Lactoferrin Supplementation to Holstein Calves During the Prewaning and Postweaning Phases

E. A. English,* B. A. Hopkins,*¹ J. S. Stroud,* S. Davidson,* G. Smith,† C. Brownie,‡ and L. W. Whitlow*

*Department of Animal Science,

†Department of Population Health and Pathobiology, and

‡Department of Statistics, North Carolina State University, Raleigh 27695-7621

ABSTRACT

Sixty Holstein calves (30 bulls, 30 heifers) were used to examine the effects of supplemental lactoferrin on feed intake, growth, and health during the preweaning and postweaning periods. One of 3 levels of lactoferrin was supplemented from 3 to 56 d in either whole milk or water to produce 3 dietary treatments: 1) 0 g/d, 2) 0.5 g/d, and 3) 1 g/d. Whole milk (3.8 L/d) containing lactoferrin supplements was fed from bottles until weaning at 35 d. From d 36 to 56, lactoferrin supplements were added to water (15 to 25 mL) and fed from bottles. Lactoferrin supplementation had no effect on feed intake, body weight, average daily gain, heart girth, body temperature, fecal scores, respiratory scores, or haptoglobin concentrations. Calves were housed in individual pens in either an open-sided barn or hutches. Calves raised in the barn consumed more calf starter and therefore grew better than calves raised in hutches. Under the conditions of this study, lactoferrin supplementation was not beneficial. Further research is needed to fully elucidate the role of lactoferrin, and possible benefits during different feeding conditions or milk sources.

Key words: calf, lactoferrin, weaning

INTRODUCTION

Lactoferrin (LF) is an iron-binding glycoprotein found in a variety of body secretions including tears, bronchial mucus, and saliva (Brock, 1985). Kidney, endometrium, and seminal vesicles also secrete LF (Masson and Heremans, 1971) and it is found in high concentrations in the mammary secretions of nonlactating dairy cattle (Rejman et al., 1989). Lactoferrin is also present in milk and colostrum. Lactoferrin concentration in bovine colostrum is approximately 2 mg/mL (Tsuji et al., 1990), whereas bovine milk contains 20 to 200 µg/mL (Masson and Heremans, 1971). Lactoferrin has been shown to

have antiparasitic, antifungal, and antibacterial activities (Wakabayashi et al., 1996; Omata et al., 2001; Hara et al., 2002). One of LF's most prevalent functions is its role in the inhibition of bacterial growth. Lactoferrin primarily inhibits proliferation of gram-negative bacteria, such as *Escherichia coli* (Ellison et al., 1988; Ellison and Giehl, 1991; Teraguchi et al., 1997). Reducing bacterial growth, especially that of *E. coli*, is of particular interest to dairy producers, because *E. coli* is a common pathogen associated with calf diarrhea.

Lactoferrin has been supplemented to preweaned dairy calves to improve calf health and growth. Joslin et al. (2002) supplemented milk replacer with 0, 1, or 10 g/d of LF. Lactoferrin supplementation increased preweaning starter DMI, ADG, and average daily heart girth gain. Calves were weaned at 35 d and the trial continued through 56 d; however, LF was not fed postweaning. No effects were seen after LF supplementation ended. Robblee et al. (2003) also supplemented milk replacer with 0, 1, 2, or 3 g/d of LF. A quadratic effect was observed for fecal scores and number of days medicated. Calves fed 1 g/d of supplemental LF had the lowest preweaning fecal scores and number of days medicated, whereas calves fed no supplemental LF had the highest values. Preweaning ADG and feed efficiency increased as the amount of supplemental LF fed to calves increased. Lactoferrin supplementation concluded at weaning, but the trial continued through 14 d postweaning. Effects of supplemental LF observed preweaning did not carry over to the postweaning period. Cowles et al. (2006) supplemented conventional and intensified milk replacer with either 0 or 1 g/d of supplemental LF. Calves were weaned at 42 d. Lactoferrin was not supplemented after weaning, but calves remained on trial through 14 d postweaning. There were no significant effects of LF supplementation on growth or health parameters at any time during the study.

To these authors' knowledge, LF supplementation of whole milk and supplementation of LF postweaning have not been evaluated. In previous work, LF was equally effective when supplemented to milk replacer at 1 g/d compared with higher levels and has not been

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¹Corresponding author: Brinton_Hopkins@ncsu.edu

supplemented at levels <1 g/d. To provide supplemental LF as an economical part of a successful calf health program, the lowest effective level needs to be determined. The objective of this study was to determine the effect of feeding whole milk supplemented with either 0.5 or 1 g/d of LF vs. whole milk with no added LF on growth and health of Holstein calves weaned at 35 d of age with postweaning supplementation of LF continued through 56 d of age.

MATERIALS AND METHODS

Calves and Treatments

This experiment was reviewed and approved by the North Carolina State University Institutional Animal Care and Use Committee.

At birth, 60 Holstein calves (30 bulls and 30 heifers) from the Piedmont Research Station (Salisbury, NC) were randomly assigned to 1 of 3 treatments: 0 (control), 0.5, or 1 g/d of supplemental LF. Calves were not twins and weighed a minimum of 34 kg at birth. Newborn calves were removed from their dams as soon as possible and fed 3.8 L of good quality colostrum, as indicated by a colostrometer using the procedure outlined by the manufacturer (Biogenics, Mapleton, OR). Calves were fed 3.8 L of colostrum via nipple bottle or esophageal feeder on the first day of life and transition milk on d 2. Calves were housed individually in outdoor fiberglass hutches with an attached, fenced outside exercise area or in individual pens in an open-sided barn (228.6 cm height of opening) with a roof height of 381 cm. The same numbers of calves were housed in the barn and in hutches, with calf numbers balanced by treatment. The housing area per calf was similar. The hutches and pens were bedded with a sand and wood chip mixture. Calves remained in their hutches or pens for the duration of the trial. Dehorning and castration were performed after the trial was completed. The trial began in January and ended in October.

Beginning at 3 d, calves were fed 3.8 L of saleable whole milk (average 3.48% fat and 2.95% protein) from cows tested negative for bovine leukosis virus (BLV) and John's disease once per day. If the calf did not consume the total amount of milk, it was offered again throughout the day until all milk was consumed. The appropriate weighed amount of supplemental LF was dissolved into a small amount of milk and brought to a volume of 1.9 L for each calf and fed from a bottle, followed by feeding an additional 1.9 L of milk without supplemental LF from the same bottle. Supplemental LF was a commercially prepared product (DMV International, Veghel, the Netherlands). The amount of supplemental LF product fed was adjusted for ingredient composition to provide 0.5 or 1 g of pure supplemental LF per calf daily. All

Table 1. Chemical analysis of calf starter^{1,2}

Item	
DM, %	92.76
CP, % of DM	25.56
TDN, ³ % of DM	77.37
ADF, % of DM	8.45
NDF, % of DM	17.69
Ca, % of DM	1.26
P, % of DM	0.79
Mg, % of DM	0.31
Fe, mg/kg	322.12

¹Analyses conducted by Cumberland Valley Analytical Services, Inc. (Maugansville, MD).

²A vitamin-trace mineral pack was added to provide the following supplemental nutrients: vitamin A (6.8 kIU/kg), vitamin D₃ (2.0 kIU/kg), vitamin E (19.5 IU/kg), cobalt 0.86 mg/kg, copper 21.5 mg/kg, iodine 1.2 mg/kg, iron, 44.2 mg/kg, manganese 120.8 mg/kg, selenium 0.73 mg/kg, zinc 129.5 mg/kg.

³The Ohio Agricultural Research and Development Center Ohio Summative Energy equation was used to estimate total digestible nutrients (TDN).

calves were successfully weaned at 35 d of age with no obvious detrimental effects. From weaning through 56 d, supplemental LF was mixed with approximately 15 to 25 mL of water and fed from bottles daily. Control calves were fed the same amount of water without supplemental LF from bottles. Starting at 3 d and continuing until 56 d, calves had unlimited access to a nonmedicated commercial calf starter (Deal-Rite Feeds, Inc., Statesville, NC; Table 1) and fresh water. No hay was fed.

Calf Starter Analysis

Starter was sampled weekly and composited monthly. Starter orts were sampled 3 times a week and composited monthly. All samples were dried and ground through a 1-mm screen using a Wiley mill (Arthur H. Thomas, Philadelphia, PA) and analyzed by Cumberland Valley Analytical Services, Inc. (Maugansville, MD). Dry matter, CP, ADF, and mineral concentrations were analyzed using AOAC (2000) methods. Neutral-detergent fiber concentration was determined using the procedure of Goering and Van Soest (1970), as modified by Mertens (1992). The Ohio Agricultural Research and Development Center Ohio Summative Energy equation was used to estimate total digestible nutrients.

Measurements

Within 24 h of birth, and every week thereafter, measurements were made for calf BW, hip height, and heart girth. Fecal scores were recorded daily using a scale of 1 to 4, with 1 = normal, 2 = soft, 3 = runny, and 4 = watery. Respiratory scores were recorded daily on a scale

Table 2. Least squares means for overall, preweaning, and postweaning daily calf starter DM, CP, ADF, and NDF intake for calves fed differing amounts of supplemental lactoferrin and for calves reared in different types of housing

Item	Lactoferrin ¹ (g/d)			SEM	P-value	Housing		SEM	P-value
	0	0.5	1			Barn	Hutch		
DMI, ² kg/d									
Overall	0.54	0.54	0.57	0.04	0.86	0.61 ^a	0.48 ^b	0.03	0.01
Preweaning	0.17	0.20	0.19	0.02	0.81	0.20	0.16	0.02	0.17
Postweaning	1.14	1.13	1.21	0.07	0.72	1.30 ^a	1.02 ^b	0.06	0.001
CP intake, kg/d									
Overall	0.14	0.14	0.14	0.01	0.83	0.16 ^a	0.12 ^b	0.01	0.005
Preweaning	0.04	0.05	0.05	0.01	0.80	0.05	0.04	0.01	0.17
Postweaning	0.29	0.29	0.31	0.02	0.68	0.33 ^a	0.26 ^b	0.02	0.0007
NDF intake, kg/d									
Overall	0.09	0.10	0.10	0.01	0.86	0.11 ^a	0.09 ^b	0.01	0.005
Preweaning	0.03	0.04	0.03	0.004	0.72	0.04	0.03	0.003	0.16
Postweaning	0.20	0.20	0.21	0.01	0.75	0.23 ^a	0.18 ^b	0.01	0.0007

^{a,b}Means within a row with different superscripts differ ($P < 0.05$).

¹Calves were supplemented with 0, 0.5, or 1 g/d of lactoferrin added to the liquid portion of the diet during the preweaning and postweaning phase.

²DMI includes intake of calf starter only.

of 1 to 4, with 1 = normal, 2 = runny nose, 3 = heavy breathing, and 4 = coughing. Rectal temperatures were taken each morning.

Once between d 2 and 4, and on 28 d and 56 d, blood was collected via jugular venipuncture into vacutainers containing EDTA (Tyco/Healthcare, Mansfield, MA) and placed on ice for transport to the laboratory. These samples were centrifuged for 15 min at $2,500 \times g$; plasma was harvested and samples were frozen until analysis. Plasma from d 2 to 4 was analyzed for total protein using a refractometer (Reichert Inc., Depew, NY). Plasma from all blood samples was analyzed for haptoglobin (HP) concentration using single radial immunodiffusion (Cardiotech Services Inc., Louisville, KY). Each day, a sample of milk fed to calves, before supplemental LF addition, was collected and analyzed for LF concentration using single radial immunodiffusion (Cardiotech Services Inc.).

Statistical Analysis

The experimental design was a randomized complete block design with calves blocked by date of birth, sex, and housing type. Preweaning and postweaning data were analyzed using the GLM procedure of SAS (SAS Institute, 2004). Calf was a random effect, and treatment, sex, and housing type were fixed effects. Overall data, which included all preweaning and postweaning data, were analyzed using PROC MIXED (SAS Institute, 2004). The main factors of the model used for both procedures were type of housing, sex, LF treatment, and season of birth. Significance was declared at $P \leq 0.05$.

RESULTS AND DISCUSSION

Diet Composition

Nutrient composition of starter is presented in Table 1. Average LF concentration of the whole milk fed to calves before supplemental LF addition was 190 $\mu\text{g/mL}$. The amount of LF supplied by 3.8 L of whole milk, with no supplemental LF, was 0.72 g/d. Robblee et al. (2003) reported that the LF concentration of milk replacer fed to calves was 14 $\mu\text{g/mL}$; therefore, calves fed 4 L of milk replacer were provided only 0.06 g/d of LF in their base diet.

Passive Transfer

Fifty-eight of 60 calves had a plasma total protein level of at least 5.0 g/dL. Fifty-three of 60 calves had a plasma total protein level of 5.5 g/dL or greater. These data indicated that calves in this study received adequate colostrum because plasma total protein levels within the range of 5.0 to 5.5 g/dL have been established as indicators of successful passive transfer of immunity (Wilson et al., 1994; Tyler et al., 1996).

Performance Results

Dry matter intake from starter only (Table 2) was not affected by LF supplementation. Calves housed in the barn had greater overall and postweaning starter DMI than calves housed in hutches. Type of housing had no effect on preweaning starter DMI. Intake of starter CP and NDF (Table 2) followed the same pattern. Dry matter intake from starter and whole milk (Table 3) (12.5%

Table 3. Least squares means for overall, preweaning, and postweaning daily DMI for calves fed differing amounts of supplemental lactoferrin and for calves reared in different types of housing

DMI, ² kg/d	Lactoferrin ¹ (g/d)			SEM	P-value	Housing		SEM	P-value
	0	0.5	1			Barn	Hutch		
Overall	0.84	0.84	0.86	0.04	0.86	0.90 ^a	0.78 ^b	0.03	0.01
Preweaning	0.65	0.67	0.65	0.02	0.81	0.68	0.64	0.02	0.17
Postweaning	1.14	1.13	1.21	0.07	0.72	1.30 ^a	1.02 ^b	0.06	0.001

^{a,b}Means within a row with different superscripts differ ($P < 0.05$).

¹Calves were supplemented with 0, 0.5, or 1 g/d of lactoferrin added to the liquid portion of the diet during the preweaning and postweaning phase.

²DMI includes intake of whole milk and calf starter.

DM) was also not affected by LF supplementation. Calves housed in the barn had greater starter and milk DMI than calves housed in hutches for both the overall and postweaning periods. Although we did not measure temperatures within the hutch or barn, we speculate that there may have been higher temperatures and less ventilation in the hutches, which may have resulted in reduced feed intake.

Body weight (Table 4) was not affected by LF supplementation. Postweaning BW for barn-housed calves was greater ($P = 0.02$) than that of hutch-housed calves. No differences were observed overall or preweaning. Overall hip height (Table 4) was greater for control calves than for calves fed 0.5 g/d of supplemental LF. Overall hip height for control calves and calves fed 1 g/d of supplemental LF were not significantly different. Overall hip heights were not different for calves fed 0.5 g/d of supplemental LF and calves fed 1 g/d of supplemental LF. There were no observed effects of supplemental LF or housing on hip height preweaning. However, postweaning hip height was greater for control calves and calves fed 1 g/d of supplemental LF than for calves fed 0.5 g/d

of supplemental LF. Housing type had no significant effect on hip height overall or preweaning, but postweaning hip height was greater for barn-housed calves than for hutch-housed calves. Heart girth (Table 4) was not affected by LF supplementation. Postweaning heart girth was greater for barn-housed calves than for hutch-housed calves.

Average daily gain (Table 5) was not affected by LF supplementation. Overall ADG and postweaning ADG were greater for barn-housed calves than for hutch-housed calves. When feed efficiency was calculated using DMI of starter only, no effects from housing type were observed, and effects from LF supplementation were observed only during the preweaning period (Table 5) such that control calves had greater feed efficiency than calves fed 1 g/d of LF. When feed efficiency was calculated using DMI of milk and starter, no effects from LF supplementation or housing type were observed (Table 5). In this trial, LF supplementation had no positive effects on calf intake, growth, or health. Dry matter intake from starter only, as well as DMI from starter and milk, was similar

Table 4. Least squares means for overall, preweaning, and postweaning BW, hip height, and heart girth for calves fed differing amounts of supplemental lactoferrin and for calves reared in different types of housing

Item	Lactoferrin ¹ (g/d)			SEM	P-value	Housing		SEM	P-value
	0	0.5	1			Barn	Hutch		
BW, kg									
Overall	48.59	48.24	49.60	1.26	0.72	50.18	47.44	1.05	0.06
Preweaning	44.40	44.06	44.61	0.96	0.92	44.85	43.86	0.79	0.38
Postweaning	56.91	57.01	58.81	1.64	0.65	59.89 ^x	55.26 ^y	1.37	0.02
Hip height, cm									
Overall	84.53 ^a	83.05 ^b	84.07 ^{ab}	0.44	0.05	84.32	83.44	0.37	0.09
Preweaning	82.58	81.38	82.60	0.50	0.16	82.27	82.10	0.40	0.77
Postweaning	87.62 ^a	85.79 ^b	87.33 ^a	0.50	0.03	87.37 ^x	86.45 ^y	0.41	0.008
Heart girth, cm									
Overall	86.23	85.40	85.98	0.59	0.58	86.35	85.39	0.51	0.19
Preweaning	84.16	83.52	83.64	0.58	0.70	84.28	83.27	0.48	0.14
Postweaning	91.35	90.32	91.10	0.82	0.64	92.44 ^x	89.41 ^y	0.68	0.002

^{a,b,x,y}Means within a row with different superscripts differ ($P < 0.05$).

¹Calves were supplemented with 0, 0.5, or 1 g/d of lactoferrin added to the liquid portion of the diet during the preweaning and postweaning phase.

Table 5. Least squares means for overall, preweaning, and postweaning ADG and feed efficiency for calves fed differing amounts of supplemental lactoferrin and for calves reared in different types of housing

Item	Lactoferrin ¹ (g/d)			SEM	P-value	Housing		SEM	P-value
	0	0.5	1			Barn	Hutch		
ADG, kg									
Overall	0.37	0.38	0.41	0.03	0.67	0.44 ^x	0.34 ^y	0.02	0.004
Preweaning	0.25	0.26	0.25	0.03	0.98	0.28	0.23	0.02	0.09
Postweaning	0.58	0.60	0.67	0.05	0.40	0.70 ^x	0.53 ^y	0.04	0.002
Feed efficiency, ² kg/kg									
Overall	0.72	0.70	0.70	0.03	0.86	0.72	0.70	0.71	0.62
Preweaning	2.04 ^a	1.64 ^{ab}	1.30 ^b	0.21	0.05	1.81	1.51	0.18	0.21
Postweaning	0.51	0.52	0.53	0.03	0.82	0.53	0.51	0.02	0.42
Feed efficiency, ³ kg/kg									
Overall	0.44	0.44	0.46	0.02	0.86	0.47	0.42	0.02	0.06
Preweaning	0.38	0.37	0.37	0.04	0.98	0.41	0.34	0.03	0.12
Postweaning	0.51	0.52	0.53	0.03	0.78	0.53	0.51	0.02	0.46

^{a,b,x,y}Means within a row with different superscripts differ ($P < 0.05$).

¹Calves were supplemented with 0, 0.5, or 1 g/d of lactoferrin added to the liquid portion of the diet during the preweaning and postweaning phase.

²Feed efficiency is calculated using DMI of calf starter only.

³Feed efficiency is calculated using DMI of whole milk and calf starter.

across all treatments, and thus supporting similar ADG across all treatments.

Body weight and heart girth were not different across treatments. Overall and postweaning hip heights were greater for control calves than for calves fed 0.5 g/d of supplemental LF, but not significantly different from calves fed 1 g/d of supplemental LF.

Health Results

Body temperatures, fecal scores, respiratory scores, and plasma HP concentrations (Table 6) were not af-

fected by LF supplementation. Pre- and postweaning body temperatures were greater for barn-housed calves than for hutch-housed calves. Fecal and respiratory scores were not affected by type of housing. Plasma HP concentrations were greater for hutch-housed calves than for barn-housed calves. Haptoglobin is an acute phase protein produced by the liver and has been shown to increase during infection, inflammation, and tissue damage (Skinner et al., 1991; Horadagoda et al., 1999; Ganheim et al., 2003). The normal HP concentration for adult cattle is approximately 100 µg/mL (Skinner et al., 1991). However, Ganheim et al. (2003) observed HP con-

Table 6. Least squares means for overall, preweaning, and postweaning body temperature, fecal score, respiratory score, and plasma haptoglobin concentration for calves fed differing amounts of supplemental lactoferrin and for calves reared in different types of housing

Item	Lactoferrin ¹ (g/d)			SEM	P-value	Housing		SEM	P-value
	0	0.5	1			Barn	Hutch		
Body temperature, °C									
Overall	38.60	38.60	38.61	0.03	0.96	38.66 ^a	38.55 ^b	0.03	0.008
Preweaning	38.59	38.57	38.59	0.03	0.88	38.63 ^a	38.54 ^b	0.03	0.03
Postweaning	38.61	38.63	38.65	0.05	0.79	38.70 ^a	38.56 ^b	0.05	0.03
Fecal score ²									
Overall	1.52	1.55	1.51	0.04	0.76	1.52	1.53	0.04	0.94
Preweaning	1.56	1.58	1.56	0.05	0.91	1.56	1.56	0.04	0.99
Postweaning	1.45	1.52	1.41	0.07	0.51	1.46	1.46	0.06	0.99
Respiratory score ³									
Overall	1.04	1.02	1.00	0.02	0.29	1.02	1.03	0.01	0.66
Preweaning	1.01	1.02	1.01	0.01	0.70	1.01	1.02	0.01	0.11
Postweaning	1.09	1.02	0.99	0.04	0.26	1.03	1.04	0.04	0.95
Plasma haptoglobin, µg/mL	33.42	22.60	13.96	12.19	0.52	7.82 ^b	38.82 ^a	10.11	0.03

^{a,b}Means within a row with different superscripts differ ($P < 0.05$).

¹Calves were supplemented with 0, 0.5, or 1 g/d of lactoferrin added to the liquid portion of the diet during the preweaning and postweaning phase.

²1 = normal, 2 = soft, 3 = runny, 4 = watery.

³1 = normal, 2 = runny nose, 3 = heavy breathing, 4 = coughing.

centrations of 890 to 1,770 $\mu\text{g/mL}$ in calves infected with bovine viral diarrhoea virus. Haptoglobin concentrations observed in this study were lower than those reported in other studies, but appropriate levels for normal or stressed calves have not been established.

Lactoferrin supplementation had no significant benefit on calf health. Body temperatures for all treatment groups were within the normal range (Table 6). Fecal and respiratory scores were low, which also indicates that all calves were healthy throughout the trial (Table 6). Any effect due to LF supplementation may not have been evident because calves did not appear to be stressed and remained healthy while on trial.

CONCLUSIONS

Results of this trial are in contrast to results of previous reports. Although Cowles et al. (2006) observed that LF supplementation had no effect on calf growth or health, Robblee et al. (2003) and Joslin et al. (2002) found that LF supplementation improved starter intake, growth, and health of calves, which was not observed in this experiment. The present study differed from previous trials in that LF was added to milk replacer in previous trials, whereas in this trial, LF was added to whole milk and fed throughout the postweaning phase via water. Lactoferrin may function differently when fed with whole milk, or may be supplied by whole milk in sufficient amounts. All calves consumed 0.72 g/d of LF provided by the whole milk. In previous trials, milk replacer has provided 0.06 g/d of LF (Robblee et al., 2003). The amount of LF may have influenced the results observed in this study. It is also possible that no effect of LF supplementation was observed because there was no imposed challenge and calves remained healthy throughout the trial. All calves had low fecal and respiratory scores, as well as low HP concentrations. This study also suggested that housing type may affect performance and stress in calves. Further research is needed to evaluate the role of supplemental LF when supplemented in whole milk or milk replacer.

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