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Assessment of lactoferrin supplementation in milk replacer on calf growth, feed efficiency and scouring incidence preweaning and postweaning

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Abstract

We investigated the hypothesis that supplementing milk replacer (MR) with exogenous lactoferrin (LF) would improve average daily gain (ADG) and feed efficiency and decrease scouring incidence in dairy calves. Lactoferrin is an antimicrobial and anti-inflammatory glycoprotein naturally found in bovine colostrum and milk that is low in MR. Previous studies suggest that supplementing LF to MR enhances ADG and feed efficiency while reducing disease occurrence in pre-weaning dairy calves. In our experiment, 103 Holstein heifer calves were randomly assigned to 1 of 4 treatments in a randomized complete block design from birth to d56 of age. Each calf received 340.1 g/d of 24% protein, 20% fat basal MR fed twice daily from d1 to 42 and once daily from d43 to 49, supplemented with 0 (L0), 1 (L1), 2 (L2) or 4 (L4) g/hd/d of LF treatment (45% purity). Calves were weaned at 49d of age. Body weight was measured at d1, 14, 28, 42, 49 and 56 of age. Faecal scores were measured weekly. Milk replacer and calf starter intake was measured daily and calculated biweekly. Data were analyzed using a linear mixed model with fixed effects of LF inclusion, and random effects of source herd and nursery room. In the first two weeks of life, ADG and gain-to-feed ratio (G:F) were numerically (non-statistically) increased in L4 tended compared with L1 and L2, but this effect was not maintained throughout the rest of the pre-weaning period or entire experiment. Average faecal score during the entire 56d experiment was greater in L2 compared with L0, L1 and L4, although faecal scores of all treatment groups were generally low. Under the conditions of the present study, LF supplementation at the inclusion levels provided showed minimal effects on feed intake, growth rate or calf health.

The growth and development of pre-weaned calves is arguably one of the most important investments of a dairy farm, as the cost of raising replacement heifers accounts for approximately 15 to 20% of dairy farm expenses (Ockenden *et al.*, 2023). Consequently, an elevated plane of nutrition and care is optimal to maximize a calf's potential in this time of maturation. Measurements such as average daily gain (ADG), body weight (BW), and feed intake are important indicators of weaning, age at first calving, and first lactation milk yield, whereas parameters such as feed efficiency are highly linked to profitability (Soberon *et al.*, 2012; Gelsinger *et al.*, 2016; Boulton *et al.*, 2017). These assessments impact short and long-term expenses for producers, as poorly performing heifers can be costly, whether that be in the form of delayed breeding, increased feeding expenses, culling, or genetic impact on future generations. The importance of receiving adequate nutrition for maintaining proper immune function is further reinforced by the neonate's high susceptibility to gastrointestinal and respiratory disease, particularly in the first few weeks of life. As calf morbidity and mortality reside among the leading causes of financial loss on dairy farms worldwide, feed supplements that can mitigate these concerns and improve long-term performance outcomes would cater to the economic interests of producers (Abebe *et al.*, 2023).

One potential feed supplement that may be beneficial to calf health and growth rate is lactoferrin (LF). Lactoferrin is an antimicrobial glycoprotein that is naturally found in colostrum, milk and most exocrine secretions that helps regulate iron absorption in the body (Legrand *et al.*, 2008; Smulski *et al.*, 2020). As a member of the transferrin family, LF sequesters free iron necessary for bacterial growth and prevents both the adhesion of bacteria to epithelial host cells and the formation of biofilms in the intestine (Smulski *et al.*, 2020). Lactoferrin is also a natural immunomodulator that protects the gut by exerting anti-inflammatory effects during bacterial infections (Drago-Serrano *et al.*, 2017; Superti, 2020). In human medicine, the therapeutic use of LF is of high interest, as it decreases diarrhea and improves immunity (Drago-Serrano *et al.*, 2017). Previous research on the effect of LF supplementation within MR for calves is inconsistent. Joslin *et al.* (2002) and Robblee *et al.* (2003) both observed increased ADG and feed efficiency in pre-weaned Holstein calves supplemented with LF in MR at 1 g/d. However, more recent studies have shown no impacts of LF fed at similar amounts per day (Cowles *et al.*, 2006; English *et al.*, 2007; Pempek *et al.*, 2018). Although LF supplementation has since been shown to reduce the overgrowth of *E. coli* in the small intestine, as well as instances and severity of diarrhea and faecal shedding in calves (Prenner *et al.*, 2007; Habing *et al.*, 2017; Rybarczyk *et al.*, 2017), there has been little evidence to corroborate the improvements in growth and performance outcomes as observed by Joslin *et al.* (2002) and Robblee *et al.* (2003).

The objective of this study was to further explore effects of increased levels of LF supplementation in MR from 3 to 5 days of age to 7 weeks of age on pre- and post-weaning feed intake, growth and scouring incidence of Holstein dairy heifer calves. Whereas previous studies have been limited to a small sample size, an advantage of the present study is its access to a large sample of Holstein heifers from three commercial dairies. We hypothesized that LF supplementation would increase growth rates and feed efficiency due to its immunomodulatory properties and antimicrobial properties within the gastrointestinal tract.

Materials and methods

Animals and housing

One-hundred and four Holstein heifer calves were obtained from three commercial dairy farms in southeastern Minnesota and transported to the University of Minnesota Southern Research and Outreach Center (SROC) Calf and Heifer Facility in Waseca, MN at 2 to 5 days of age. Sample size was determined based on an 80% power of observing a $P < 0.05$ difference in ADG based on a standard deviation of 0.02 kg and an expected difference of 0.1 kg observed in previous experiments (Chester-Jones *et al.*, 2016; Jaeger *et al.*, 2020). Calves were delivered to the research farm twice per week and upon arrival, were blocked by source herd and randomly assigned to one of four treatments in a randomized block design. Calves were enrolled in the experiment between October 21, 2021 and December 17, 2022, with the experiment ending on February 11, 2022. Prior to arriving at SROC, all calves received a minimum of three feedings of 4 L colostrum each, within 48 h of birth at their source farm.

Upon arrival (d1 of experiment), calves were vaccinated intranasally for infectious bovine rhinotracheitis, parainfluenza 3 and bovine respiratory syncytial virus (Inforce-3; Zoetis Inc.; Parsippany-Troy Hills, NJ). Ten mL of blood were collected via jugular venipuncture into serum vacutainer tubes (BD; Franklin Lakes, NJ), allowed to clot, centrifuged at 1500 x *g* and serum total protein (STP) was analyzed using a Brix refractometer (Spartan Refractometer, model A 300 CL, Spartan, Tokyo, Japan). Body weight (BW) was measured using a livestock scale (VS-660; A and A Scales LLC; Wyckoff, NJ), and hip height (HH) was measured using a wooden measuring stick with sliding bar (Nasco Education; Fort Atkison, WI). Enrollment criteria required calves have a serum total protein concentration (STP) greater than 4.8 and have an initial BW between 34.0 and 47.6 kg. Serum total protein concentration (μ = 6.10; SEM = 0.16; P=0.72) and initial BW (P=0.99; Table 2) did not differ among calves assigned to each of the four treatments prior to treatment administration. Each calf was placed in an individual pen (2.3 x 1.2 m) bedded with wood shavings and straw, located inside one of four naturally-ventilated nursey rooms with two-curtain sidewalls (Chester-Jones *et al.*, 2016; Jaeger *et al.*, 2020). Calves were placed into nursery rooms sequentially by arrival date. All procedures for animal care and handling were approved by the University of Minnesota Institutional Animal Use and Care Committee (Protocol number: 2110-39487A), with feeding, BW measurements, and faecal scoring conducted by trained research staff at the University of Minnesota SROC.

Diets and treatments

Treatments (n = 26 per treatment) included 340.2 g/d of 24% protein, 20% fat basal milk replacer (12.5% wt/vol in water) supplemented with 0 (L0), 1 (L1), 2 (L2) or 4 (L4) g/calf/d of LF (45%purity: Table 1). The diet was fed 2x/d (every 12 h) between d1 to 42, then switched to 1x/feeding in the morning from d43 to 49. The LF was a commercially prepared product (Milk Specialties Global, Eden Prairie, MN; iron saturation: 13.2 mg/100 g) added directly to the MR powder, with treatment concentrations confirmed by the manufacturer. Beginning on d1, calves were offered a commercial 18% CP texturized calf starter (CS; Hubbard Feeds, Mankato, MN) containing decoquinate at 50.04 mg/kg (Table 1), fed to maintain a 5% refusal rate. Damp CS was replaced daily. Water was available *ad libitum* throughout the entirety of the study. Samples were taken directly from all bags of MR powder and CS weekly, composited by treatment, stored at − 20°C and later analyzed for nutrient composition using AOAC approved wet chemistry analysis methods (Dairyland Laboratories, Inc, Sauk Rapids, MN). Intake and refusals of MR were measured daily and composited biweekly. Intake and refusals of CS were measured weekly and composited biweekly. Calves were weaned at d49 and removed from the experiment on d56.

Data collection

Body weight was measured at d1, 14, 28, 42, 49 and 56 of the experiment in the afternoon before the evening feeding on each of those days and ADG was calculated for each interval within BW measurements. Gain-to-feed ratio (G:F) was calculated as kg of BW gain divided by kg of total feed intake over the same time period. Hip height (HH) was measured on d1 and 56, and HH gain during the experiment was calculated. Faecal scores were recorded daily by trained SROC personnel using a 4-point scale, in which 1 = normal consistency, 2 = semi-formed or pasty, 3 = loose, and 4 = very loose with watery separation (Larson *et al.*, 1977). Calves were considered positive for scouring when the faecal consistency score was ≥ 3. The frequency of scouring was determined by averaging the number of days that each calf had a faecal score of ≥ 3. Milk replacer intake, CS intake, total feed intake, ADG, G:F and average faecal score were determined for each of the intervals between d1 to 14, 15 to 28, 42 to 49 and 49 to 56.

Statistical analysis

Data were analyzed as a linear mixed effects model using the PROC MIXED procedure of SAS 9.4 with fixed effects of treatment and the random effects of source herd and nursery room. The effects of LF concentration on daily MR intake, CS intake, total intake, ADG

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Table 1. Nutrient composition of milk replacer and calf starter

	Milk Replacer[1]				
Item	L0	L1	L2	L4	Calf Starter All Treatments
Dry matter, %	96.9	96.8	96.8	96.8	87.5
CP, % of diet DM	25.4	25.5	24.5	27.3	21.9
ADF, % of diet DM	-	-	-	-	10.2
aNDF, % of diet DM	0.16	0.27	0.09	0.05	22.1
aNDFom, % of diet DM	-	-	-	-	19.9
Starch, % of diet DM	-	-	-	-	32.5
Fat, % of diet DM	20.5	20.0	20.5	19.3	3.55
Ash, % of diet DM	8.37	8.41	8.55	8.40	7.78
Ca, % of diet DM	1.34	1.34	1.34	1.41	1.50
P % of diet DM	0.79	0.81	0.81	0.83	0.62
Mg, % of diet DM	0.15	0.14	0.14	0.13	0.31
K, % of diet DM	1.63	1.59	1.63	1.51	1.43
S, % of diet DM	0.38	0.37	0.37	0.35	0.38

[1]Treatments included 680.25 g/d of 24% protein, 20% fat basal MR (12.5% w/v in water) supplemented with 0 (L0), 1 (L1), 2 (L2) or 4 (L4) g/d of lactoferrin (45% purity).

and average faecal score over the entire pre-weaning period (d1 to 49) and the entire experimental period (d1 to 56) were analyzed using repeated measures in the MIXED procedure of SAS with the fixed effect of LF concentration, random effects of source herd and nursery room as well as the repeated effect of time interval. The heterogeneous autoregressive (ARH1) covariance structure was used. Initial BW was used as a covariate for ADG in the repeated measures analysis. Linear and quadratic orthogonal contrasts were tested for all responses, and preplanned contrasts were used to determine differences between the least squares means of individual treatments. For all analyses, data points with studentized residuals outside ± 3.5 were removed as outliers. Statistical significance was declared at $P < 0.05$. Heteroscedasticity was determined by generating a histogram and normal Q-Q plot of residuals in PROC MIXED.

Results

Body weight, ADG and Hip height

Data are shown in Table 2. Average daily gain was quadratically affected by LF inclusion during the first two weeks of life ($P = 0.02$), with greater ADG in L4 and L0 compared to L1 and L2. This resulted in a greater BW at d14 in L4 compared to L1 and L2 ($P < 0.05$). A similar effect was also observed from d 43 to 49, where L4 and L0 had numerically (non-significantly) greater ADG ($P = 0.07$) compared to L1 and L2 during that period. Average daily gain was unaffected by LF treatment during all other intervals of the study and did not differ ($P > 0.05$) during the full pre-weaning period or throughout the whole experiment. Similarly, no differences in BW were observed at the end of the experiment (d56; $P = 0.39$). Furthermore, there were no differences ($P > 0.05$) in the initial HH, d56 HH or HH gain among treatments.

Feed intake

Data are shown in Table 3. Milk replacer intake during days 15 to 28 differed among treatments ($P < 0.05$) in a numerically linear inverse fashion ($P = 0.07$), and a quadratic effect ($P = 0.03$) with L1, L2, and L3 all reducing feed intake by 0.3% compared to L0 ($P < 0.05$; Table 3). Furthermore, CS intake per day across the entire pre-weaning period was affected by treatment ($P = 0.05$), with L2 decreasing feed intake compared to L0 ($P < 0.01$), but no differences occurring among the other treatments ($P > 0.05$). Calf starter intake did not differ among groups for any other 2-week period of the experiment, during the entire pre-weaning period or during the entire study (all $P > 0.05$; Table 3). During the first two weeks of the study, total feed intake was decreased by 3.2% and 3.9% in L1 and L2, respectively, compared to L0 and L4 ($P < 0.05$). However, total feed intake did not differ across treatments during any other period of the experiment, the entire pre-weaning period or the entire experiment (all $P > 0.05$).

Feed efficiency

Data are shown in Table 4. During the first two weeks of the experiment LF treatment affected G:F ($P = 0.02$), and a quadratic effect was observed ($P = 0.01$), with L0 and L4 having greater G:F compared to L1 and L2 ($P < 0.05$; Table 4). Between d43 to 49, L1 showed a numerical (non-significant) lower efficiency ($P = 0.08$) than L0, L2 and L4. However, LF treatment did not affect G:F throughout the pre-weaning period or the overall study (both $P > 0.05$).

Scouring incidence

Data are shown in Table 5. Average faecal score was numerically (non-significantly) higher in L2 compared to L0, L1 and L4 during the pre-weaning period ($P = 0.07$; Table 5). Similarly, L2 increased average faecal score compared to the other three treatments during the post-weaning period ($P < 0.01$) resulting in a 7.8%, 5.5% and 5.5% greater average faecal score than L0, L1 and L4, respectively, across the entire experiment ($P = 0.02$). However, no differences in scouring frequency (days with faecal score ≥ 3) were observed

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Table 2. Effects of increasing supplementation of lactoferrin in milk replacer on growth of dairy calves

Item	Treatment[1]				SEM	*P*- Value[2]		
	L0	L1	L2	L4		Trt	Linear	Quad
N	26	25	26	26	–	–	–	–
BW, kg								
d 1	39.7	39.9	39.9	39.7	0.7	0.99	0.99	0.77
d 14	44.5[xy]	44.1[y]	43.9[y]	44.8[x]	0.3	0.07	0.48	0.02
d 28	53.7	53.5	52.5	53.9	0.5	0.17	0.93	0.09
d 42	66.0	66.5	65.4	67.4	0.7	0.17	0.31	0.22
d 49	74.1	73.9	73.4	75.8	0.8	0.14	0.17	0.10
d 56	82.4	82.5	81.6	83.8	1.0	0.39	0.38	0.27
ADG, kg								
d 1 to 14	0.33[xy]	0.30[y]	0.29[y]	0.35[x]	0.02	0.07	0.48	0.02
d 15 to 28	0.66	0.67	0.62	0.65	0.03	0.62	0.59	0.61
d 29 to 42	0.88[y]	0.93[xy]	0.92[xy]	0.96[x]	0.03	0.22	0.06	0.84
d 43 to 49	1.16[xy]	1.08[y]	1.16[y]	1.21[x]	0.04	0.07	0.14	0.07
d 50 to 56	1.17	1.22	1.17	1.14	0.06	0.82	0.55	0.54
d 1 to 49	0.70	0.69	0.68	0.73	0.02	0.14	0.17	0.10
d 1 to 56	0.76	0.76	0.74	0.78	0.02	0.38	0.38	0.26
HH, cm								
d 1	82.6	81.5	81.0	81.3	0.8	0.37	0.15	0.33
d 56	94.5	93.5	93.2	93.7	0.5	0.10	0.10	0.06
HH Gain	12.0	11.9	12.2	12.2	0.5	0.91	0.58	0.94

[1]Treatments included 680.25 g/d of 24% protein, 20% fat basal MR (12.5% w/v in water) supplemented with either 0 (L0), 1 (L1), 2 (L2) or 4 (L4) g/d of lactoferrin (45% purity). Treatments with different superscripts were considered different at ($P < 0.05$; a, b, c), or numerically but non-significantly different ($0.05 < P < 0.10$; x, y, z)
[2]*P*-Value of the overall model (Trt) and linear and quadratic (Quad) contrasts.

during the pre-weaning period ($P > 0.05$), with no calves across any treatments experiencing scours post-weaning.

Discussion

Increased ADG during the pre-weaning period is correlated with increased BW at calving and greater first lactation milk yield (Van De Stroet *et al.*, 2016; Chester-Jones *et al.*, 2017). Therefore, producers are likely to receive long-term benefits from investing in strategies that can improve the growth of neonatal calves. Joslin *et al.* (2002) observed that supplementing LF at 1 or 10 g/d in MR increased ADG of Holstein calves by 0.12 and 0.03 kg/d respectively. Furthermore, they observed the addition of 1 g/d of LF increased intake of CS and reduced the number of days to weaning. Similarly, Robblee *et al.* (2003) observed increased ADG and improved feed efficiency in preweaning Holstein calves when MR was supplemented with 1 g/d of LF. In contrast, more recent research has failed to observe improved ADG when 0.5 to 1.0 g of LF were fed per calf per day (Cowles *et al.*, 2006; English *et al.*, 2007). Pempek *et al.* (2019) similarly observed no improvements in ADG when calves were orally administered 3 g LF/d in a 30 mL aqueous solution for 3 consecutive days on five commercial dairy farms.

We observed minimal effects of LF on ADG. During the first two weeks of life, LF at 4 g/d increased ADG compared to the 1 and 2 g/d treatments, but not compared to the control, which contained no supplemental LF. However, this effect was numerically small and did not persist throughout the pre-weaning period or the entire experiment. One potential explanation for the disparity in effects of LF on ADG between the current study and those conducted by Joslin *et al.* (2002) and Robblee *et al.* (2003) is dietary CP concentrations. In both earleir studies the MR had a lower basal protein concentration (22.4% and 21.9% of diet DM, respectively) than ours (25.4%). Similarly, English *et al.* (2007) fed a 25.6% CP MR and also failed to observe a difference in ADG due to LF addition, which may suggest that benefits of LF are less evident with higher dietary CP concentrations. However, Cowles *et al.* (2006) conducted a 2 × 2 factorial examining the effect of LF and MR CP concentration (20.5% vs 27.4%) and failed to observe an interaction between CP concentration and LF inclusion.

Intake of MR and CS during the pre-weaning period can be used to monitor calf progress and achieve target growth rates, as grain intake stimulates rumen development. Joslin *et al.* (2002) observed increased CS intake in Holstein calves during the pre-weaning period (first 35d of life) when supplementing LF to MR with both 1 and 10 g/d LF. However, in agreement with Robblee *et al.* (2003) and English *et al.* (2007), we found no difference in CS intake when offering supplemental LF. We did observe a slight decrease in both MR intake and total feed intake in L1 and L2 compared to L0 and L4 during the first two weeks of life. The reduction in MR intake by L2 was maintained during the entirety of the pre-weaning period. However, this difference was numerically small

Table 3. Effects of increasing supplementation of lactoferrin in milk replacer on milk replacer, calf starter, and total feed intake of dairy calves

	Treatment[1]					P- Value[2]		
Item	L0	L1	L2	L4	SEM	Trt	Linear	Quad
Milk Replacer Intake, g/d								
d 1 to 14	643	632	623	633	5.87	0.12	0.14	0.07
d 15 to 28	660[a]	658[b]	658[b]	658[b]	0.37	0.04	0.07	0.03
d 29 to 42	660[a]	658[c]	659[b]	658[c]	0.070	<0.001	<0.001	<0.001
d 43 to 49[3]	329	329	329	329	0.000	–	–	–
d 1 to 49	608[a]	604[ab]	601[b]	604[ab]	2.06	0.05	0.08	0.04
Calf Starter Intake, g/d								
d 1 to 14	46.9	35.4	41.2	53.6	5.69	0.08	0.26	0.02
d 15 to 28	360	316	284	333	31.0	0.28	0.36	0.10
d 29 to 42	786	790	788	860	48.0	0.52	0.24	0.41
d 43 to 49	1,637	1,603	1,625	1,719	59.6	0.45	0.27	0.24
d 50 to 56	2,480	2,394	2,485	2,546	80.9	0.56	0.37	0.34
d 1 to 49	576	558	549	603	28.5	0.49	0.51	0.17
d 1 to 56	812	785	793	844	32.5	0.49	0.43	0.18
Total Feed Intake, g/d								
d 1 to 14	693[a]	671[b]	666[b]	689[a]	36.5	0.05	0.77	0.01
d 15 to 28	1,018	975	942	993	125	0.27	0.36	0.10
d 29 to 42	984	966	975	1,026	121	0.45	0.27	0.24
d 43 to 49	1,970	1,937	1,955	2,050	238	0.45	0.27	0.24
d 50 to 56	2,480	2,394	2,485	2,546	323	0.56	0.37	0.34
d 1 to 49	1,184	1,161	1,151	1,206	114	0.44	0.57	0.14
d 1 to 56	1,349	1,315	1,320	1,375	130	0.46	0.47	0.15

[1]Treatments included 680.25 g/d of 24% protein, 20% fat basal milk replacer (12.5% w/v in water) supplemented with either 0 (L0), 1 (L1), 2 (L2) or 4 (L4) g/d of lactoferrin (45% purity). Treatments with different superscripts were considered different at ($P < 0.05$; a, b, c), or numerically but non-significantly different ($0.05 < P < 0.10$; x, y, z)
[2]*P*-Value of the overall model (Trt) and linear and quadratic (Quad) contrasts.
[3]Calves across all treatments consumed the same amount of milk replacer for days 43 to 49. Therefore, we were unable to statistically compare milk replacer intake during this period.

Table 4. Effects of increasing supplementation of lactoferrin in milk replacer on feed efficiency of dairy calves

	Treatment[1]					P- Value[2]		
Item	L0	L1	L2	L4	SEM	Trt	Linear	Quad
Gain per feed, kg/kg								
d 1 to 14	0.48[a]	0.46[b]	0.42[c]	0.52[a]	0.03	0.02	0.39	0.01
d 15 to 28	0.64	0.68	0.64	0.66	0.02	0.62	0.93	0.65
d 29 to 42	0.61	0.64	0.65	0.63	0.01	0.32	0.32	0.11
d 43 to 49	0.59[x]	0.55[y]	0.60[x]	0.59[x]	0.02	0.08	0.49	0.21
d 1 to 49	0.59	0.60	0.60	0.61	0.01	0.31	0.08	0.59
d 1 to 56	0.57	0.57	0.57	0.57	0.01	0.90	0.47	0.94

[1]Treatments included 680.25 g/d of 24% protein, 20% fat basal MR (12.5% w/v in water) supplemented with 0 (L0), 1 (L1), 2 (L2) or 4 (L4) g/d of LF (45% purity). Treatments with different superscripts were considered different at ($P < 0.05$; a, b, c), or numerically but non-significantly different ($0.05 < P < 0.10$; x, y, z)
[2]*P*-Value of the overall model (Trt) and linear and quadratic (Quad) contrasts.

(7 g/d lower than L0) and unlikely to be of biological or economic consequence.

Optimizing feed efficiency in dairy calves is key for reducing overall costs of raising replacement heifers. It was hypothesized that increasing levels of LF administration would improve feed efficiency, consistent with reports by Robblee *et al.* (2003) who reported a linear increase in feed efficiency during the pre-weaning period (first 28 d of life) with inclusion of 1, 2 and 3 g/d LF to MR. In the current study, feed efficiency increased in L4 by 15% and 24% compared to L1 and L2, respectively. However, LF inclusion did not

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Table 5. Effects of increasing supplementation of lactoferrin in milk replacer on scouring incidence of dairy calves

Item	Treatment[1]				SEM	*P*-Value[2]		
	L0	L1	L2	L4		Trt	Linear	Quad
Average Faecal Score								
d 1 to 14	1.90	1.94	1.97	1.90	0.07	0.79	0.95	0.33
d 15 to 28	1.48[b]	1.60[ab]	1.69[a]	1.51[b]	0.06	0.03	0.44	0.01
d 29 to 42	1.23	1.16	1.26	1.27	0.05	0.38	0.26	0.42
d 43 to 49	1.02[b]	1.06[b]	1.17[a]	1.11[ab]	0.05	0.15	0.09	0.30
d 50 to 56	1.02[b]	1.03[b]	1.18[a]	1.04[b]	0.04	0.004	0.22	0.03
d 1 to 49	1.46[b]	1.50[b]	1.57[a]	1.50[b]	0.03	0.07	0.19	0.07
d 1 to 56	1.41[b]	1.44[b]	1.52[a]	1.44[b]	0.03	0.02	0.14	0.03
Scouring Frequency, d $\geq$ 3								
d 1 to 49[3]	2.53	2.78	3.85	0.46	0.46	0.13	0.28	0.14

[1]Treatments included 680.25 g/d of 24% protein, 20% fat basal MR (12.5% w/v in water) supplemented with 0 (L0), 1 (L1), 2 (L2) or 4 (L4) g/d of LF (45% purity). Treatments with different superscripts were considered different at ($P < 0.05$; a, b, c), or numerically but non-significantly different ($0.05 < P < 0.10$; x, y, z)
[2]P-Value of the overall model (Trt) and linear and quadratic (Quad) contrasts.
[3]Scouring did not occur during the post-weaning period (d 50 to 56) for any calves.

improve feed efficiency compared to the basal MR. This disparity among treatment groups within the first two weeks of life may be due to the naivety of the neonatal immune and digestive system at this early stage. In the initial weeks of life, a calf's immune system is still developing, and many functional antibody components do not develop until between two to four weeks of age. The 4 g/d level of supplementation may have provided additional immune support against subclinical disease during this period when the neonatal immune system is particularly susceptible. Additionally, during the first two weeks of life, the underdeveloped digestive tract of the calf and enterocytes in the intestines are more susceptible to change (Meale *et al.*, 2017). In human and animal models, LF supplementation at high levels has been shown to positively affect intestinal development by increasing intestinal epithelial cell proliferation and enhancing maturation of intestinal mucosa (Conesa *et al.*, 2023). Therefore, it is possible that LF supplementation at 4 g/d aided in intestinal growth resulting in slightly improved G:F and BW values that were not observed in groups receiving LF supplemented at lower doses.

Our failure to see major differences may have been partially due to the robust health of the calves and the fact that the CS provided was medicated with decoquinate. Decoquinate is a coccidiostatic agent that inhibits the growth of enteric parasites (Keeton and Navarre, 2018). Given that one of the major potential mechanisms of LF supplementation is limiting colonization and proliferation of enteric pathogens, the presence of decoquinate may have masked potential effects of LF (Hulbert and Moisá, 2016). Heinrichs *et al.* (1990) demonstrated that addition of decoquinate into CS increased ADG when calves were naturally exposed to sporulated coccidial oocysts. Previous studies demonstrating increased gain due to LF addition were fed non-medicated MR and CS (Joslin *et al.*, 2002; Robblee *et al.*, 2003). However, several other studies that failed to show impacts of LF addition also used unmedicated CS (Cowles *et al.*, 2006; English *et al.*, 2007). Disparity in results could be related to disease exposure in calves, but this was not directly quantified in any of these reports. Our average faecal scores across all treatments were low ($\mu = 1.51$; SEM = 0.07), compared to studies reporting improved growth due to LF (Joslin *et al.*, 2002: $\mu = 2.50$; SEM = 0.09; Robblee *et al.*, 2003: $\mu = 2.34$; SEM = 0.05). These results further support the idea that general health of calves was high and may have mitigated potential benefits of LF. Our calves also received MR up until 49d of age, compared to earlier studies where calves were weaned at 35d of age and as early as 21d of age (Joslin *et al.*, 2002: Robblee *et al.*, 2003, respectively). This may explain why we did not see as pronounced effects from LF supplementation. Our calves consumed liquid feed for a longer period, which may have supplied a greater and more consistent plane of nutrition, which is often associated with a stronger immune system (Leal *et al.*, 2018; Lorenz *et al.*, 2021). This theory is limited by the absence of microbiome and blood metabolite data that would have provided a better assessment of health status. Future research studying the potential benefits of LF in MR should consider pathogen exposure, possible interactions with other medications, and the collection of additional health parameters.

Previous research suggests that bovine LF becomes less capable of effectively reducing disease occurrence and supporting intestinal maturation when supplemented at high doses, and instead induces a pro-inflammatory response (Drago-Serrano *et al.*, 2017; Superti, 2020). The results of Robblee *et al.* (2003) demonstrated that average faecal scores increased linearly with LF dose. In the present study, there is no indication that increasing levels of LF supplementation induced a pro-inflammatory response in the subjects, as there were no differences among groups in scouring frequency. Instead, calves receiving 2 g/d LF had greater faecal consistency scores compared to those receiving 4 g/d. Cowles *et al.* (2006) suggests similar findings, where LF treatment had no effect on faecal consistency score, and scores of all calves were altogether low.

In conclusion, supplementing MR with LF did not provide consequential benefits to the neonatal calf, having minimal effects on feed intake, growth rate and calf health. Between d1 to 14, calves receiving 4 g/d LF showed numercially (non-significantly) increased G:F and ADG compared to 1 and 2 g/d, but these increased values were similar to calves receiving no LF. Supplementing MR with 2 g/d LF produced a numerical (non-significant) increase in average faecal scores compared to all other treatments during the pre-weaning period and overall duration of the study, however, no differences were seen in scouring frequency, and faecal scores of all groups were generally low. Considering the antimicrobial and immunomodulatory properties of LF, and the robust health of the calves enrolled in the study, future studies to determine the optimal level of LF may benefit by using a challenge models to understand potential benefits of LF supplementation in MR during stress or disease.

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