

East Friesian cross-bred lambs (n=294) were sourced from naturally mated ewes lambed outdoors on pasture on a commercial dairy sheep farm in the central North Island. Lambs were separated from their dams at 2-3 days of age to provide sufficient time for colostral intake and were transferred immediately to the rearing facility between the 21st and 26th of August 2016. Upon entry to the dedicated commercial rearing facility, all lambs were weighed, tagged for identification and their navels dipped in iodine to reduce the risk of infection. The lambs were randomly allocated to one of two treatment groups balanced for sex, birth rank (single-twin or triplet-quadruplet) and date of birth to reflect commercial rearing practices. The lambs were group housed in 3 × 3 metre pens with eight pens per treatment group (16-20 lambs/pen with two teats per pen). Treatments started upon entry to the rearing shed and consisted of either a casein-based milk replacer formulated from 100% bovine milk proteins and fat (CBMR; n=151: 93 female, 58 male, 61 single-twin, 90 triplet-quad) or whey-based milk replacer formulated from bovine milk proteins, hydrolyzed wheat protein and vegetable oil (WBMR; n=143: 82 female, 61 male, 74 single-twin, 69 triplet-quad). All lambs were fed using *ad-libitum* warm reconstituted milk replacer (MR) at a mixing rate of 200 g/L using automatic feeders (DeLaval LKF1200, DeLaval, Hamilton, NZ). Lambs within each pen received the same treatment. Lamb starter meal and fresh water were available in the pens *ad-libitum.* Lambs within a treatment group were fed MR using a single automatic feeder. Therefore, individual intake of MR was not recorded. However, total amount of MR per group was recorded. The total amount of starter diet offered per pen was recorded and total intake per group calculated. All pens were bedded with wood chip and the rearing facility had good ventilation and dedicated rearing staff.

All CBMR and WBMR was purchased as a single batch. Whole sheep milk powder (WSMP) was also sourced from a commercial dairy sheep operation and used to enable the comparison of the nutritional composition profile between the two milk replacers evaluated in this study compared to whole sheep milk. Three samples of each batch of MR were pooled and composition analysed (Table 1) for ash (Furnace 550oC AOAC 942.05), crude protein (AOAC 968.06 Dumas method, N-P = 6.38), fat (Mojonnier, dairy AOAC989.05), lactose (Enzymatic method), minerals (Vacuum oven, AOAC 990.19, 990.21), and carbohydrate (by difference) by the Massey University Nutrition Laboratory (Palmerston North, New Zealand). Metabolisable energy and lactose content of the MR and WSMP was calculated using NRC (2002) equations. The milk protein composition of each diet was determined using high-throughput liquid chromatography (Day et al. 2015) using a Phenomenex Aeris™ 3.6 µm WIDEPORE XB-C18 200 Å, LC Column 250 x 4.6 mm Part Number:00G-4482-E0 (Phenomenex, Torrance CA, USA). Bovine milk protein standards were used for quantitation as sheep protein standards are not available (Sigma Aldrich, Auckland, New Zealand: κ-CN (C-0406), α-CN (C-6780), β-CN (C-6905), α-LA (L-5385), β-LG B (L-8005), and β-LG A (L-7880), β-LG (L3908). Amino acid profiling was performed as described by McCoard et al. (2021).

Dry matter (DM), crude protein (CP), acid detergent fibre (ADF), neutral detergent fibre (NDF), soluble sugars (SS), starch and ash in the starter diet were determined using the following procedures: DM (Method 945.15, AOAC, 2010), CP (Method 992.15, AOAC, 2010), ADF and NDF (Method 7.074, AOAC, 1990), soluble sugars (Method 942.05, AOAC, 2010), starch (Method 996.11, AOAC, 2010), and ash (Method 942.05, AOAC, 2012) by a commercial laboratory (Massey Nutrition Laboratory, Palmerston North, New Zealand).

Lamb live weight was measured upon entry to the rearing shed and exit from the rear shed, approximately three weeks later. After this time, the lambs were managed as a single mob and, therefore, there was no replication which prevented statistical analysis of the performance data. Therefore, our focus was on the first three weeks of rearing. All animal health issues that required animal health treatment intervention were recorded as part of the daily health-check regime for all individual animals. All animal health treatments were administered by trained staff according to the manufacturer's instructions and under guidance from the farm veterinarian as previously described (McCoard et al. 2021). All lamb deaths were recorded.

Lamb growth data were analysed using a linear mixed model (LMM). Lamb sex, date of birth, rank (single-twin vs triplet-quad), diet (MR type), and an interaction term between diet and rank were included in the model as fixed effects. Pen was used as a random effect (random intercept) to account for between-pen heterogeneity.

Generalised linear mixed models (GLMM) were used to model the effects of diet on lamb mortality, health events (pneumonia and lameness) and drug use (antibiotics and anti-inflammatories). A logit link function with a binomial distribution was used for modelling lamb mortality. A log link function with a negative binomial distribution and an offset using the natural logarithm of time (rearing period) was used for modelling health events and drug use. Diet was used as a fixed effect, and a random intercept was used for the pen to account for between-pen heterogeneity.

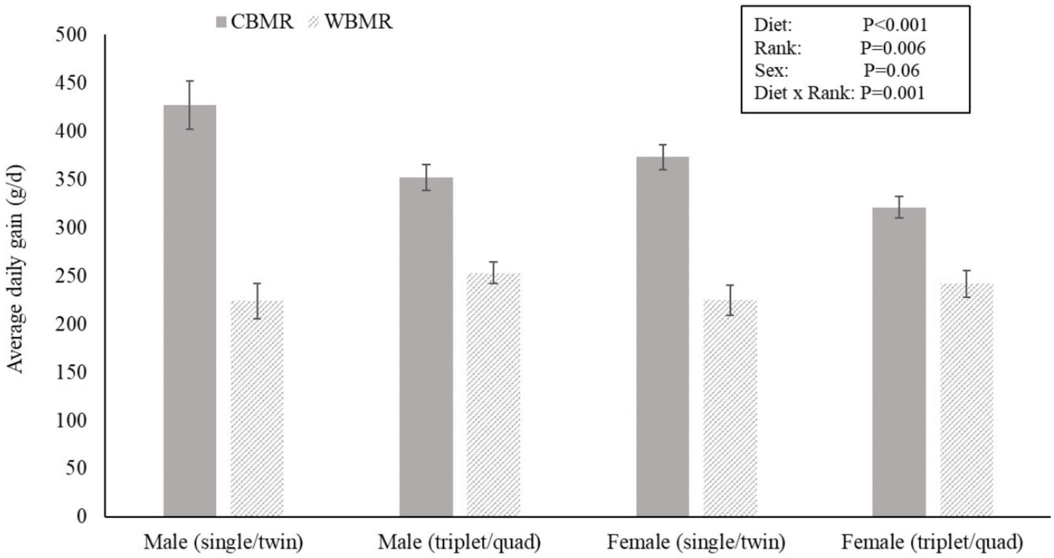
Statistical analysis was performed using R (R Core Team. 2021). GLMM modelling was performed using glmmTMB, and model fit was assessed using simulated quantile residuals from the DHARMa package. LMM modelling was performed using lme4 and model fit was assessed using residual plots (QQ-plots, residual vs fitted plots). Treatment contrasts, P values and confidence intervals were generated from the emmeans package.

Table 1 Mortality rate (%) and incidence rate and ratio[1] of drug use and selected animal health issues in lambs reared on casein-based MR (CBMR) or whey-based milk replacer (WBMR) in the first three weeks of life.

	WBMR	CBMR	Ratio (CI)	P value
Mortality (%)	11	4	-	0.055
Total antibiotics	4.43	1.27	3.5 (0.90, 13.80)	0.073
Total anti-inflammatories	4.59	1.59	2.89 (0.80, 10.2)	0.099
Pneumonia	1.89	0.70	2.70 (0.54, 13.50)	0.225
Lameness	1.50	0.26	5.82 (0.40, 85.10)	0.197

[1] Incidence rate and incidence ratio per 100 animal weeks. In this study, incidence rate is a measure of new cases of disease per unit of time at risk (i.e., animal week at risk), e.g., incidence rate of 4.43 per 100 animal weeks means we expect to see an average of 4.43 cases for every 100 animals observed during a one-week period within a treatment group. The incidence ratio is a measure of the incidence rates between two treatment groups, e.g., an incidence ratio of 3.5 means that the rate of total antibiotic use is 3.5 times greater in lambs exposed to WBMR compared with lambs exposed to CBMR.

Figure 1 Effect of milk replacer protein source (casein-based milk replacer, CBMR; whey-based milk replacer, WBMR), birth rank (single-twin vs. triplet-quad) and sex, and their interaction, on average daily gain. Data are presented as means ± standard error of the mean.



Results

Growth and DMI

Overall, lambs reared with *ad-libitum* CBMR grew faster than those reared on WBMR (116g/d per lamb difference). A diet-by-rank interaction was evident for average daily gain (ADG) whereby single/twin-born lambs reared on CBMR grew faster than triplet/quad-born lambs, while the opposite was observed for lambs reared on WBMR (Figure 1).

On average, the CBMR group consumed 6.8 kg compared to 5.3 kg total MR dry matter intake (DMI) per lamb in the WBMR. Similar intake of starter meal per lamb (0.8-0.9 kg/lamb) was recorded during the rearing period in both groups.

Health

During the three-week rearing period, mortality rate tended to be greater in lambs reared on *ad-libitum* WBMR compared to CBMR (11% vs. 4%, P=0.055). Lambs reared with WBMR tended to be 3.5 times (P=0.073) more likely to be treated with antibiotics and 2.9 times (P=0.099) more likely to be treated with anti-inflammatories than lambs reared with CBMR (Table 1). The main health issues observed were lameness and pneumonia with numerically higher incidence rates in the WBMR than CBMR group.

Milk and starter composition

The gross milk composition of both the CMBR and WBMR differed from WSMP (Table 2). Compared to WSMP, CBMR had greater carbohydrate and lactose content and lower protein, and fat content, while WBMR had a greater carbohydrate and lower protein, fat and lactose. The CBMR had greater protein, fat and lactose content, and lower carbohydrate content relative to the WBMR.

Compared to WSMP, the CBMR had lower levels of all the individual milk proteins except α-casein which was similar with the largest differences observed for β-casein (Table 2). Overall, CBMR had 12% lower total casein, 51% lower whey protein and 16% lower total milk protein compared to WSMP. Compared to WSMP, the WBMR had no casein milk proteins, almost four-fold more α-lactalbumin and almost two-fold more β-lactoglobulin, leading to just over two-fold more total whey protein but 78% less total milk protein. The CBMR contained 22% less total whey proteins compared to the WBMR, and WBMR contained no casein-protein with the CBMR having almost four times as much total milk protein compared to the WBMR. Overall, based on milk protein content, CBMR was more similar to WSMP than WBMR.

The amino acid content of the WSMP and the MR evaluated in this study are presented in Table 2. Compared to WSMP, CBMR had 9-23% lower EAA, 14-38% lower conditionally essential AA (CEAA) levels and 12-27% lower NEAA content leading to 18%, 20% and 18% lower overall EAA, CEAA and non-essential AA (NEAA) levels respectively and 19% lower total AA content. Compared to WSMP, WBMR had 7-50% lower EAA, 10-56% lower CEAA except cystine which was 77% greater, and 23-35% lower NEAA leading to 40%, 26% and 32% lower overall EAA, CEAA and NEAA levels respectively and 32% lower total AA content. Compared to WBMR, CBMR had 21% greater EAA levels driven by 8-62% higher levels of all EAA except threonine which was 13% lower and similar tryptophan content. CBMR had 32% greater CEAA levels relative to WBMR driven by 47-94% greater levels of arginine, proline and tyrosine while CBMR had lower levels of cystine and tyrosine compared to WMBR. Total AA content of CBMR was 19% greater than that of WBMR. Composition (%DM) of the starter diet was 20% CP, 6.4 % ADF, 15.7% NDF, 5.8% SS, 26% starch.

Table 2 Gross milk composition, individual milk proteins and amino acid composition of whole sheep milk powder (WSMP), casein-based milk replacer (CBMR), whey-based milk replacer (WBMR) and the percentage differences in amino acid composition between each component.

	WSMP	CBMR	WBMR
Composition			
Moisture % DM	1.3	2.6	2.6
Ash % DM	5.4	5.4	9.1
Protein % DM	32.1	25.9	22.8
Fat % DM	35.0	27.1	23.5
Carbohydrate % DM	28.8	41.6	44.5
Lactose g/100g DM	24.4	35.4	19.2
Calcium g/kg DM	10.7	9.2	10.2
Sodium g/kg DM	2.6	2.9	7.2
ME MJ/kg DM	23.8	21.8	20.3
Milk proteins (mg/g)			
κ-casein	41.8	35.2	0
α-casein	100.8	102.1	0
β-casein	248.6	207.5	0
α-lactalbumin	7.2	2.8	27.7
β-lactoglobulin	33.9	17.5	65.8
Total casein protein	391.2	344.8	0
Total whey protein	41.1	20.3	93.5
Total milk protein	432.2	365.1	93.5
Amino acids (mg/ml)			
Essential AA			
Histidine	8.4	6.8	4.2
Isoleucine	15.5	12.6	10.6
Leucine	30.2	24.1	20.0
Lysine	21.9	19.9	18.4
Methionine	6.8	5.5	3.5
Phenylalanine	14.5	12.1	8.2
Threonine	13.9	11.2	12.9
Tryptophan	4.4	3.4	3.4
Valine	20.9	16.0	11.3
Conditionally essential AA			
Arginine	10.2	8.7	5.9
Cystine	2.6	1.6	4.6
Glycine	6.2	4.8	5.6
Proline	31.0	24.0	16.3
Tyrosine	14.0	12.0	6.2
Non-essential AA			
Alanine	11.8	8.6	8.9
Aspartic acid	24.7	19.5	18.1
Glutamic Acid	66.5	55.5	51.3
Serine	16.2	14.2	10.5
Total EAA	136.5	111.6	92.5
Total CEAA	64.0	51.1	28.6
Total NEAA	119.2	97.8	88.8
Total AA	319.7	260.5	219.9

Note: Glutamine and Asparagine were not detected

Discussion

For the first few weeks of life, the diet of pre-ruminant lambs consists primarily of milk, and the gastrointestinal tract functions in a similar manner to that of monogastric species. This development period is characterised by rapid growth which is supported by high rates of skeletal muscle protein synthesis (Chien et al. 1993; Denne & Kalhan 1987). The neonatal period is also one of the most vulnerable for the establishment of pathogenic gastrointestinal infections which can impact growth and health, especially in intensive indoor-lambing systems where pathogen loads are higher than in outdoor environments (Tzipori et al. 1981). Therefore, the neonatal period is critical for the growth and health of pre-ruminants.

Antimicrobials, particularly in intensive livestock farming systems (ESVAC, 2013) are widely used to improve animal health, welfare and productivity. Such use has driven the growing concern around increased risk of development of resistance in animal and human pathogens (Van De Sande-Bruinsma et al. 2008). In the present study antibiotics were only used therapeutically. While neonates are particularly vulnerable to infectious pathogens and other animal health issues due to the immaturity of their immune system (Jones et al. 2010), this study illustrated that MR formulation can influence drug use in artificially-reared lambs consistent with our prior observations (McCoard et al. 2021). The results from this study suggest that use of WBMR is likely to increase the use of therapeutic drugs and negatively affect growth and health of lambs compared to CBMR.

Rapid pre-weaning growth rates in artificial lamb-rearing systems are desirable to reduce the time to reach weaning weights to reduce the cost of rearing. In the present study, one of the biggest changes in animal performance was the difference in growth rates and lamb mortality. While overall growth rates were lower in lambs reared in WBMR than CBMR, single- and twin-born lambs reared on WBMR were particularly compromised. Capacity for skeletal muscle protein synthesis is reduced in *in-utero* growth-restricted lambs such as multiple-born lambs (Sales et al. 2014) therefore, lower growth rates of triplets/quadruplets compared to singles and twins is expected, as was observed in the CBMR group. However, the lack of difference in growth rates between birth ranks, and lower overall growth rates compared to the CBMR group, indicates that the WBMR was unable to meet the requirements for growth, especially in single/twin-born lambs, which are heavier at birth and, therefore, have greater energy requirements for maintenance (Greenwood et al. 1998). The authors are not aware of prior studies that compare the growth rates of single-twin and triplet-quadruplet artificially reared lambs so comparisons are unable to be made. We have recently reported that growth and health is compromised in lambs artificially reared on a CBMR containing a greater proportion of whey protein, hydrolysed wheat and vegetable oil compared to a CBMR formulated from 100% milk proteins and fats (the same formulation used in the present study), while mortality was similar between groups (McCoard et al. 2021). In the present study, growth rates were substantially lower and mortality was higher than those in the aforementioned study.

Lower growth rates of the lambs fed the WBMR versus CBMR was likely due, at least in part, to lower average DMI of MR. Average DMI of MR per lamb for the CBMR group (322 g/lamb/d) were similar to other studies in which lambs were artificially reared indoors with *ad-libitum* milk allowance on automatic feeders (McCoard et al. 2021, ~300 g DM/d; Belanche et al. 2019, 300g DM/d; David et al. 2014, 329 g DM/d; Bimzcok et al. 2005, ~319 g DM/d) while MR intake per lamb in the WBMR group was lower (253 g/hd/d). Similar DMI were observed in our previous study independent of MR formulation (McCoard et al. 2021) despite lower inclusion rates of whey and hydrolysed wheat protein. Therefore, the lower DMI of WBMR than CBMR in the present study suggests the WBMR formulation may have negatively affected intake. In both the present study, and that of McCoard et al. (2021), the incidences of drug use and health issues were higher in lambs reared with a MR containing vegetable proteins and fats, suggesting health issues may also be a contributor to differences in DMI. However, incidence of health issues that did not require therapeutic drug use (e.g., scours) were not recorded in the present study and increased scour incidence was associated with lower ADG in lambs reared on a MR containing vegetable proteins and oils (McCoard et al. 2021). Other factors that can influence DMI include, competition at the feeder or adaptation to automatic feeders (David et al. 2014) and palatability of the diet (Miller-Cushon et al. 2014). Direct evaluation of these factors was not undertaken in the current study but warrant further investigation. Starter intake can also influence MR intake in calves (Khan et al. 2016) but to the authors' knowledge has not been evaluated in lambs. However, starter intake was very low and similar between groups (0.8-0.9 kg/lamb total intake during the study), which is consistent with low intake of starter observed in young lambs (McCoard et al. 2019; Belanche et al. 2019) and calves (Gerrits, 2019). It is important to note that individual intakes were not measured in this study due to group housing. Therefore, the reported overall differences in MR and starter intake cannot be evaluated statistically. However, that the size and shape of the pens, stocking density, number of teats per pen, and animal husbandry was consistent across all pens and treatments suggests that the differences in growth rate are more likely due to differences in the composition of the MR.

Neonates and growing animals have a high demand for amino acids for synthesis of protein, glucose and other cellular metabolites and have been demonstrated to be involved in functional roles that influence growth, development, health and survival (McCoard et al. 2016). The use of vegetable proteins as an alternative cheaper protein source to milk proteins for formulation of MR is growing in popularity (Gerrits 2019). In general, the digestive system of the young ruminant is poorly developed and can only digest a limited range of carbohydrates, fats and proteins (Davis & Drackley 1998). Substitution of skim milk powder with a mix of solubilised wheat protein concentrate and whey powder increases abomasal emptying of fat and protein and decreases digestibility of AA and total nitrogen, but not fat (Toullec & Formal, 1998). In the present study, the source of both milk protein and fats differed considerably so it is not possible to determine whether substitution of milk protein or milk fat contributed to the observed differences in animal performance. However, CP, milk protein type (casein *vs.* whey) and AA content differed considerably between WBMR and CBMR, with CBMR more closely reflecting the profile of WSMP. In the present study, the CBMR contained almost four times as much total milk protein compared to the WBMR. The similar protein content of the MR (25.2 *vs.* 22.2 % DM for CBMR and WBMR respectively) implies that the WBMR contained a much greater proportion of non-milk proteins than the CBMR. According to the product label, the WBMR contained hydrolysed wheat protein. In calves, the use of wheat gluten and rice protein concentration (80% CP content) as a source of protein for calf diets to substitute for milk protein has been associated with a reduction in growth rate as the proportion of vegetable protein increased (Hill et al. 2008). The type of vegetable protein is also important in calves as soybean concentrate and rice-protein isolate delivered similar growth performance to that of full milk protein, while lower growth rates were observed when hydrolysed wheat-protein or peanut-protein concentrate were used at the same nutrient (AA) levels (Li et al. 2008). Thus, the high inclusion rate of hydrolysed wheat protein at the expense of milk protein, coupled with lower DMI and supply and/or balance of AA including specific functional AA (e.g., arginine, leucine, methionine; McCoard et al. 2016), are likely major contributing factors to the reduction in growth rate of the lambs in the current study, as previously observed in lambs (McCoard et al. 2021) and calves (Huang et al. 2015). Future studies to establish the optimal AA profile to support the growth and health of artificially-reared lambs, compared to naturally-reared lambs, is warranted to provide insights into strategies to improve the formulation of MR to more closely mimic whole sheep milk. The impact of these MR formulations beyond three weeks of rearing is also warranted.

This study illustrates that young lambs can be reared on both casein- and whey-based milk replacers, albeit at a cost in terms of liveweight gain, health and welfare of lambs reared using the WBMR. The key result from this study was that CBMR, under an *ad-libitum* milk-feeding regime using automatic feeders, supports greater ADG independent of birth-rank, and tends to reduce mortality and incidence of disease requiring therapeutic drug use in the first three weeks of life compared to WBMR. Furthermore, compared to CBMR, WBMR under an *ad-libitum* milk feeding regime results in lower ADG of single-twin-born lambs compared to their triplet-quad counterparts. These effects are likely driven by a combination of lower ME and CP intake from MR and lower nutritional quality of the WBMR resulting from a lower inclusion rate of milk proteins which are highly digestible and have a balanced

AA profile for growth. We conclude that selection of MR for lamb rearing should consider not just feed cost, but also the impact on growth, health and mortality rates. Based on the observations from this study, feeding CBMR formulated from 100% milk proteins and fats is recommended for artificial rearing of lambs in the first three weeks of life.

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