OXFORD

AMERICAN SOCIETY OF **ANIMAL SCIENCE**

Effects of milk replacer composition on growth and development of beef × dairy crossbred calves

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Abstract

The production of crossbred beef × dairy (B × D) calves is increasing; however, evaluation of pre-weaning feeding strategies for B × D calves is limited. We hypothesized that both male and female B × D calves fed added fat and protein through milk replacer would have increased muscle growth, muscle fiber cross-sectional area, average daily gain, BW, and morphometric measurements. We also hypothesized that calves fed the additional fat and protein milk replacer would have upregulation of regulatory genes involved in muscle hypertrophy. SimAngus × Holstein calves ($n = 42$) were assigned to one of two milk replacers: 30.0% crude protein (CP), 32% crude fat (CF) milk replacer (HPHF, $n = 11$ males, 11 females), or 22% CP, 20% CF milk replacer (CON, $n = 10$ males, 10 females) from 0 to 8 wk of age. B × D calves were weighed at birth and weekly thereafter. At week 2 and 8, longissimus muscle biopsies were collected for muscle fiber cross-sectional area (CSA) or gene expression analysis. Ultrasounds were performed at 4 and 8 wk of age to quantify ribeye area (REA), and backfat and rump fat thickness. Morphometric measurements, BW, CSA, and ultrasound data were analyzed with PROC MIXED with animal as the subject and fixed effects of milk replacer, age, sex, and their interactions. Gene expression data were analyzed in R Studio. Calves that consumed the HPHF milk replacer were heavier than calves consuming the CON milk replacer (HPHF: 70.7 ± 0.39 kg; CON: 68.5 ± 0.41 kg; $P < 0.01$). At 8 wk of age, HPHF calves tended to have 14% larger muscle fiber CSA than CON calves ($P = 0.06$). No differences due to diet were observed for REA or fat thickness ($P \geq 0.38$). Expression of MyoD tended to be 34% greater in CON females than HPHF females at 2 wk ($P = 0.06$), but at 8 wk, HPHF females tended to express 39% more MyoD than CON females ($P = 0.09$). Myogenin expression was 3% greater in CON calves than HPHF calves at 2 wk ($P = 0.02$), and CON females tended to express 52% more IGF-1 than HPHF females ($P = 0.07$). Feeding a milk replacer with a protein and fat content similar to beef cow milk improves B × D calf growth compared with a conventional milk replacer with less protein and fat. Improvements in early growth may improve B × D carcass quality and quantity, with the potential to increase return to the producer.

Lay Summary

The production of crossbred beef × dairy (B × D) calves is increasing; however, evaluation of pre-weaning feeding strategies for B × D calves is limited. Many B × D calves are raised on milk replacer that does not replicate the nutritional content of beef cow milk. Feeding a milk replacer with a protein and fat content similar to beef cow milk improved calf growth and muscle growth.

Key words: beef × dairy, cattle, growth, milk replacer, muscle

INTRODUCTION

With increased use and decreased cost of sexed semen in the dairy industry, there is a greater opportunity to breed beef sires to dairy dams to generate crossbred beef × dairy (B × D) calves to add value to surplus calves coming from dairy farms. Crossbreeding beef and dairy animals is not a novel concept (Pahnish et al., 1969). However, increased use of sexed dairy semen and the added value calves has incentivized the breeding programs of dairy farms in recent years.

Despite in-depth reviews on B × D production, selection, and genetics by Basiel and Felix (Basiel and Felix, 2022) and Berry (Berry, 2021), there are few studies regarding the nutritional requirements to optimize feeding in B × D cattle in the United States, especially during the pre-weaning period. In most beef cattle rearing systems, healthy calves are allowed ad libitum access to maternal milk throughout the pre-weaning period. In most dairy systems, calves are housed separately from their dams and milk harvested for sale. To facilitate milking their dairy dams, B × D calves are housed separately and provided with an alternative source of nutrition. However, feeding B × D calves similar to purebred dairy calves may not support optimal calf growth for beef production systems. The pre-weaning diet provided to a calf can influence the growth and production of the calf through metabolic imprinting (Khounsaknalath et al., 2021). Additionally, the negative impacts of the early life environment (particularly nutrition) on growth can increase risk of metabolic disturbances later in life (Patel and Srinivasan, 2011). For example, Wagyu calves fed greater volume of milk replacer pre-weaning and a high-concentrate grower ration until 10 mo of age had greater subcutaneous fat, perirenal

fat, and body weights (BW) when slaughtered at 30 mo of age than animals fed less milk replacer and roughage from 4 to 10 mo of age (Khounsaknalath et al., 2021). Thus, the feeding strategy during the early postnatal period in B × D calves may be an opportunity to enhance future growth and carcass composition to increase similarity to purebred beef calves.

Despite the widespread adoption of B × D crossbreeding, there is a lack of standardized feeding practices for B × D calves that has led to variability between farms. A survey reported that a majority (>60%) of dairy farmers in the Northeastern United States fed at least some waste milk from the dairy to B × D calves and 41% of operations fed, or also fed, standard milk replacer (Felix et al., 2023). Similarly, 52% of farms in the Midwest region of the United States reported feeding whole milk from the dairy to their B × D calves, with 76% pasteurizing the milk before consumption (Sterry et al., 2023). Whole milk or waste milk from the dairy can be economically efficient for farmers but can contain pathogens if not pasteurized. Further, milk composition can vary farm to farm and by season (reviewed in Schwendel et al., 2015). Beef cow milk is approximately 29% crude protein (CP) and 31% CF on a DM basis (National Academies of Sciences and Medicine, 2016), while Holstein cow milk is approximately 24% CP and 28% CF on a DM basis (Zhou et al., 2016). However, the optimal preweaning diet to support B × D crossbred calf growth is currently unknown. Improving preweaning B × D calf growth may result in calves that are more similar in muscle mass and body weight to purebred beef calves at weaning, ultimately improving carcass metrics and quality.

Although production of B × D calves has occurred for decades, studies regarding the effects of diet on neonatal growth in more intensely-managed production settings are limited. Therefore, the objective of this project was to determine how two different milk replacers affected calf growth body morphometrics and gene expression in B × D calves. We hypothesized that both male and female B × D calves fed added fat and protein through milk replacer would have increased muscle growth, muscle fiber cross-sectional area, average daily gain, BW, and morphometric measurements. We also hypothesized that calves fed the additional fat and protein milk replacer would have upregulation of regulatory genes involved in muscle hypertrophy.

MATERIALS AND METHODS

Animal Care

All procedures involving animals were reviewed and approved by the Institutional Animal Care and Use Committee at University of Connecticut (Protocol# A21-023).

Multiparous Holstein dams ($n = 42$) were inseminated with SimAngus beef semen (ABS Global, DeForest, WI) at natural estrus. Upon parturition, B × D crossbred calves ($n = 21$ males, 21 females) were separated from the dam and provided 3.8 L of colostrum from their dam by bottle at the initial postpartum feeding within 2 h of birth. At the second and third feedings (approximately 8 and 16 h after birth), 1.9 L of colostrum from their dam was provided. Calves were balanced for sex and assigned 1 of 2 milk replacers: an increased nutritional plane milk replacer (Table 1; HPHF, Bovine Innovations Group, Cortland, NY) containing 30.0% crude protein (CP), 32% crude fat (CF), or a control milk replacer with 22% CP, 20% CF (Table 1; CON, Authentic CertiFeeD

Nutritional Products, Conklin, NY). Calves were housed individually in hutch-systems and fed milk replacer three times per day by pail. Both milk replacers were fed using a step up-step down program (Table 2) recommended by Hoober Feeds (Gordonville, PA). Animals were supplied with ad libitum calf starter grain (Table 1; Bovine Innovations Group, Cortland, NY), hay, and access to fresh water. The starter grain is composed of processed grain byproducts, plant protein products, grain products, forage products, linseed meal, roughage products, Bovine Innovations Group calf base 100, Bovine Innovations Group sugar pack, molasses products, diflubenzuron and 6% decoquinate. Refusals of milk replacer were recorded. Calves were weaned at 8 wk of age.

Sample Collection

Within 24 h of parturition (day 0), BW, crown rump length (CRL), heart girth (HG) and hip height (HH) were collected from each calf. Measurements were repeated weekly thereafter until 8 wk of age. Calves were weighed again at 180 d of age. Body weight data were used to calculate the average daily gain (ADG) of each animal. Average daily gain was evaluated between day 0 and week 2, and day 0 and week 8 to account for the entire pre-weaning period.

Muscle biopsy samples were collected from the left longissimus muscle (LM) at 2 and 8 wk of age. Muscle biopsies were performed using a 14-gauge × 9 cm SuperCore Biopsy Instrument (Argon Medical Devices, Athens, TX). Before collection, the sample site was shaved, cleaned, and locally blocked with 1 mL lidocaine 2% (Covetrus, Portland, ME). Muscle samples used for RNA isolation were snap frozen in liquid nitrogen and stored at –80 °C until analysis. Muscle samples used for histology were fixed in Tissue-Plus O.C.T. Compound (Fisher Scientific, Waltham, MA). Samples were then frozen in a cooled bath of methylbutane and dry ice and stored at –80 °C until analysis. Upon finishing collection, the sample site was stapled and AluShield Aluminum Powder (Neogen, Lansing, MI) was administered. Calves

Table 1. Chemical composition of milk replacer[1] and starter[2]

Component[3]	HPHF	CON	Calf starter
Crude Protein, min (%)	30.00	22.00	22.00
Crude Fat, min (%)	32.00	20.00	2.00
Crude Fiber, max (%)	0.15	0.15	13.00
Acid Detergent Fiber, max (%)	n/a	n/a	14.00
Calcium, min (%)	0.75	0.75	n/a
Calcium, max (%)	1.25	1.25	n/a
Phosphorus, min (%)	0.70	0.70	n/a
Vitamin A, min (IU/kg)	33,000	66,000	n/a
Vitamin D, min (IU/kg)	11,000	22,000	n/a
Vitamin E, min (IU/kg)	220	330	n/a
Vitamin B12, min (mg/kg)	n/a	1.76	n/a
Ascorbic Acid, min (mg/kg)	n/a	220	n/a
Lasalocid (g/ton)	72.00	n/a	n/a
Diflubenzuron (g/ton)	13.60	n/a	n/a

[1]Calves were provided either fortified milk replacer (HPHF; 30% CP; 32% CF; $n = 20$) or a control milk replacer (CON; 22% CP, 20% CF: 20; $n = 20$).
[2]Calf starter was provided ad libitum to both groups ($n = 42$).
[3]Component is defined as ingredients within milk replacer or calf starter on a dry matter basis.

Table 2. Beef × dairy calf milk replacer feeding program[1]

Time point	L per feeding	L per d	g MR/d	HPHF		CON	
				Protein, g	Fat, g	Protein, g	Fat, g
Week 1 to 2	1.42	4.26	637.98	191.4	204.2	140.4	127.6
Week 3 to 6	1.90	5.70	853.63	256.1	273.2	187.8	170.7
Week 7	1.42	4.26	637.98	191.4	204.2	140.4	127.6
Week 8	0.95	2.85	426.81	128.0	136.6	93.9	85.4
Week 9	0.95	0.95	124.27	42.7	45.5	31.3	28.5
Week 10	--	--	--	--	--	--	--

[1]Calves were provided either fortified milk replacer (HPHF; 30% CP; 32% CF; $n = 20$) or a control milk replacer (CON; 22% CP, 20% CF: 20; $n = 20$). Amounts presented are relative to amount offered. Refusals were recorded.

were administered 15 mg per 45.4 kg BW meloxicam tablets orally (Zydus Pharmaceuticals, Pennington, NJ) immediately and 24 h after biopsy. Staples were removed 7 to 12 d post collection.

Ultrasound imaging was performed at 4 and 8 wk of age. All images were taken from the right side of the animal for determination of ribeye area (REA), backfat thickness (BFT) and rump fat thickness. Ribeye area and BFT were measured between the 12th and 13th rib, intersecting with the medial end of the LM. Rump fat was measured between the hooks and pins of the calf. Ultrasounds were performed using an Aloka500V (Corometrics Medical Systems, Wallingford, CT) ultrasound machine. A 17 cm, 3.5 mHZ linear probe was used both with and without a standoff pad to prevent misrepresentation of rib images. Images were taken at 1.5× magnification and collected with UICS2 software (UltraInsights, Inc., Maryville, MO). Images were interpreted by the CUP Lab, Inc. (Ames, IA).

Immunohistochemistry

Muscle biopsy samples were cryosectioned with a Microm HM 525 cryostat (Thermo Fisher Scientific, Waltham, MA) at 8 μm. Samples were stained with Alexa Fluor 350 Wheat Germ Agglutinin (WGA; 1:400, Thermo Fisher Scientific) to visualize muscle fiber membranes as previously described (Reed et al., 2014). An AxioCam camera mounted to an AxioObserver microscope (Zeiss, Inc., Jena, Germany) was used for imaging. For each sample, 5 to 10 pictures were taken from three to four sections. ImageJ (National Institutes of Health, Bethesda, MD) was used to analyze images for muscle fiber cross-sectional area (CSA).

Gene Expression

Isolation of RNA was performed as previously described (Reed et al., 2014; Smith et al., 2021). Longissimus muscle was homogenized using the Qiagen Tissuelyser system with 1 mL Qiazol (Qiagen,Valencia, CA) and a Qiagen Mini Kit was used to extract RNA according to the manufacturer's protocol. RNA quantity was assessed using the NanoDrop2000 UV Visible Spectrophotometer (Thermo Fisher Scientific) and quality was assessed using an Agilent Tapestation 2200 analysis system (Agilent Technologies, Santa Clara, CA). Reverse transcription (RT) and real time polymerase chain reaction (PCR) were performed as described previously (Hoffman et al., 2014). Briefly, 200 ng total RNA was combined with 1 μL random primers, 1 μL dNTP (Promega, Madison, WI), and 12 μL of RNase free water and incubated at 65 °C for

5 min. Four microliters 5× buffer (250 mM Tris-HCl [pH 8.3], 375 mM KCl, 15 mM $MgCl_2$; Invitrogen), 2.0 μL dl-dithiothreitol, and 1 μL of RNaseOUT (Invitrogen) was added and incubated at 37 °C for 2 min. One μL of M-MLV reverse transcriptase buffer was added and reverse transcription was performed with a standard protocol of 65 °C for 5 min, 37 °C for 2 min, 25 °C for 10 min, 37 °C for 50 min, 70 °C for 15 min, and then cooled to 4 °C. Primers (Supplemental Table 1) were synthesized by Integrated DNA Technologies (Coralville, IA). Real-time RT-PCR was performed using PowerUp SyberGreen Master Mix (Invitrogen) and an ABI 7500 real-time PCR system (Applied Biosystems, Foster City, CA) using standard cycling conditions (stage 1: 50 °C for 2 min and 95 °C for 10 min, stage 2: 95 °C for 15 s and 60 °C for 1 min; repeated for 40 cycles). Gene expression was determined using the 2-ΔΔCT method (Livak and Schmittgen, 2001) and changes in gene expression are expressed relative to CON calves. Glyceraldehyde-3 phosphate dehydrogenase (GAPDH) was used as the housekeeping gene for LM tissue.

Statistical Analysis

Body weight, morphometric measurements, CSA, and ultrasound data were analyzed with PROC MIXED in SAS with animal as the subject and fixed effects of milk replacer, age, sex, and their interactions. Birth weight was used as a covariate for body weight and morphometric analyses, as birth weights were significantly different between treatment groups. Age was used as a repeated measure when analyzing BW, morphometric measurements, CSA, and ultrasound data. Covariance structure was determined by the lowest Akaike's information criterion. Statistical significance was considered at $P \leq 0.05$ and a tendency at $0.05 < P \leq 0.10$. Data are presented as LSMeans ± SEM.

For gene expression, data were analyzed using the R programming language in the R Studio (version 4.2.2; R Core Team, 2021) on "Spotted Wakerobin" release for Windows, using the packages car (Fox and Weisberg, 2019), emmeans (Lenth, 2023), nlme (Pinheiro et al., 2022), ggplot2 (Wickham, 2016), ggeffects (Lüdecke, 2018) and tidyverse (Wickham et al., 2019). Gene expression data were analyzed using a three-way mixed effects analysis of variance (ANOVA) to account for repeated measures with animal (random), milk replacer (fixed), sex (fixed), and age (continuous) included in the model. Where appropriate, post hoc pairwise comparisons were made using emmeans. Statistical significance was considered at $P \leq 0.05$ and a tendency at $0.05 < P \leq 0.10$. Data are presented as LSMeans ± SEM.

RESULTS

Effects of Milk Replacer on B × D Calf BW and Morphometric Measurements

At birth, CON females were 12.5% lighter than CON males ($P = 0.02$), but not different from HPHF males or females ($P \geq 0.12$). There were main effects of age and milk replacer on offspring body weight between 1 and 8 wk of age ($P < 0.01$; Table 3). Overall, calves that consumed the HPHF milk replacer were heavier than calves consuming the CON milk replacer (HPHF: 70.7 ± 0.39 kg; CON: 68.5 ± 0.41 kg; $P < 0.01$). As expected, BW increased with age ($P < 0.01$; Table 3). There were no observed effects of sex or interactions on calf body weight ($P > 0.17$). There was a tendency for an interaction between age and sex for ADG in the first two weeks of life ($P = 0.09$; Table 4), where male HPHF calves had greater ADG than male CON calves. There were no differences observed in ADG from day 0 to day 56 ($P > 0.10$; Table 4).

An age effect was observed for CRL and HH where each variable increased from day 0 to day 56 (Table 3). There

were no observed milk replacer or sex effects, or interactions of milk replacer, sex, or age on calf CRL ($P \geq 0.10$) or HH ($P \geq 0.46$). A tendency for an age by diet interaction was observed for heart girth ($P = 0.10$; Table 5), however there were no differences between dietary treatments within a day of age ($P > 0.10$).

Effects of Milk Replacer on B × D Calf Muscle Size

There was a tendency for an interaction of milk replacer and age on muscle fiber CSA in B × D calves ($P = 0.06$; Table 6). At 8 wk of age, HPHF calves tended to have larger muscle fiber CSA than CON calves (HPHF: 1652.8 ± 87.6 μm^2; CON: 1429.3 ± 91.9 μm^2). An age effect was also observed, where calves at 2 wk of age had smaller CSA than at 8 wk of age ($P < 0.001$). No main effects of milk replacer or sex, or interactions between milk replacer and sex, age and sex, or milk replacer, age, and sex ($P \geq 0.38$) were observed. Rib eye area was 29% greater at week 8 than at week 4 ($P < 0.001$; Table 7) and there was a tendency for an age by sex interaction

Table 3. Effects of age on beef × dairy calf body weight, hip height, and crown rump length

Item[1]	Age[2]	Mean ± SEM	P-value Diet[3]	Sex	Age	Diet × Sex	Diet × Age	Sex × Age	Diet × Sex × Age
BW			<0.01	0.29	<0.01	0.92	0.31	0.17	0.42
	7	49.0 ± 0.81[a]							
	14	53.2 ± 0.82[b]							
	21	29.2 ± 0.80[c]							
	28	64.9 ± 0.79[d]							
	35	71.4 ± 0.78[e]							
	42	79.4 ± 0.79[f]							
	49	86.2 ± 0.85[g]							
	56	93.4 ± 0.78[h]							
HH			0.95	0.68	<0.01	0.90	0.53	0.54	0.18
	0	81.0 ± 0.8[a]							
	7	81.3 ± 0.8[a]							
	14	81.8 ± 0.9[a]							
	21	83.2 ± 0.6[b]							
	28	85.2 ± 0.7[c]							
	35	87.1 ± 0.8[d]							
	42	89.1 ± 0.7[e]							
	49	91.1 ± 0.8[f]							
	56	93.4 ± 1.0[g]							
CRL			0.33	0.45	<0.01	0.58	0.35	0.59	0.60
	0	87.5 ± 0.9[a]							
	7	85.7 ± 0.9[a]							
	14	88.1 ± 1.1[a]							
	21	89.3 ± 0.7[a]							
	28	92.6 ± 0.9[b]							
	35	94.9 ± 0.9[c]							
	42	99.4 ± 0.9[d]							
	49	100.9 ± 0.9[e]							
	56	104.3 ± 1.0[f]							

[1]Body weight (BW) in kg, hip height (HH) and crown rump length (CRL) in cm.
[2]Day of age.
[3]Calves were provided one of two milk replacers: HPHF (30% CP, 32% CF; *n* = 22) or CON (22% CP, 20% CF; *n* = 20).
[a–h]Indicates *P* < 0.05 within item.

(P = 0.06) although no differences were observed between sexes at week 4 or 8 of age (P > 0.30). No main effects of milk replacer, sex, or interactions of diet and sex or diet and age, or diet, sex, and age were observed (P > 0.44). No effects of age, sex, or milk replacer, or their interactions on ultrasound backfat thickness were observed (P ≥ 0.40). Rump fat thickness was undetectable in all animals.

Effects of Milk Replacer on Muscle Gene Expression

Genes related to myogenic regulation were assessed in B × D calves (Table 8). There was a tendency for a sex effect in Pax7 gene expression (P = 0.06) where males expressed 35% more Pax7 than females (P < 0.1; Table 8), however, there were no observed effects of milk replacer, age, milk replacer by age, milk replacer by sex, age by sex or milk replacer by age by sex interactions (P ≥ 0.40). A tendency for a milk replacer by age by sex interaction was observed in MyoD expression (P = 0.07), where CON females tended to express 34% more MyoD than HPHF females at 2 wk of age (P = 0.06). There was also a tendency for HPHF females to express 39% more MyoD than CON females at 8 wk of age (P = 0.09). There were no observed effects of milk replacer, age, sex, milk replacer by sex or age by sex (P > 0.33) on MyoD expression. Myogenin expression was affected by an interaction of milk replacer and age (P = 0.02), where myogenin gene expression

was 3% greater in CON calves than HPHF calves at 2 wk of age. There were no observed effects of milk replacer, sex, age, milk replacer by sex, age by sex, or milk replacer by age by sex (P ≥ 0.25) on myogenin gene expression. There were no main or interaction effects observed on MRF4 gene expression (P ≥ 0.14). There was a milk replacer by sex interaction for IGF1 gene expression (P = 0.02), where CON females tended to express 52% more IGF-1 than HPHF females (P = 0.07), with CON and HPHF males intermediate and not different from CON or HPHF females. However, there were no observed milk replacer, age, milk replacer by age, age by sex or milk replacer by age by sex interactions (P ≥ 0.14).

DISCUSSION

There is growing interest in raising B × D crossbred calves; however, little attention has been given to management practices to support growth. This is particularly important during the pre-weaning phase when B × D calves are raised on milk replacer instead of in conventional beef production systems, with ad libitum milk supplied via the dam and consumption of other feeds. Thus, the objective of this project was to determine how two different milk replacers affected calf growth body morphometrics and gene expression.

The diet fed to calves during the pre-weaning period of life can impact calf growth. Both milk replacer composition

Table 4. Effects of milk replacer, sex, and age on beef × dairy calf average daily gain[1]

| Age[2] | HPHF | | CON | | SEM[3] | P-value | | |
	Male	Female	Male	Female		Diet	Sex	Diet × Sex
0 to 14						0.59	0.80	0.09
	0.52[X]	0.41[X,Y]	0.36[Y]	0.50[X,Y]	0.06			
0 to 56						0.13	0.15	0.29
	0.88	0.86	0.85	0.73	0.06			

[1]Calves were provided one of two milk replacers: HPHF (30% CP, 32% CF; n = 22) or CON (22% CP, 20% CF; n = 20). ADG = average daily gain in kg/d.
[2]Average daily gain from day 0 to day of age indicated.
[3]Largest SEM across treatment by sex for each variable.
[X,Y]indicates tendency for a difference within a row, P < 0.10.

Table 5. Effects of milk replacer and age on beef × dairy calf heart girth[1]

| Age[2] | HPHF | CON | SEM[3] | P-value | | | | | | |
				Diet	Sex	Age	Diet × Sex	Diet × Age	Sex × Age	Diet × Sex × Age
				0.32	0.74	<0.01	0.31	0.10	0.13	0.40
0	83.5[a]	83.4[a]	0.9							
7	84.3[a]	83.8[a]	0.6							
14	86.7[b]	86.6[b]	0.7							
21	89.1[c]	88.9[c]	0.8							
28	92.7[d]	89.7[c]	1.3							
35	94.5[d]	94.6[d]	0.9							
42	99.6[e]	98.6[e]	0.8							
49	101.2[f]	101.5[f]	0.9							
56	137.5[g]	103.1[g]	0.9							

[1]Calves were provided with one of two milk replacers: HPHF (30% CP, 32% CF; n = 22) or CON (22% CP, 20% CF; n = 20). Heart girth in cm.
[2]Day of age.
[3]Largest SEM across treatment by age for each variable.
[a–g]Indicates effect within column; P < 0.05.

Table 6. Effects of milk replacer[1] and age on muscle fiber cross-sectional area[2] in beef × dairy calves

Age[3]	HPHF	CON	SEM[4]	P-value						
				Diet	Sex	Age	Diet × Sex	Diet × Age	Sex × Age	Diet × Sex × Age
				0.55	0.36	<0.01	0.39	0.06	0.99	0.50
2	827.52[a]	944.65[a]	87.6							
8	1652.8[b,X]	1429.3[b,Y]	91.9							

[1]Calves were provided one of two milk replacers: HPHF (30% CP, 32% CF; *n* = 22) or CON (22% CP, 20% CF; *n* = 20).
[2]Cross-sectional area measured in µm².
[3]Week of age.
[4]Largest SEM across treatment by age for each variable.
[a,b]Indicates differences between means *P* < 0.05.
[X,Y]Indicates tendencies between means 0.01 ≥ *P* > 0.05 within a row.

Table 7. Effects of sex and age on rib eye area[1] in beef × dairy calves

Age[2]	Female	Male	SEM[3]	P-value						
				Diet	Sex	Age	Diet × Sex	Diet × Age	Sex × Age	Diet × Sex × Age
				0.89	0.88	<0.01	0.67	0.46	0.06	0.44
2	3.51[a]	3.17[a]	0.23							
8	4.20[b]	4.44[b]	0.29							

[1]Rib eye area measured in square inches.
[2]Week of age.
[3]Largest SEM across sex by age for each variable.
[a,b]Indicates differences between means *P* < 0.05.

Table 8. Effects of milk replacer on muscle gene expression of beef × dairy calves

Item[2]	Age[3]	Milk replacer[1]				SEM[4]	P-value						
		HPHF		CON									
		Male	Female	Male	Female		Trt	Sex	Age	Trt × Sex	Trt × Age	Sex × Age	Trt × Sex × Age
Pax7							0.72	0.06	0.84	0.61	0.99	0.40	0.58
	2	1.42	1.18	2.35	0.88	0.53							
	8	1.39	1.23	1.02	1.48	0.38							
MyoD							0.63	0.75	0.33	0.74	0.06	0.95	0.07
	2	1.10[c,d]	0.73[c]	1.20[c,d]	1.10[d]	0.25							
	8	0.71[c,d]	1.02[d]	0.85[c,d]	0.62[c]	0.13							
MyoG							0.42	0.39	0.36	0.63	0.02	0.43	0.25
	2	0.88[a]	0.80[a]	1.03[b]	1.28[b]	0.34							
	8	1.02[a,b]	1.26[a,b]	0.94[a,b]	0.80[a,b]	0.17							
MRF4							0.41	0.70	0.21	0.86	0.14	0.65	0.33
	2	1.03	1.61	1.03	1.18	0.63							
	8	1.00	1.10	0.57	0.69	0.22							
IGF-1							0.14	0.36	0.19	0.02	0.16	0.41	0.15
	2	0.76[a,b]	0.69[a]	0.69[a,b]	2.20[b]	0.63							
	8	1.02[a,b]	1.01[a]	1.20[a,b]	1.04[b]	0.26							

[1]Calves were provided either fortified milk replacer (HPHF; 30% CP, 32% CF; *n* = 22) or a control milk replacer (CON; 22% CP, 20% CF; *n* = 20).
[2]Gene expression presented in fold change. Abbreviations: *IGF-1* = insulin-like growth factor 1; *MRF4* = myogenic regulatory factor 4; *MyoD* = myoblast determination protein 1; *MyoG* = myogenin; *Pax7* = Paired box 7.
[3]Age in weeks.
[4]Largest SEM across treatment × sex × age for each variable.
[a,b]Indicates *P* < 0.05.
[c,d]Indicates 0.10 ≥ *P* > 0.05.

(Blome et al., 2003) and milk replacer feeding rate (Bartlett et al., 2006) can influence calf growth rates, lean tissue deposition and body composition. In the current work, calves fed a 22% CP, 20% CF milk replacer were lighter than calves fed a 30% CP, 32% CF milk replacer throughout the preweaning period. Beef cow milk is approximately 29% CP and 31% CF

on a DM basis (National Academies of Sciences and Medicine, 2016), similar to the HPHF milk replacer fed in the current study, whereas Holstein cow milk is approximately 24% CP and 28% CF on a DM basis (Zhou et al., 2016). Feeding increased CP through milk replacer can increase the rate of gain for lean tissue, leading to a linear increase in BW within male dairy calves during the pre-weaning period (Blome et al., 2003; Bartlett et al., 2006). Thus, the increased nutrient availability likely led to better growth than control-fed calves.

Postnatal muscle growth in livestock occurs through muscle fiber hypertrophy, as the number of muscle fibers is fixed at birth (Greenwood et al., 2000). MacGhee et al. (2017) reported an increase in LM CSA in Holstein bull calves fed a milk replacer containing 27% CP and 10% CF compared with a milk replacer containing 20% CP and 20% CF. Similarly, in our B × D crossbred calves, calves fed the 30% CP, 32% CF milk replacer had greater muscle fiber CSA than B × D calves fed a 22% CP, 20% CF milk replacer. This suggests that early life nutrition can improve muscle hypertrophy, which may increase later postnatal growth and result in a better quality and quantity of product at slaughter.

Skeletal muscle hypertrophy is a highly regulated process involving multiple pathways (Schiaffino et al., 2013). Insulin-like growth factor-1 (IGF-1) is a regulator of myogenesis and muscle hypertrophy (Ahmad et al., 2020). In muscle, IGF-1 works in an autocrine/paracrine manner to activate several downstream pathways such as the mitogen-activated protein kinase (MAPK) pathway responsible for proliferation of myogenic precursor cells. Insulin-like growth factor-1 is also responsible for regulation of the phosphatidylinositol-3 kinase/protein kinase B (Akt) cascade, leading to activation of the mammalian target of rapamycin (mTOR) pathway and inducing muscle growth and differentiation (reviewed in Schiaffino et al., 2013; Ahmad et al., 2020). In a study by Haisan et al. (2018), calves fed a milk replacer at a higher plane of nutrition expressed more circulating IGF-1 and had heavier BW and increased ADG in the preweaning period compared with calves fed a lower plane of nutrition. Schaff et al. (2016) observed similar results in circulating IGF-1 concentrations in calves fed ad libitum milk replacer during the first 5 wk of life. In the current study, female CON calves expressed more IGF-1 than female HPHF calves. However, this did not translate into increased muscle hypertrophy based on muscle fiber CSA. Expression of MyoD was increased in female CON calves at 2 wk of age but reduced at 8 wk of age compared with HPHF female calves. These data suggest that there may have been some attempts at compensatory muscle growth in the first 2 wk, but they were not sustained or sufficient to support growth similar to those calves on the HPHF milk replacer.

Paired box protein 7 is one of several upstream regulators involved in myogenesis, specifically proliferation of satellite cells, which contribute heavily to postnatal muscle hypertrophy (Esteves De Lima and Relaix, 2021). Furthermore, expression of Pax7 in male yak was significantly upregulated compared with females (Wu et al., 2016). Our study reflected similar results, where male calves tended to produce more Pax7 than female calves. Myogenin and MyoD are both activated downstream of IGF-1 signaling and regulate the proliferation and differentiation of myoblasts, as well as muscle hypertrophy. Expression of MyoD and myogenin increases expression of MRF4, which is responsible for the maturation of multinucleated fibers (Buckingham and Rigby, 2014). No

effect of milk replacer on MRF4 expression was observed within our study, despite differences in BW. Increased expression of MRF4 may not occur within the first 8 wk of life, as full maturation of calves is not yet complete (Day and Nogueira, 2013).

SUMMARY AND CONCLUSION

Supporting early growth in B × D calves has the potential to increase later growth, and improving muscle mass during early postnatal life may increase carcass yield at slaughter. We demonstrated that feeding B × D calves milk replacer with 30% protein and 32% fat, similar to that of beef cow milk, improves body weight gain and muscle fiber growth compared with feeding B × D calves milk replacer containing 22% protein and 20% fat.

Supplementary Data

Supplementary data are available at *Translational Animal Science* online.

Acknowledgments

Conflict of Interest Statement

The authors report no real or perceived conflict of interest.

Author Contributions

Rachel Carter (Conceptualization, Formal analysis, Investigation, Writing—original draft, Writing—review & editing), Joesph Emenheiser (Conceptualization, Formal analysis, Funding acquisition, Investigation, Resources, Writing—review & editing), Steven Zinn (Conceptualization, Data curation, Formal analysis, Funding acquisition, Writing—review & editing), Kristen Govoni (Conceptualization, Data curation, Formal analysis, Funding acquisition, Writing—review & editing), Tara Felix (Conceptualization, Formal analysis, Funding acquisition, Writing—review & editing), and Sarah Reed (Conceptualization, Data curation, Formal analysis, Funding acquisition, Project administration, Writing—original draft, Writing—review & editing)

Funding

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