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THE IMPORTANCE OF LACTOFERRIN IN CALVES NUTRITION

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ABSTRACT

Due to the fact that the majority of preweaned dairy calf mortality can be attributed to diarrhea, or scours and to the fact that calf loss can be controlled by either treatment or prevention of the illness, research focus on prevention has increased. Lactoferrin (LF), a naturally occurring protein from milk, has proven antibacterial properties. It has been shown to not only improve calf health, but calf growth as well. LF's ability to maintain calf health may establish it as a preventative agent against calf gastrointestinal illness, including diarrhea in calves.

Milk and colostrum contain the highest amounts of LF of any bodily secretion. Lactoferrin concentration in bovine colostrum is approximately ten to one hundred times higher than in mature bovine milk (2 mg/mL vs. 20 to 200 µg/mL). In comparison, human colostrum and mature milk contain 7 mg/mL and 1 mg/mL respectfully.

The primary function of LF is supposed to be the inhibition of microbial growth. Lactoferrin reduces the proliferation of bacteria by damaging the bacterial cell wall. While LF is most active against gram-negative bacteria, it also displays some activity against gram-positive bacteria.

Since the bovine milk has a low LF concentration, calves may benefit from supplemental LF. Studies have been conducted with calves fed supplemental doses of LF in order to evaluate its beneficial effects of supplementation to calf milk replacers (MR) or to whole milk fed calves. LF has proven to promote calf health and performances in milk replacer fed calves. Therefore, its addition to feed in the calf rearing process might have health and economical reasons. However, additional research needs to be conducted in order to clarify LF's role in different types of calf raising programs (esspecialy in calves feed whole milk), or during periods of pathogen challenge.

KEY WORDS*: Lactoferrin, Calves, Feed, Milk Replacer, Performances*

INTRODUCTION

Since majority of preweaned dairy calf mortality can be attributed to diarrhea, or scours and the fact that calf loss can be controlled by either treatment or prevention of the illness, has lead to an increase in the research focus on prevention. Lactoferrin (LF), a naturally occurring protein found in milk, has proven antibacterial properties and been shown to not only improve calf health, but calf growth as well. LF's ability to maintain calf health may establish it as a preventative agent against calf gastrointestinal illness, including diarrhea in calves.

Lactoferrin is an iron binding protein found in many body secretions, including milk and colostrum. Milk and colostrum contain the highest amounts of LF of any bodily secretion. Lactoferrin concentration in bovine colostrum is approximately 2 mg per milliliter while bovine

mature milk contains 20 to 200 µg/mL. In comparison, human colostrum and mature milk contain 7 mg/mL and 1 mg/mL. The primary function of LF is supposed to be the inhibition of microbial growth. Lactoferrin reduces the proliferation of bacteria by damaging the bacterial cell wall. While LF is most active against gram-negative bacteria, it also displays some activity against gram-positive bacteria.

Bovine milk has a low LF concentration, therefore, calves may benefit from supplemental LF. Because of the mentioned, studies have been conducted with calves fed supplemental doses of LF in order to evaluate its beneficial effects of supplementation to milk replacers (MR) or to whole milk fed calves. Since, LF promotes calf health and performance in milk replacer fed calves, 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.

The roles of lactoferrin

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 (English et al., 2007).

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, such as E. coli, and gram-positive bacteria, such as *C. ramosum* (English et al., 2007). 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 (English et al., 2007). Although LF does bind iron from its environment, some bacterial species have developed systems of recovering the bound iron (Ekins et al., 2004). Therefore lactoferrin's primary method of microbial inhibition is believed to be accomplished by causing damage to the cell membrane of gram-negative bacteria, particularly the cell wall. By increasing membrane permeability LF enhance the susceptibility of gram-negative bacteria to lysozyme importance (English et al. 2007).

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 (Heinrichs et al., 2003). One of the antibacterial mechanisms 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 in milk replacer fed calves

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.

Dawes et al. (2004) fed calves supplemental LF to establish its effect on serum LF concentrations. Within 4 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. They have found 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.

The absorption of colostral LF has also been investigated by Hurley and Sixiang (2000). These authors have found that increasing the total consumption of LF at the first feeding results 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. They suggested that LF clearance from the blood is increased within 12 h after the first feeding.

Talukder et al. (2003) infused calves intraperitonealy with LF in order to monitor the LF concentrations in plasma and cerebrospinal fluid (CSF). They reported that LF concentration increased after application and peaked at 4 h in both plasma (seven times higher than 0h) and CSF (four times higher than 0h).

Kume and Tanabe (1996) investigated the efficiency of feed supplementation of LF and $FeSO_4$ on the Fe status of calves during the first ten d after birth. Calves were fed colostrum with the following addition: 1) control, 2) 40 mg Fe as $FeSO_4$, 3) 40 mg Fe as $FeSO_4$ + 5g LF. They suggested that LF may not be an Fe source to calves, however supplemental LF with ferrous Fe may be a more efficient means to accelerate the shift of Fe into hemoglobin.

Muri et al.(2005) also conducted research on LF and Fe status of calves fed milk based formula. The calves were fed following treatments: a) milk based formula only (F); b) formula + LF (FL); c) formula + vitamin A (FA); d) colostrum only (C). Hematocrit and hemoglobin were not affected by treatment, even in groups 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 it needs to be mentioned that 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. Simmilarly, the research with genetically modified mice supported these findings (Ward et al., 2003).

Joslin et al. (2002) fed calves supplemental LF to determine effects on growth, health, serum Fe concentration, hematocrit, and starter DMI (Dry Matter Intake). Colostrum, milk, and milk replacer were supplemented with either 0 (control), 1, or 10 g/d of LF. They have found that calves fed LF in the preweaning phase, had higher starter DMI than control calves, however that 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 (Average Daily Gain) than control calves. Calves fed 1 g LF had greater ADG than calves fed 10 g LF. 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. Once again, there were no differences postweaning. 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 the 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 milk replacer fed 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. Calves fed 1 g/d LF had the lowest scores and control calves had the highest scores. No differences were observed postweaning. Calves fed 1 g/d LF had the lowest number of days medicated and control calves had the highest. No differences were observed postweaning. Starter DMI, body weight, and weaning age were no different among treatments. Preweaning ADG increased linearly with LF supplementation. Postweaning ADG was similar among treatments. Preweaning feed efficiency also increased linearly with LF supplementation, however postweaning feed efficiency decreased linearly. 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). 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.

Lactoferrin supplementation in whole milk fed calves

Other than the work of English et al. (2007) we have not found other works describing lactoferrin supplementation in the whole milk fed calves. Since some producers still 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.

English et al. (2007) investigated the effects of supplemental lactoferrin on feed intake, growth, and health during the preweaning and postweaning periods in milk fed calves. They supplemented whole milk with three levels of lactoferrin 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. 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. DMI from starter only was not affected by LF supplementation.

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. The results of the trial conducted by English et al. (2007) 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 the trial of English et al. (2007). The study of English et al. (2007) differed from the other 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. Therefore English et al. (2007) proposed that lactoferrin may function differently when fed with whole milk, which may

explain the different results. 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.

CONCLUSION

Based on the results of studies conducted by numerous authors lactoferrin supplementation has positive effects in dairy calves. These beneficial effects are more prominent in cases when calves are fed milk replacer formulas that do not contain or contain small amounts of lactoferrin. The fact that lactoferrin supplementation to calves fed formulas containing high amounts of lactoferrin or fed whole milk did not give to significant results only confirms its necessity in feed. Therefore 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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