## ABSTRACT

**ENGLISH, ELIZABETH ANNE. Lactoferrin Supplementation to Holstein Calves during the Preweaning and Postweaning Phases. (Under the direction of Dr. B. A. Hopkins and Dr. L. W. Whitlow).**

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 three levels of lactoferrin was added to whole milk in order to produce three dietary treatments: 1.) 0 g/d, 2.) 0.5 g/d, 3.) 1 g/d. Milk (3.8 L/d) was fed from bottles until weaning at 35 days. From days 36 to 56, lactoferrin supplements were added to water (15-25 mL) and fed from bottles. Lactoferrin supplementation did not have any significant 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. In this study, lactoferrin supplementation was not beneficial. Further research is needed to fully elucidate lactoferrin's effects when supplemented to whole milk as well as any potential benefit for continued lactoferrin feeding postweaning.

# LACTOFERRIN SUPPLEMENTATION TO HOLSTEIN CALVES DURING THE PREWEANING AND POSTWEANING PHASES

By

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# BIOGRAPHY

Elizabeth Anne English was born in Marion, North Carolina on October 21, 1981 to Terry and Susan English. She graduated from McDowell High School in 2000, and entered North Carolina State University in the fall of 2000. Elizabeth received a B. S. degree in Animal Science in the spring of 2004. She remained at North Carolina State University to earn a M. S. degree in animal science and nutrition under the direction of Dr. Brinton Hopkins and Dr. Lon Whitlow.

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## ABBREVIATIONS

ADG……average daily gain

ADF…….acid detergent fiber

CP……crude protein

DM……..dry matter

DMI…….dry matter intake

HP……haptoglobin

LF……lactoferrin

NDF……neutral detergent fiber

# LITERATURE REVIEW

## INTRODUCTION

In the United States the majority of preweaned dairy calf mortality can be attributed to diarrhea, or scours (NAHMS, 1993; NAHMS, 1996). Calf loss can be controlled by either treatment or prevention of the illness. Recently, the focus on prevention has increased. Lactoferrin (LF) has been shown to not only improve calf health, but calf growth as well (Joslin et al., 2002; Robblee et al., 2003). LF's ability to maintain calf health may establish it as a preventative agent against calf gastrointestinal illness, including diarrhea.

Lactoferrin is an iron binding glycoprotein found in many body secretions, including milk and colostrum (Moore et al., 1997; Brock 1985; Masson and Heremans, 1971). The primary function of LF is the inhibition of microbial growth. Lactoferrin reduces the proliferation of bacteria by damaging the bacterial cell wall (Ellison et al., 1988; Ellison and Giehl, 1991). While LF is most active against gram-negative bacteria, it also displays some activity against gram-positive bacteria (Teraguchi et al., 1997).

Because of milk's naturally low LF concentration, research has been conducted with calves fed supplemental doses of LF. Lactoferrin research focusing on calf performance is limited. Joslin et al. (2002) supplemented milk replacer with 0g, 1g, or 10g/d of LF in the preweaning phase. Calves fed LF had increased starter DMI, ADG, and heart girth gain. Robblee et al. (2003) added 0g, 1g, 2g, or 3g/d of LF to milk replacer in the preweaning phase. Calves fed LF had higher ADG and reduced fecal scores and number of days medicated. In neither trial was LF fed postweaning and thus no information is available concerning LF feeding to postweaned calves.

This experiment was designed to investigate the effects of LF added to whole milk. In previous trials, LF was added to milk replacer, and thus the value of adding LF in whole milk has yet to be determined. This experiment also tested LF's effect when fed postweaning which has not been done in previous experimental work. Research suggests that lower doses of LF (1g/d) are effective and may be more beneficial (Joslin et al., 2002; Robblee et al., 2003). Lactoferrin was fed in this experiment at 0g, 0.5g, or 1g/d, which was equal to or below the low dosages fed previously.

## LACTOFERRIN STRUCTURE AND FUNCTION

Lactoferrin is found in a wide variety of body secretions. Tears, bronchial mucus, and saliva contain LF, as does the neutrophilic granules of leukocytes (Brock, 1985). The kidney, endometrium, and seminal vesicles also secrete LF (Masson and Heremans, 1971). Blood concentrations of LF are very low (Brock, 2002). Lactoferrin is present in high concentrations in the mammary secretions of nonlactating cattle (Rejman et al., 1989). Milk and colostrum contain the highest amounts of LF of any bodily secretion. Lactoferrin concentration in bovine colostrum is approximately 2 mg per milliliter (Tsuji et al., 1990) while bovine mature milk contains 20 to 200 ug/ml (Masson and Heremans, 1971). In comparison, human colostrum and mature milk contain 7 mg/ml and 1 mg/ml, respectively (Bernt and Walker, 2001).

Lactoferrin, transferrin, and ovotransferrin make up the transferrin family of proteins. These are glycoproteins with molecular weights of approximately 78 kDa (Moore et al., 1997). Bovine LF is composed of 689 amino acid residues; human LF is composed of 691 residues. Bovine and human LF have 69% sequence identity (Moore et al., 1997). The

polypeptide chain of bovine LF is folded into two lobes and each lobe is divided by two domains (Moore et al., 1997). Between the two domains is a deep cleft that contains a metal binding site (Baker et al., 1994). A single LF molecule reversibly binds two $Fe^{3+}$ ions along with two $CO_3^{2-}$ ions. Synergistic binding of a metal ion and an anion is a unique characteristic of the transferrin family (Baker et al., 1994; Moore et. al, 1997).

Due to its similarity to transferrin, LF was originally thought to function as an iron transporter. However, research has failed to produce results that support this theory (Brock, 2002). Lactoferrin has been shown to have several different functions in the body. The inflammatory response has been shown to be somewhat regulated by LF (Baynes and Bezwoda, 1994). Lactoferrin can bind to bacterial endotoxin, leading to decreased stimulation of cytokines (Miyazawa et al., 1991). Lactoferrin also exhibits antiparasitic, antifungal, and antiviral activity. Incubation of *Toxoplasma gondii* and *Eimeria stieda* spores with LF decreased their infectivity in animals (Omata et al., 2001). Lactoferrin inhibits activity of the fungus *Candida* by directly binding to the cell surface and affecting the cell membrane (Wakabayashi et al., 1996). Several viruses, including herpes simplex 1 and 2 as well as hepatitis B and C, have been found to be sensitive to LF *in vitro* (Hasegawa et al., 1994; Ikeda et al., 1998; Hara et al., 2002).

The most prevalent function of LF is its role in the inhibition of bacterial growth. This effect has been observed in all types of bacteria, but is most prevalent in gram-negative bacteria. Teraguchi et al (1997) demonstrated that LF displays activity against gram-positive and gram-negative bacteria. Two different sets of three to four week old pathogen-free mice were used to determine if LF suppresses *in vivo* proliferation of endogenous *Enterobacteriaceae* and orally administered *Clostridium*. One group of mice was fed bovine

milk and another was fed bovine milk containing 2% bovine LF. Each group contained five mice and each diet was fed for 7 d. Feces were collected and assayed for *Enterobacteriaceae*. To examine LF's effect on *Clostridium*, ten groups containing five to ten mice were fed experimental diets for 14 d. Five groups were fed bovine milk only and five were fed bovine milk with 2% bovine LF. After 7 d, one group from the milk only diet and one group from the LF diet were given *C. ramosum* at a dose of 5.2 $\log_{10}$ CFU. A second group from the milk only diet and a second group from the LF diet were given *C. ramosum* at a dose of 7.1 $\log_{10}$ CFU. The doses were applied directly into the stomach via catheters.

In mice fed milk only, *Enterobacteriaceae* in feces increased to a level that was 100 times greater than levels in feces of the mice before milk feeding. In mice fed milk with added bovine LF, proliferation of *Enterobacteriaceae* was significantly suppressed (Teraguchi et al., 1997). *Escherichia coli* was the primary species of *Enterobacteriaceae*. In mice inoculated with *C. ramosum*, levels of the bacteria in feces of milk fed mice increased to 10 $\log_{10}$ CFU/g, regardless of inoculation dose. Bacterial proliferation was suppressed in mice fed milk with bovine LF. Fecal levels of *C. ramosum* in the low inoculation dose group were significantly less than those in the high dose group (Teraguchi et al., 1997). This data suggests that LF has antibacterial activity against gram-negative bacteria, such as *E. coli*, and gram-positive bacteria, such as *C. ramosum*.

Lactoferrin can sequester iron from its environment, thereby reducing the amount of iron available for bacterial growth. This ability was originally believed to be responsible for its antimicrobial activity (Orsi, 2004). However, subsequent research has shown the iron binding mechanism to be of little importance. Arnold et al. (1977) incubated a *Vibrio*

*cholerae* suspension with 200 μl of various concentrations of apolactoferrin. This mixture was then grown in an iron rich medium. *V. cholerae* growth was completely inhibited by 100 μg of apolactoferrin. The broth used to grow the bacteria contained 4.0 to 7.0 μM Fe. It would require 2 to 3.5 μM LF to bind all the iron in the growth medium, however bacterial growth was totally inhibited with only 0.4 μM LF (Arnold et al., 1977). The author suggests that *V. cholerae* inhibition was due to another mechanism besides LF's capacity to sequester iron.

Although LF does bind iron from its environment, some bacterial species have developed systems of recovering the bound iron. Many bacteria produce siderophores, which are small iron chelating molecules. In gram-negative species, iron binds to the siderophore, forming a complex that is moved into the bacterial cell's periplasmic space via outer membrane receptors (Ekins et al., 2004). These siderophores may stimulate the production of antibodies, which may enhance the antimicrobial activity of IgG, IgM, or secretory IgA (Ellison, 1994). However, this theory has not yet been confirmed. Highly host-adapted bacterial species, such as those in the families *Neisseria*, *Moraxella*, and *Pasteurella*, have specific outer membrane receptors that directly bind to LF and remove any bound iron. The receptor then transports iron into the cell's periplasmic space (Orsi, 2004; Ekins et al., 2004).

Lactoferrin's primary method of microbial inhibition is accomplished by causing damage to the cell membrane of gram-negative bacteria, particularly the cell wall. The cell wall is composed of an inner and outer membrane with periplasmic space between the membranes. The inner membrane is a phospholipid bilayer with membrane transport proteins, the periplasmic space contains a peptidoglycan matrix, and the outer membrane is also a phospholipid bilayer with transport proteins and transmembrane pore proteins

(Beveridge, 1999; Costerton et al., 1974; Ellison, 1994). The outer membrane itself has two layers. The inner leaflet is composed of neutral phospholipids, while the outer leaflet is made of lipopolysaccharide (LPS) molecules (Beveridge, 1999; Costerton et al., 1974). Each LPS molecule has three regions. A lipid A region inserts into the membrane bilayer. There is an area of charged sugar molecules with free phosphate groups at the membrane surface, and a polysaccharide chain that extends beyond the membrane surface. In order to balance the charged sugar molecules, the outer leaflet contains divalent cations (Ellison, 1994). EDTA, a synthetic chelator, binds and removes the cations, causing LPS release from the outer membrane, which increases the cell's membrane permeability (Leive, 1974; Nikaido and Vaara, 1985).

Ellison et al. (1988) have shown that LF behaves similarly to EDTA by causing LPS release and thereby altering bacterial membrane permeability. An *E. Coli* inoculum of $10^4$ CFU/ml was added to a nutrient broth with one of the following: 0.001 M EDTA, 2 mg of apolactoferrin, and 2 mg of iron saturated LF. Apolactoferrin was 3% saturated and saturated LF was 10% saturated. Apolactoferrin caused significant LPS release, but less than the amount released by EDTA. Saturated LF did not cause LPS release (Ellison et al., 1988). Additional studies were performed to establish the optimum conditions for LPS release. It was found that LF produced LPS release at pH 6.0 to 8.0 and EDTA released LPS at pH 5.5 to 8.0. At pH 7, LF and EDTA caused comparable LPS release for bacterial concentrations of $10^4$ to $10^7$ CFU/ml. A final study was conducted to determine if LPS release by LF is associated with a change in outer membrane permeability. The effects of EDTA and LF on the susceptibility of *E. Coli* to the antibiotic rifampin were observed. EDTA and LF alone were bacteriostatic. The addition of rifampin increased the bacteriostatic capacity of EDTA

and LF (Ellison et al., 1988). These results confirm that at physiologic pH, LF releases LPS from enteric gram-negative bacteria. The LPS release caused the bacterial cell membrane's permeability to be altered. Rifampin normally does not cross the gram-negative cell membrane (Ellison et al., 1988). However, since rifampin added to the bacteriostatic effects of LF, it is possible that the membrane was altered in such a way that it could cross into the cell.

Ellison and Giehl (1991) conducted further experiments comparing LF and agents that increase membrane permeability, such as EDTA and polycationic compounds. By increasing membrane permeability, these agents enhance the susceptibility of gram-negative bacteria to lysozyme. Therefore, it was hypothesized that LF would act in a similar manner. A $5 \times 10^5$ CFU inoculum of E. *Coli* was added to 500 µl of growth media and incubated with 2 mg/ml of human LF, 0.5 mg/ml of human lysozyme, or $8 \times 10^{-5}$ M EDTA. A portion of the mixture was then plated (Ellison and Giehl, 1991). Lactoferrin enhanced the antimicrobial activity of lysozyme, and the effect was dose dependent. Lactoferrin's additive effect began to decrease when bacterial concentrations reached $5 \times 10^7$ CFU/ml (Ellison and Giehl, 1991). These results suggest that like EDTA, LF potentiates lysozyme's antimicrobial activity by increasing bacterial membrane permeability.

Further studies were performed in order to determine the mechanisms by which LF affects the bacterial membrane. The addition of 1.3 mM $CaCl_2$ or 0.8 mM $MgCl_2$ to the growth media inhibited the activity of LF and lysozyme. Lower concentrations of $CaCl_2$ were also evaluated. The inhibition of LF and lysozyme activity was dose dependent, with smaller Ca concentrations leading to less inhibition (Ellison and Giehl, 1991). A dialysis chamber study was carried out to define if LF required direct bacterial cell contact in order to

be effective. Lactoferrin only enhanced the activity of lysozyme when it was in direct contact with cells. Finally, Ellison and Giehl (1991) conducted a trial to determine if LF directly binds LPS molecules. Lactoferrin, polycationic agents known to bind LPS, and a soybean trypsin inhibitor known to not bind LPS were linked to Tris-blocked Sepharose beads. The beads were then incubated with differing concentrations of LPS prepared from *E. Coli*. Lactoferrin and polycationic agents had a similar ability to bind LPS and each had a greater ability to bind LPS than the trypsin inhibitor (Ellison and Giehl, 1991).

The majority of studies have examined the effects of LF as a whole molecule. However, research has been conducted to determine if the entire LF molecule is required for antimicrobial activity. Tomita et al. (1991) utilized commercial proteases to characterize hydrolysates of LF. Proteases such as porcine pepsin, porcine trypsin, cod pepsin, and PD acid protease were used to hydrolyze bovine LF. Lactoferrin was dissolved in distilled water and the pH was adjusted to 2.5 or 7.0. Each protease was then added at concentrations of 3% of the weight of the substrate. *E. Coli* was grown on media containing solutions of either undigested LF or LF hydrolysates. The minimum amount of hydrolysate required for complete inhibition of bacterial growth was 100 μg/ml. The minimum amount of undigested LF required to produce the same inhibitory effect was 2000 μg/ml (Tomita et al., 1994). The hydrolysates produced by porcine pepsin, cod pepsin or PD protease had a greater effect against *E. Coli* than those produced from other protease (Tomita et al., 1991). These results indicate that when enzymes similar to gastric pepsin act upon LF, active peptides that are more potent than LF itself are produced, suggesting that the active sites are concentrated in certain sections of the LF molecule and remain active in the LF peptide fragment. In order to isolate the active peptides, the pepsin hydrolysates were fractionated by reverse phase HPLC.

Only one peptide peak was found to have antimicrobial activity against *E. Coli*. This peptide was named lactoferricin B, with the B denoting bovine lactoferricin (Tomita et al., 1994).

Female rats were fed a diet containing 40% bovine LF for four days. Gastrointestinal contents were sampled at 2, 4, and 8 hours postfeeding. The samples were analyzed using HPLC. An active LF peptide in the GI content was sequenced. The primary structure was confirmed to be identical to lactoferricin B (Tomita et al., 1994). This suggests that active peptides of LF (lactoferricin B) can be produced by pepsin digestion *in vivo*. Lactoferricin B has also been tested against a variety of bacterial strains. The differing strains were incubated with lactoferricin B for 60 minutes and the number of remaining viable cells was measured. Viable cell loss was in strains of *E. Coli, Klebsiella, Bacteroides, Staphylococcus, Streptococcus, Corynebacterium,* and *Listeria* (Tomita et al., 1991).

The effect of lactoferricin B on bacterial cell morphology has also been investigated. Shin et al. (1998) tested bovine LF, bovine LF pepsin hydrolysate, and lactoferricin B for activity against *E. Coli*. Bovine LF hydrolysate (bLFH) was prepared by exposing LF to porcine pepsin. This protein was then purified by reverse phase HPLC to produce lactoferricin B (Shin et al., 1998). Lactoferrin, bLFH, and lactoferricin B was incubated with *E. Coli* in either 1% Bactopeptone or PYG growth medium. PYG media contains 1% Bactopeptone, 1% glucose, and 0.05% yeast extract (Shin et al., 1998). In the Bactopeptone media the minimal inhibitory concentration (MIC) of LF was 3 mg/ml. The MIC of bLFH was 0.1 mg/ml and the MIC of lactoferricin B was 0.008 mg/ml. Each peptide had higher MIC in the PYG media, but lactoferricin B still had the strongest antibacterial activity (Shin et al., 1998). Since lactoferricin B was the most potent peptide, its effect on bacterial cell morphology was investigated. *E. Coli* was exposed to lactoferricin B for 30 minutes. The

cells were then isolated and observed using a transmission electron microscope. Some membrane blisters were seen on the cells. Also, alterations in the cytoplasmic content were observed. When *E. Coli* was exposed to lactoferricin B for 60 to 120 minutes, increasing amounts of cytoplasmic debris were seen (Shin et al., 1998). These results indicated that lactoferricin B exerts a bactericidal effect by acting initially on the bacterial cell membrane and then on the cytoplasm.

In recent years, considerable research focusing on antibiotic alternatives has been conducted. Mannan oligosaccharides (MOS), a component of yeast cell walls, have been shown to improve growth and health of many species, including dairy calves (Dvorak and Jacques, 1997; Heinrichs et al., 2003). One of the antibacterial mechansisms of MOS is similar to that of lactoferrin. Mannan oligosaccharides bind to the cell wall of bacteria, which prevents the bacteria from attaching to intestinal epithelial cells (Spring, et al., 2000). Since LF and MOS have similar antibacterial mechanisms, research concerning supplemental LF's effect on calf health and growth has performed.

## LACTOFERRIN SUPPLEMENTATION TO CALVES

Supplemental LF has been fed to calves in order to determine its effects on several different parameters, including blood LF levels, Fe status, health status, and growth measurements. Dawes et al. (2004) fed calves supplemental LF to establish its effect on serum LF concentrations. Twenty-two Holstein bull calves were separated from their dam after birth, prior to colostrum ingestion. Within four h of birth calves were fed four liters of one of the following treatments: 1) colostrum (control), 2) colostrum + 1g/kg BW of LF, 3) milk replacer + 1g/kg BW of LF. After 24 h of age all calves were fed two liters of milk

replacer twice per d until weaning. Serum samples were taken at birth before feeding and at d 1, 9, 30, and 60 and analyzed for LF concentration.

At birth, LF concentrations were similar in all calves. At d 1, LF concentrations were significantly higher in calves supplemented with LF compared to control calves ($P<0.05$). No significant differences were observed among treatments on d 9, 30, and 60. Lactoferrin concentrations for all calves at d 60 were within the range of pretreatment concentrations (Dawes et al.; 2004).

These results show that calves have low serum LF concentrations immediately after birth and that serum LF concentrations are increased following colostrum ingestion. Lactoferrin concentrations increased 24 h after calves were fed colostrum or the supplemental LF treatments, but calves receiving LF had significantly higher LF concentrations than control calves. This suggests that LF is absorbed from colostrum and supplemental LF augments serum LF concentrations (Dawes et al.; 2004). However, this effect does not appear to continue after LF supplementation has stopped. After d 2 calves did not receive any supplemental LF, and subsequent serum LF concentrations were not different among calves.

Absorption of colostral LF has also been observed by Hurley and Sixiang (2000). At birth, 17 Holstein bull calves were assigned to one of three treatment groups. Calves in group A were fed pooled colostrum from the second and third milkings of postpartum cows. The LF concentration of the colostrum was 0.58 mg/ml. Calves were fed two liters of colostrum at birth and 12 h after birth. Total colostrum fed at birth was 1,160 mg. Calves in groups B and C were fed pooled colostrum from the first milking of postpartum cows. Lactoferrin concentration of the colostrum was 0.73 mg/ml. Calves in group B were fed two

liters of colostrum at birth and 12 h after birth, with 1,460 mg of LF fed at birth. Calves in group C were fed four liters of colostrum at birth and two liters 12 h after birth, with 2,920 mg of LF fed at birth. Blood samples were taken 0, 4, 8, 12, 24, and 48 h after the first feeding.

Increasing the total consumption of LF at the first feeding resulted in increased peak serum LF concentrations. Increasing the volume of colostrum fed at the first feeding resulted in a longer time to reach peak serum LF concentration. It was also observed that feeding additional LF 12 h after the first feeding did not further increase serum LF concentrations. The results suggest that LF clearance from the blood is increased within 12 h after the first feeding (Hurley and Sixiang, 2000) (Figure 1). The main tissue of LF clearance in other species is the liver, and some may be excreted in the urine (Hurley and Sixiang, 2000). Little is known about LF clearance in the calf

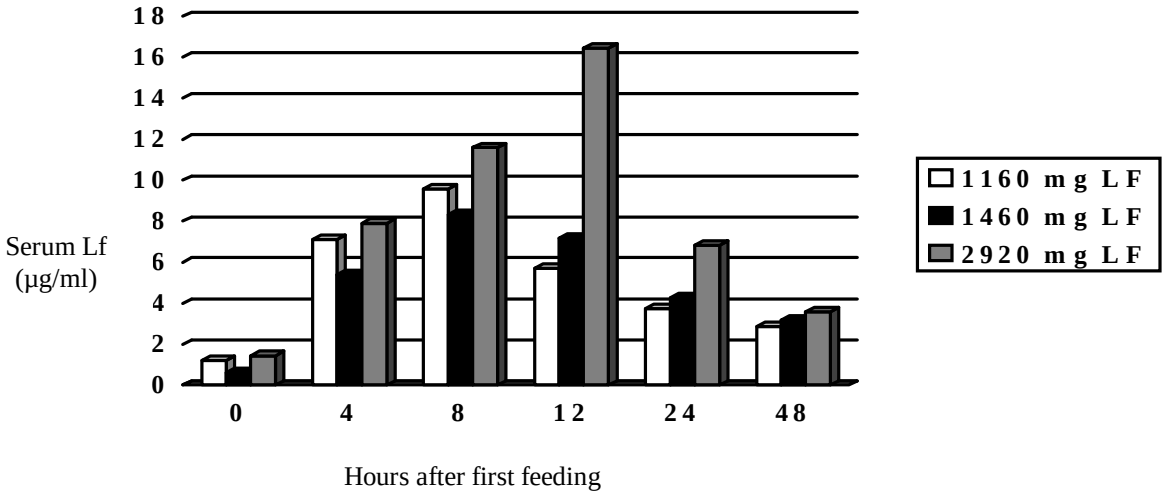


**Figure 1.** Serum concentrations of lactoferrin (LF) in calves after consuming differing amounts o f LF from colostrum at birth (Hurley and Sixiang, 2000).

Some research has shown that after LF is absorbed into the blood, it is transported into the cerebrospinal fluid (CSF) (Harada et al., 1999; Talukder et al., 2002). Talukder et al. (2003) further investigated the absorption and transportation of colostral LF in plasma and CSF of newborn calves. To examine LF transport into CSF via plasma, four newborn Holstein calves were fed colostrum through d four of life. Milk replacer was then fed through week four of life. Blood samples were taken before and 12 h after feeding on d two and four, and again at weeks 1, 2, 3, and 4. Cerebrospinal fluid samples were also taken at these times.

To investigate mechanisms of LF absorption and transportation into CSF, four Japanese black calves ages three to five months were used. Three of the calves were a treatment group, while the remaining calf was used as a control. All calves were fasted overnight and water was given ad libitum. Treatment calves were infused intraduodenally with 30 ml/kg of LF. The control calf was infused with saline. Blood samples were taken before infusion and 0.5, 1, 2, 4, 8, 12, and 24 h after infusion. Cerebrospinal fluid samples were taken before infusion and 4, 8, 12, and 24 h after infusion. Treament calves were euthanized and the choroids plexus was collected. The choroids plexus, located in the brain, is the main producer of CSF (Talukder et al., 2003). The choroids plexus was then subjected to a LF binding assay.

In calves fed colostrum, average prefeeding plasma LF concentration was 238 ng/ml and CSF LF concentration was 9.8 ng/ml. After feeding, LF concentration increased in both fluids, with plasma concentrations peaking at 12 h at 1,696 ng/ml and CSF concentrations peaking at 12 h at 74 ng/ml ($P<0.01$) (Talukder et al., 2003). In calves infused with LF, plasma and CSF LF concentrations gradually increased and peaked at four h. At four h,

average plasma LF concentration was 1,968.3 ng/ml and CSF LF concentration was 756.3 ng/ml ($P<0.05$), which is seven and four times higher than the concentrations before infusion. It was also shown that LF binds to the epithelial membrane of the choroids plexus, suggesting that a LF receptor is present through which LF is transferred into the CSF (Talukder et al., 2003). These results indicate that LF is absorbed in the gut and transferred into the CSF via plasma in a time dependent manner in young calves.

Research has also been conducted on the effect of LF on the Fe status of calves. Kume and Tanabe (1996) compared the efficiency of supplemental LF and $FeSO_4$ on the Fe status of calves during the first ten d after birth. Thirty-six calves were fed 1 kg of colostrum at birth and then 2.5 kg twice daily through 1 week of life. After 1 week, calves were fed 2.5 kg of whole milk twice per d. The following three treatments were added to the colostrum that was fed from d 1 to 5: 1) control, 2) 40 mg Fe as $FeSO_4$, 3) 40 mg Fe as $FeSO_4$ + 5g LF. Blood samples were collected on d 1, 2, 6, and 10 before feeding. Blood was analyzed for hematocrit, hemoglobin, and plasma Fe.

In control calves, hematocrit and hemoglobin both decreased from d 1 to 10 ($P<0.001$). Hemoglobin in treatment calves increased from d 1 to 10 ($P<0.001$). Hematocrit in calves supplemented with Fe was significantly decreased at d 2 ($P<0.01$). Hematocrit in calves supplemented with Fe + LF significantly increased from d 2 to 10 ($P<0.001$). Hematocrit and hemoglobin levels were significantly higher at d 6 and 10 (after supplementation had stopped) in calves fed Fe + LF than in calves fed Fe only ($P<0.01$). Plasma Fe in calves supplemented with Fe and Fe + LF significantly increased at d 2 ($P<0.001$). Concentrations were higher at d 2 for calves fed Fe than for calves fed Fe + LF

($P<0.01$), however, at d 6 and 10 plasma Fe was significantly lower for Fe supplemented calves than in Fe + LF supplemented calves.

From d 1 to 5, while calves were being supplemented, both Fe and Fe + LF improved hematocrit and hemoglobin, but hematocrit and hemoglobin in calves fed Fe + LF were higher even after supplementation stopped. Also, plasma Fe in calves fed Fe + LF remained higher at d 6 and 10. This suggests that while LF may not be an Fe source to calves, supplemental LF with ferrous Fe may be a more efficient means to accelerate the shift of Fe into hemoglobin (Kume and Tanabe, 1996).

Muri et al.(2005) also conducted research on LF and Fe status of calves. Thirty-five newborn Holstein calves were assigned to one of the following treatments: 1) milk based formula only (F), 2) formula + LF ($F_L$), 3) formula + vitamin A ($F_A$), 4) formula + vitamin A + LF ($F_{AL}$), 5) colostrum only (C). All treatments were fed d 1 through 4 of life. In group $F_L$, 3850, 1990, 660, and 660 mg LF/kg DM were added to the formula fed on d 1, 2, 3, and 4 respectively. In group $F_A$, 351, 402, 490, and 490 µmol vitamin A/kg DM were added to the formula fed on d 1, 2, 3, and 4 respectively. Calves in group $F_{AL}$ were supplemented with the same amounts of LF that was fed to group $F_L$ and the same amount of vitamin A that was fed to group $F_A$. Blood samples were taken on d 1 through 5. All calves were euthanized on d 5.

Plasma LF of calves increased on d 1 in group C ($P<0.001$) and group F ($P<0.05$) and then returned to basal levels on d 4 and 2 respectively. In group $F_L$, no change was seen in plasma LF concentrations. This outcome was not expected and the causes for the lack of absorption are not known (Muri et al., 2005). Hematocrit and hemoglobin were not affected by treatment, even in groups C and F, where there was obvious LF absorption. This is in contrast to the results observed by Kume and Tanabe (1996) in which hematocrit and

hemoglobin both increased with LF supplementation, but these calves were also supplemented with Fe, therefore it is not clear if the increase in hematocrit and hemoglobin were due to the Fe, LF, or both.

Results from Kume and Tanabe (1996) and Muri et al. (2005) seem to suggest that supplemental LF does not have an effect on Fe status of newborn calves. Research with genetically modified mice support these results. Ward et al. (2003) used mice lacking the LF gene to determine if LF has an effect on Fe status. Serum Fe concentrations in LF knockout mice pups were not different from the concentrations in normal mice pups. Adult mice were fed either a basal diet or a high Fe diet. There were no differences in transferrin saturation or tissue Fe stores between normal mice and LF knockout mice (Ward et al., 2003). This trial supports the hypothesis that LF does not play a major role in the regulation of Fe homeostasis.

Research focusing on the effect of LF on calf performance is limited. Joslin et al. (2002) fed calves supplemental LF to determine effects on growth, health, serum Fe concentration, hematocrit, and starter DMI. Twenty-one Holstein calves were assigned to one of three treatments. Colostrum, milk, and milk replacer were supplemented with either 0 (control), 1, or 10 g/d of LF. At birth calves were separated from their dams and fed two liters of colostrum +/- LF. Within 12 h of birth calves received another two liters of colostrum +/- LF. On d 2, calves received two liters of milk +/- LF at 12 h intervals. Beginning on d 3, calves received a non-medicated milk replacer +/- LF twice daily. Calf starter and water were available ad libitum beginning on d 3. Feed intakes were determined daily. Calves were weaned when they consumed 0.7 kg of starter for 2 consecutive d or when they reached 35 d of age. The experiment was concluded on d 56. At birth, and every

week thereafter, calves were weighed, hearth girth was measured, and hip and wither heights were measured. Blood samples were also taken at these times. Feces were evaluated and scored three times each week on a scale of 1 to 5, with 1 = firm and 5 = watery. Any day on which medication was required by a calf was designated a sick day.

In the preweaning phase, calves fed LF had higher starter DMI than control calves (Table 1). This effect did not carry over to the postweaning phase. Weaning was based on starter intake and was attained 2 to 3 d earlier when calves were fed LF ($P<0.05$). Body weight was higher during wks 2 to 6 for calves fed LF ($P<0.04$). Calves fed LF had a greater preweaning ADG than control calves. Calves fed 1 g LF had greater ADG than calves fed 10 g LF (Table 1). No differences were observed postweaning. Calves fed 1 g LF also tended to have a greater preweaning feed efficiency than calves fed 10 g LF (Table 1). Once again, there were no differences postweaning. Preweaning heart girth gain was greater in calves fed LF compared to control calves, with no effects seen postweaning (Table 1). Hip and wither height gains were similar across all treatments. Hematocrit and serum Fe concentrations were not affected by treatment. Fecal scores and days medicated did not differ throughout the experiment.

Preweaning ADG and starter DMI were increased by LF, thus decreasing weaning age. These responses may have been due to improved health status in calves (Joslin et al., 2002). Effects of LF were only observed in the preweaning phase. These effects did not carry over to the postweaning phase when no LF was being fed. This suggests that in order for LF to be effective, it must be continuously supplemented since its effects do not appear to continue once supplementation has been stopped. Also, no differences were observed in hematocrit or serum Fe concentrations. This is in agreement with results found by Muri et al.

(2005), further supporting the hypothesis that supplemental LF does not play a major role in Fe status of calves.

Robblee et al. (2003) also supplemented calves with LF to further examine its effects on health, growth, feed intake, and feed efficiency. Forty Holstein calves were assigned to one of four treatments: 0 (control), 1, 2, or 3 g/d of LF. At birth calves were removed from their dam and fed two liters of colostrum and another two liters of colostrum 8 to 12 h later. On d 2 calves were fed two liters of non-medicated milk replacer twice daily. Starting on d 3 and continuing through 14 d postweaning, calves had unlimited access to calf starter and water. Feed intake was determined daily. Starting on d 3, LF was added to the milk replacer. Calves were weaned when they met a criteria based on age and starter intake. Calves were removed from the trial at 14 d postweaning. Each calf was weighed at birth. Calves were weighed and measured for heart girth each week, with body weights also taken at weaning and 14 d postweaning. Feces were scored three times per week on a scale of 1 to 4, with 1 = firm and 4 = watery diarrhea. Sick days were recorded as any day that a calf received medication. Medication was given to calves that had a fecal score greater than or equal to 3 or calves that had a rectal temperature greater than 39$^{o}$C.

Preweaning fecal scores followed a quadratic pattern. Calves fed 1 g/d LF had the lowest scores and control calves had the highest scores (Table 2). No differences were observed postweaning. Preweaning number of days medicated also followed a quadratic pattern. Once again, calves fed 1 g/d LF had the lowest number of days medicated and control calves had the highest (Table 2). No differences were observed postweaning. Starter DMI, body weight, and weaning age were no different among treatments. Preweaning ADG increased linearly with LF supplementation (Table 2). Postweaning ADG was similar among

treatments. Preweaning feed efficiency also increased linearly with LF supplementation, however postweaning feed efficiency decreased linearly (Table 2). This differs from feed efficiency results observed by Joslin et al. (2002). It is not clear why postweaning feed efficiency decreased, but despite the decrease, postweaning ADG was not different among treatments (Robblee et al., 2003). Overall, average daily hearth girth gain increased linearly (P=0.02) with LF level (0.25, 0.24, 0.29, 0.31 cm/d; SE = 0.02) for treatments 0, 1, 2, and 3 g/d.

Supplementation with LF increased preweaning feed efficiency and ADG, which is in agreement with results found by Joslin et al. (2002). Supplementation with LF also reduced preweaning fecal scores and number of days on medication, with 1 g/d LF most effective. Robblee et al. (2003) attributes this to improved intestinal health due to LF's antibacterial activity. This trial, as in Joslin et al. (2002), also found no effect of LF postweaning for most parameters measured, suggesting that LF's effect does not continue once supplementation has stopped.

**Table 1.** Starter DMI, ADG, feed efficiency and heart girth gain of calves fed differing amounts of lactoferrin from birth to weaning (Joslin et al., 2002)

| | Lactoferrin[1] (g/d) | | | | Contrast[2] (P = ) | |
|---|---|---|---|---|---|---|
| Item | 0 | 1 | 10 | SE | 1 | 2 |
| Starter DMI, kg | | | | | | |
| Preweaning | 0.19 | 0.29 | 0.30 | 0.04 | 0.03 | 0.93 |
| Postweaning | 1.31 | 1.63 | 1.42 | 0.12 | 0.11 | 0.21 |
| ADG, kg | | | | | | |
| Preweaning | 0.21 | 0.33 | 0.24 | 0.03 | 0.02 | 0.04 |
| Postweaning | 0.54 | 0.59 | 0.53 | 0.18 | 0.87 | 0.52 |
| Feed Efficiency, gain/DMI | | | | | | |
| Preweaning | 0.29 | 0.38 | 0.31 | 0.04 | 0.12 | 0.10 |
| Postweaning | 0.37 | 0.34 | 0.34 | 0.02 | 0.25 | 0.96 |
| Heart Girth Gain, cm/d | | | | | | |
| Preweaning | 0.11 | 0.19 | 0.16 | 0.02 | 0.02 | 0.38 |
| Postweaning | 0.24 | 0.22 | 0.23 | 0.03 | 0.68 | 0.84 |

[1]Calves were supplemented with either 0, 1, or 10g/d of lactoferrin added to the liquid portion of the diet during the preweaning period.
[2]1 = 0 g of lactoferrin versus 1 g of lactoferrin and 10 g of lactoferrin, and 2 = 1 g of lactoferrin versus 10 g of lactoferrin.

**Table 2.** Least square means of preweaning and postweaning fecal score, number of days medicated, ADG, and feed efficiency for calves fed different amounts of lactoferrin during the preweaning period (Robblee et al., 2003).

| | Lactoferrin[1] (g/d) | | | | | Contt[2] (P = ) | |
|---|---|---|---|---|---|---|---|
| Item | 0 | 1 | 2 | 3 | SE | L | Q |
| Fecal Score[3] | | | | | | | |
| Preweaning | 2.38 | 2.17 | 2.23 | 2.28 | 0.05 | NS | 0.01 |
| Postweaning | 2.39 | 2.43 | 2.46 | 2.48 | 0.09 | NS | NS |
| Days Medicated | | | | | | | |
| Preweaning | 4.70 | 1.59 | 2.01 | 3.40 | 0.89 | NS | 0.02 |
| Postweaning | 1.98 | 0.86 | 1.83 | 2.01 | 0.82 | NS | NS |
| ADG, kg/d | | | | | | | |
| Preweaning | 0.28 | 0.30 | 0.35 | 0.36 | 0.03 | 0.02 | NS |
| Postweaning | 0.74 | 0.66 | 0.66 | 0.60 | 0.08 | NS | NS |
| Feed Efficiency, gain/DMI | | | | | | | |
| Preweaning | 0.38 | 0.41 | 0.48 | 0.49 | 0.04 | 0.01 | NS |
| Postweaning | 0.52 | 0.46 | 0.45 | 0.42 | 0.04 | 0.04 | NS |

[1]Calves were supplemented with either 0, 1, 2, or 3 g/d of lactoferrin added to milk replacer during the preweaning period.
[2]Contrasts: L = linear and Q = quadratic; NS = non-significant (P > 0.10).
[3]Fecal Score: 1 = firm through 4 = watery diarrhea.

## PRACTICAL IMPLICATIONS OF LACTOFERRIN

The health benefits of LF supplementation may make it an ideal addition to the calf rearing process. The National Dairy Heifer Evaluation Project, conducted by the National Animal Health Monitoring System, surveyed 1811 US dairy producers and showed average death losses of 8.4 % for heifer calves from birth to weaning (1993). Another survey conducted in 1996 reported that mortality of heifer calves was 10.8 % of all heifers born alive (NAHMS). It has been shown that the most prevalent causes of death in calves from birth to weaning are diarrhea (scours) and respiratory problems. In 1993, NAHMS showed that these 2 factors accounted for almost 75 % of all deaths in unweaned heifer calves, with diarrhea being the main cause. Of deaths in unweaned heifers, diarrhea accounted for 52.2 % (NAHMS, 1993). A more recent survey reported that diarrhea accounted for 60.5 % of calf deaths (NAHMS, 1996). These statistics show that diarrhea is a common problem that can lead to significant calf loss.

Calf diarrhea can be classified as infectious or nutritional (Roy, 1990). Nutritional diarrhea is caused by improper nutrition, while infectious diarrhea is caused by bacteria, viruses, or protozoa (Kasari, 1990). The degree to which the calf can cope with these factors is related to the extent to which it has been stressed (Heath, 1992). Depending on the severity of the diarrhea, most calves can survive with proper treatment. However, death from dehydration and electrolyte imbalances (Booth and Naylor, 1987) can occur if the calf is left untreated.

The price of raising a dairy heifer from birth to 24 months of age can range from $1000 to $1500 (Quigley, 1996). With heifers often representing the second largest expenditure on a dairy farm (Cady and Smith, 1996), it is highly economical for producers to keep calves healthy. A typical breakdown of expenses for one heifer from birth to calving is shown in Table 3 (Dairy Heifers Birth To Calving (26 Months). 2001.Virginia Cooperative Extension Farm Management). Labor and medical expenses account for approximately 33% of the total cost. During the first three months of life the primary costs are those associated with feed, labor, and health (Quigley, 1996). Obviously, labor and health expenses would increase during times of calf illness, thus further increasing operating costs. Lactoferrin supplementation at 1 g/d for 35 d would cost approximately $10.50 per calf, with LF priced at $0.30 (DMV International, 2006). If LF can offset costs associated with poor health, supplementation may be economical to the producer. Supplementation of LF may be of greatest importance during periods of stress or a pathogen challenge.

**Table 3.** Typical Cash Expenses for Raising a Heifer Calf From Birth to Calving [1]

| | Item | Cost ($) | % of Total |
|---|---|---|---|
| Operating Costs | Feed | 549.44 | 60.9 |
| | Vet and Medicine | 33.42 | 3.8 |
| | Utilities and Supplies | 15.01 | 1.6 |
| | Bedding | 18.02 | 1.9 |
| | Breeding | 19.51 | 2.2 |
| | Labor | 267.36 | 29.6 |
| Total Cost | | 902.76 | |

[1]Dairy Heifers Birth To Calving (26 Months). 2001. Virginia Cooperative Extension Farm Management.

## SUMMARY

Lactoferrin found in body secretions, such as milk and colostrum, has antibacterial properties. Bovine milk has a low LF concentration, therefore, calves may benefit from supplemental LF. Research has shown that when milk replacer is supplemented with LF, calf health, growth, and feed intake may be improved (Joslin et al., 2002; Robblee et al., 2003). Because LF promotes calf health and performance, it may be a reasonable addition to the calf rearing process. However, additional research needs to be conducted in order to clarify LF's role in different types of calf raising programs, or during periods of pathogen challenge.

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INTERPRETIVE SUMMARY

**Lactoferrin Supplementation to Dairy Calves.** *By English et al.* Lactoferrin, a naturally occurring protein found in milk, has antibacterial properties. Feeding supplemental lactoferrin to dairy calves may improve intake, growth, and health status. This study evaluates lactoferrin's effect when fed in whole milk during the preweaning phase and when fed in water during the postweaning phase.

RUNNING HEAD: LACTOFERRIN SUPPLEMENTATION TO CALVES

**Lactoferrin Supplementation to Holstein Calves during the Preweaning and Postweaning Phases.**

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## 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 three levels of lactoferrin was added to whole milk in order to produce three dietary treatments: 1.) 0 g/d, 2.) 0.5 g/d, 3.) 1 g/d. Milk (3.8 L/d) was fed from bottles until weaning at 35 days. From days 36 to 56, lactoferrin supplements were added to water (15-25 mL) and fed from bottles. Lactoferrin supplementation had no significant 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 lactoferrin's effects in whole milk as well as its role when fed postweaning.

**(Key words: calves, lactoferrin, weaning)**

**Abbreviation key: ADG =** average daily gain; **LF** = lactoferrin; **HP** = haptoglobin.

## INTRODUCTION

Lactoferrin (**LF**) is an iron-binding glycoprotein found in a variety of body secretions, including tears, bronchial mucus, and saliva (Brock, 1985). The 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) while bovine milk contains 20 to 200 μg/ml (Masson and Heremans, 1971). Lactoferrin has been shown to have antiparasitic, antifungal, and antibacterial activity (Omata et al., 2001; Wakabayashi et al., 1996; Hara et al., 2002). One of LF's most prevalent functions is its role in the inhibition of bacterial growth. Lactoferrin primarily inhibits the 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 since it is a common pathogen associated with calf diarrhea.

Lactoferrin has been supplemented to preweaned dairy calves in order 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, average daily gain (**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 the number of days medicated. Calves fed 1 g/d of LF had the lowest preweaning fecal scores and number of days medicated, while calves fed no LF had the highest values. Preweaning ADG and feed

efficiency increased as the amount of LF fed to calves increased. Lactoferrin supplementation concluded at weaning, but the trial continued through 14 d postweaning. The effects of LF observed preweaning did not carry over to the postweaning period.

Lactoferrin has not been supplemented to whole milk. Since some producers utilize whole milk as a part of their calf rearing program, LF's activity in whole milk should be evaluated. Daily supplementation of LF postweaning has not been evaluated. The lowest effective level of LF needs to be identified in order to establish LF supplementation as an economical part of a successful calf health program. 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 versus 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 in Salisbury, NC, were randomly assigned to one of three treatments: 0 (control), 0.5, or 1 g/d of LF. Calves were not twins and weighed a minimum of 34 kg at birth. Newborn calves were removed from their dam as soon as possible and fed 3.8 L of good quality colostrum, as indicated by a colostrometer (Nasco, Inc., Fort Atkinson, WI). Calves were fed 3.8 L of colostrum via nipple bottle or esophageal feeder for the first 2 d of life. Calves were housed individually in either outdoor fiberglass hutches with an attached fenced outside

exercise area or in individual pens in an open-sided barn. The housing square footage per calf was similar. The hutches and pens were bedded with a sand and wood chip mixture. Calves remained in their hutches for the duration of the trial. Dehorning and castration were performed after the trial was completed.

Beginning on d 3, calves were fed 3.8 L of saleable whole milk from cows tested negative for Bovine Leukosis Virus (BLV) and Johnes 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 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 LF from the same bottle. Lactoferrin was a commercially prepared product (DMV International, Veghel, The Netherlands). The amount of LF supplement fed was adjusted for ingredient composition in order to provide 0.5g or 1g of pure LF per calf daily. All calves were weaned at 35 d of age. From weaning through d 56, 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 LF from bottles. Starting on d 3 and continuing until d 56 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 Intake and Analysis*

Starter was sampled weekly and composited monthly. Starter orts were sampled three times weekly 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 for

DM, CP, ADF, and NDF by Cumberland Valley Analytical Services, Inc. (Maugansville, MD).

***Measurements***

Within 24 h of birth, and every week thereafter, measurements were made for calf body weight, 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 applied daily on a scale 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 d 28 and 56, blood was collected via jugular venipuncture into vacutainers containing EDTA and placed on ice for transport to the laboratory. These samples were centrifuged for 15 min at $2500 \times g$, 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 (**SRID**) (Cardiotech Services, Inc., Louisville, KY). Each day a sample of milk fed to calves prior to LF addition was collected and analyzed for LF concentration using SRID (Cardiotech Services, Inc., Louisville, KY). Following the trial, calves were weighed each month through 6 months of age.

***Statistical Analysis***

The experimental design was a randomized complete block design with calves blocked by date of birth, gender, and housing type. Preweaning and postweaning data were analyzed using the general linear methods (GLM) procedure of SAS (2004). Calf was a random effect and treatment, gender, and housing type were fixed effects. The combination

of preweaning and postweaning data, reported as overall data, were analyzed using the mixed procedure (PROC MIXED) of SAS (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

Nutrient composition of starter is presented in Table 1. Average LF concentration of the whole milk fed to calves prior to LF addition was 190 μg/ml. The amount of LF supplied by 3.8 L of whole milk, with no supplemental LF, was 0.72 g. Robblee, et al. (2003) reported that the LF concentration of milk replacer fed to calves was 14 μg/ml. Calves in this experiment were fed 4 L of milk replacer, which provided only 0.06 g of LF. 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. This data indicates that calves in this study received adequate colostrum since 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 (Naylor et al., 1977; Tyler et al., 1996; Wilson et al., 1994).

### *Performance Results*

DMI 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, ADF, and NDF (Table 2) followed the same pattern. DMI from starter and whole milk (Table 3) (12.5% 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.

Body weight (Table 4) was not affected by LF supplementation. Postweaning body weight for barn-housed calves was greater (P = 0.02) than that of hutch-housedcalves. 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 LF. Overall hip height for control calves and calves fed 1 g/d of LF were not significantly different. Overall hip height for calves fed 0.5 g/d of LF and calves fed 1 g/d of LF were also not different. There were no observed effects of LF or housing on hip height preweaning. However, postweaning hip height was greater for control calves and calves fed 1 g/d of LF than for calves fed 0.5 g/d of 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 and postweaning barn-housed calves had greater ADG than 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). Control calves had a greater feed efficiency than calves fed 1 g/d of LF. Preweaning feed efficiency was not different among control calves and calves fed 0.5 g/d of LF. There were also no differences between calves fed 0.5 g/d of LF and 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). Post trial ADG (Table 5) was not affected by treatment or type of housing.

In this trial, LF supplementation had no significant positive effects on calf intake, growth, or health. DMI from starter only, as well as DMI from starter and milk, was similar across all treatments, and thus supporting similar ADG across all treatments.
Feed efficiency, calculated with DMI from starter and milk, was not different among treatments. When feed efficiency was calculated using DMI from starter only, overall and postweaning feed efficiency was also not different among treatments. Preweaning feed efficiency, calculated with DMI from starter only, was greater for control calves versus calves fed 0.5 g/d of LF.

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

***Health Results***

Body temperatures, fecal scores, respiratory scores, and plasma HP concentrations (Table 6) were not affected by LF supplementation. Overall, preweaning, and postweaning body temperatures were higher for barn-housed calves than for hutch-housed calves. Fecal and respiratory scores were not affected by type of housing. Plasma HP concentrations were higher for hutch-housed calves than for barn-housed calves.

Lactoferrin supplementation had no significant benefit on calf health. Body temperatures for all treatment groups were within the range of normal calf temperatures. Fecal and respiratory scores were low, which also indicates that all calves were healthy

throughout the trial. Any effect due to LF supplementation may not have been evident since calves did not appear to be stressed and remained healthy while on trial. While no statistical differences were observed in plasma HP concentrations, there were numerical differences, with decreased HP concentrations as LF supplementation increased. It is likely that no statistical differences were seen because the sample size was too small. A decreased HP concentration could indicate that calves fed LF were less stressed than control calves. Concentrations of HP, an acute phase protein produced by the liver, increase during infection, inflammation, and tissue damage (Ganheim et al., 2003; Horadagoda et al., 1999; Skinner et al., 1991). Despite the numerical decrease across treatments, HP values for all calves were relatively low. It has been reported that the normal HP concentration for adult cattle is approximately 100 μg/ml (Skinner et al., 1991). Ganheim et al. (2003) observed HP concentrations of 890 to 1770 μg/ml in calves infected with Bovine Viral Diarrhea virus. These values are much higher than those observed in this study.

### *Housing Effects*

The type of housing utilized affected many of the parameters measured in this trial. This was not expected, since many conditions for both types of housing were similar. Calves were housed in either individual pens in an open-sided barn or hutches with an attached exercise area. Both types of housing were similar in size and bedded with the same material. The barn had a high roof and open side. While calves in the hutches had shade in the hutch, it was hot during summer months. The same numbers of calves were housed in the barn and in hutches.

Calves in the barn had greater intakes and increased growth compared to calves in hutches. The calves raised in the barn had higher postweaning DMI, ADG, body weight, hip height, and heart girth. There were no differences in fecal and respiratory scores. Calves in the barn had higher average body temperatures. While the difference was statistically significant, the difference was small and may not be biologically important. Calves in the barn also had significantly lower plasma HP concentrations (Table 6), indicating that these calves may have been under less stress and healthier than calves in hutches.

## CONCLUSIONS

The results of this trial are in contrast to results of previous reports. 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 these trials in that LF was added to whole milk and fed throughout the postweaning phase via water. Lactoferrin was added to whole milk, as opposed to milk replacer. Lactoferrin may function differently when fed with whole milk, which may explain the results from this trial. All calves consumed 0.72 g/d of LF provided by the whole milk. Calves in previous trials only consumed 0.06 g/d of LF provided by milk replacer (Robblee, et al., 2003). This may have influenced the results observed in this study. It is also possible that no effect of LF 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. Due to the inconclusive results of this trial, further research is needed to evaluate LF's role in whole milk and its effect when fed in the postweaning period.

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# APPENDIX

## Colostrum, Milk, and Plasma Analysis Procedures

**Colostrometer Procedure**

**(Nasco, Inc., Fort Atkinson, WI)**

A colostrometer measures the specific gravity of colostrum and estimates total gamma globulin on the basis of a statistical relationship.

1. Collect only first or second milking colostrum into a clean and dry container.
2. Transfer approximately 250 ml of room temperature (22°C) colostrum into the measuring cylinder.
3. Lower the colostrometer into the measuring cylinder, allowing excess colostrum to overflow until the instrument floats freely.
4. While the instrument floats freely, determine the quality of colostrum by reading the color coded scale that is located inside the unsubmerged portion of the instrument.
5. Only feed colostrum that registers in the green zone.
6. Rinse the colostrometer in cold water.

**Refractometer Procedure**

**(Reichert Inc., Depew, NY)**

A refractometer works by shining a beam of light through a sample of liquid. The device measures the amount of light that is refracted from the light path due to the constituents in the sample. In blood, proteins will cause light to bend. Instead of measuring IgG, the refractometer measures total serum or plasma protein. In newborn calves, there is a close correlation between total protein and IgG in the blood.

1. Hold the instrument in a horizontal position and swing up the cover plate to expose both the measuring prism and the bottom surface of the cover plate.
2. Place a drop or two of sample on the prism. To minimize evaporation, close the cover plate over the prism immediately.
3. To hold the instrument for a reading, place your finger on the cover plate and press gently, but firmly.
4. Point the instrument toward a light source. The instrument should be tilted with respect to the light source until the optimum contrast between light and dark boundaries is obtained. Take a reading at the point where the dividing line between light and dark fields crosses the scale.

**Haptoglobin SRID Procedure**

**(Cardiotech Services, Inc., Louisville, KY)**

1. Prepare 40 mM L-Cysteine solution; 24 mg of L-Csyteine is dissolved with 5 mL of Solvent for L-Cysteine
2. Plasma samples are treated with Cysteine by adding an equal volume of a buffer containing 40mM Cysteine, usually, 0.1 mL of plasma is added to 0.1 mL of 40 mM Cysteine solution and mixed well.
3. Remove plastic cover from the test plate.
4. Use a micro-dispenser (5 µl) to apply 5 µl of Standard Solution A to one test well, repeat with 5 µl of Standard Solution B to a separate well.
5. In an identical manner, apply exactly 5 µl of each Cysteine treated bovine plasma sample to separate wells. Use one well for each sample.
6. Firmly secure plate cover.
7. Maintain plate in a horizontal position. Place in a humidified box at room temperature. Incubate for 24 to 48 h before reading results.
8. Remove plate from incubator.
9. Invert the plate over a source of illumination.
10. Use the plastic scale provided to measure the external diameter of each precipitin ring.
11. Plot the diameters of the Standard Solutions on the vertical axis of the graph paper provided. The haptoglobin concentration is on the horizontal axis.
12. Make a straight line through points A and B, which is used as a reference line.

13. Plot the diameters of precipitin rings for each test sample.
14. From the reference line, the haptoglobin concentration of each test sample may be calculated
15. To obtain the actual concentration of haptoglobin, double the concentration read off the reference line.
16. If no precipitin ring appeared, the haptoglobin concentration was below 10 μg/mL, which is normal.

**Lactoferrin SRID Procedure**

**(Caridotech Services, Inc., Louisville, KY)**

1. Remove plastic cover from test plate.
2. Use a micro-dispenser (5 µl) to apply 5 µl of Standard Solution A to one test well, repeat with 5 µl of Standard Solution B in a separate test well.
3. In an identical manner, apply 5 µl of each bovine milk sample to separate wells. Use one well per sample.
4. Firmly secure plate cover.
5. Maintain the plate in a horizontal position. Place the plate in a humidified box at room temperature to incubate for 24 to 48 h before reading test results.
6. Remove plate from incubator
7. Invert the plate over a source of illumination.
8. Use the plastic scale provided to measure the external diameter of each precipitin ring.
9. Plot the diameters for the Standards on the vertical axis of the graph paper provided. Lactoferrin concentration in on the horizontal axis.
10. Make a straight line through points A and B, which can be used as a reference line.
11. Plot the diameters of the precipitin ring for each test sample.
12. From the reference line, the lactoferrin concentration of each sample may be calculated

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**Table 1.** Chemical analysis of calf starter[1].

| Item | Starter |
|---|---|
| DM, % | 92.76 |
| CP, % DM | 25.56 |
| TDN, % DM | 77.37 |
| ADF, % DM | 8.45 |
| NDF, % DM | 17.69 |
| Ca, % DM | 1.26 |
| P, % DM | 0.79 |
| Mg, % DM | 0.31 |
| Fe, ppm | 322.12 |

[1]Deal-Rite Feeds, Inc., Statesville, NC.

**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 lactoferrin and for calves reared in different types of housing.

| | Lactoferrin[1] (g/d) | | | | | Housing | | | |
|---|---|---|---|---|---|---|---|---|---|
| Item | 0 | 0.5 | 1 | SEM | P = | Barn | Hutch | SEM | P = |
| DM Intake[2],kg/d | | | | | | | | | |
| Overall | 0.54 | 0.54 | 0.57 | 0.04 | 0.86 | 0.61[x] | 0.48[y] | 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[x] | 1.02[y] | 0.06 | 0.001 |
| CP Intake, kg/d | | | | | | | | | |
| Overall | 0.14 | 0.14 | 0.14 | 0.01 | 0.83 | 0.16[x] | 0.12[y] | 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[x] | 0.26[y] | 0.02 | 0.0007 |
| ADF Intake, kg/d | | | | | | | | | |
| Overall | 0.05 | 0.05 | 0.05 | 0.004 | 0.87 | 0.05[x] | 0.04[y] | 0.003 | 0.005 |
| Preweaning | 0.01 | 0.02 | 0.02 | 0.002 | 0.64 | 0.02 | 0.01 | 0.002 | 0.12 |
| Postweaning | 0.10 | 0.10 | 0.10 | 0.01 | 0.75 | 0.11[x] | 0.09[y] | 0.01 | 0.0006 |
| NDF Intake, kg/d | | | | | | | | | |
| Overall | 0.09 | 0.10 | 0.10 | 0.01 | 0.86 | 0.11[x] | 0.09[y] | 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[x] | 0.18[y] | 0.01 | 0.0007 |

[x,y]Means within a row with different superscripts differ ($P < 0.05$).
[1]Calves were supplemented with either 0, 0.5, or 1 g/d of lactoferrin added to the liquid portion of the diet during the preweaning and postweaning phase.
[2]DM intake includes intake of calf starter only.

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

| | Lactoferrin[1] (g/d) | | | | | Housing | | | |
|---|---|---|---|---|---|---|---|---|---|
| Item | 0 | 0.5 | 1 | SEM | P = | Barn | Hutch | SEM | P = |
| DM Intake[2],kg/d | | | | | | | | | |
| Overall | 0.84 | 0.84 | 0.86 | 0.04 | 0.86 | 0.90[x] | 0.78[y] | 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 [x] | 1.02 [y] | 0.06 | 0.001 |

[x,y]Means within a row with different superscripts differ (P < 0.05).
[1]Calves were supplemented with either 0, 0.5, or 1 g/d of lactoferrin added to the liquid portion of the diet during the preweaning and postweaning phase.
[2]DM intake includes intake of whole milk and calf starter.

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

| | Lactoferrin[1] (g/d) | | | | | Housing | | | |
|---|---|---|---|---|---|---|---|---|---|
| Item | 0 | 0.5 | 1 | SEM | P = | Barn | Hutch | SEM | P = |
| Body Weight, 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[a,b] | 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 |
| Hearth 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 ($P < 0.05$).

[1]Calves were supplemented with either 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 average daily gain and feed efficiency and post trial average daily gain for calves fed differing amounts of lactoferrin and for calves reared in different types of housing.

| | Lactoferrin[1] (g/d) | | | | | Housing | | | |
|---|---|---|---|---|---|---|---|---|---|
| Item | 0 | 0.5 | 1 | SEM | P = | Barn | Hutch | SEM | P = |
| 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 [a,b] | 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 |
| Post Trial ADG, kg | 1.02 | 1.08 | 1.09 | 0.19 | 0.53 | 1.04 | 1.09 | 0.04 | 0.37 |

[a,b]Means within a row with different superscripts differ ($P < 0.05$).

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

[2]Feed efficiency is calculated using DM intakes of calf starter only.

[3]Feed efficiency is calculated using DM intakes of whole milk and calf starter.

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

| | Lactoferrin[1] (g/d) | | | | | Housing | | | |
|---|---|---|---|---|---|---|---|---|---|
| Item | 0 | 0.5 | 1 | SEM | P = | Barn | Hutch | SEM | P = |
| Body Temperature, ˚C | | | | | | | | | |
| Overall | 38.60 | 38.60 | 38.61 | 0.03 | 0.96 | 38.66[x] | 38.55[y] | 0.03 | 0.008 |
| Preweaning | 38.59 | 38.57 | 38.59 | 0.03 | 0.88 | 38.63[x] | 38.54[y] | 0.03 | 0.03 |
| Postweaning | 38.61 | 38.63 | 38.65 | 0.05 | 0.79 | 38.70[x] | 38.56[y] | 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[y] | 38.82[x] | 10.11 | 0.03 |

[x,y]Means within a row with different superscripts differ ($P < 0.05$).

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

[2]1 = normal, 2 = soft, 3 = runny, 4 = watery.

[3]1 = normal, 2 = runny nose, 3 = heavy breathing, 4 = coughing