

EFFECT OF SUPPLEMENTAL *LACTOBACILLUS ACIDOPHILUS* ON THE
GROWTH AND PERFORMANCE OF DAIRY CALVES

by

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(Under the Direction of John K. Bernard)

ABSTRACT

Eighty Holstein calves were used in a 56 day trial to determine the effect of including *Lactobacillus acidophilus*, LA 51, in milk replacer at increasing concentrations (control, 5×10^4 , 5×10^6 , and 5×10^8 cfu LA 51) on their overall health and growth rate. Intake of milk replacer and calf starter was similar for all treatments, but the control and 5×10^8 LA 51 groups tended to have higher starter intake than the other two treatments, both before and after weaning. Total body weight and average daily gain were higher ($P < 0.08$) for the control and 5×10^8 LA 51 than the other two treatments. Excluding the control, a positive linear relationship was observed for all variables with increasing LA 51 at the end of six weeks. No differences were observed for incidence of scours or respiratory illness among the treatments. Results of this trial demonstrate that supplementation of 5×10^8 LA 51 has the potential to support higher rates of gain, which persist after weaning and supplementation ceases.

INDEX WORDS: Calf, Probiotic, *Lactobacillus acidophilus*, Milk Replacer, Body Weight Gain, Microbial Inoculant

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DEDICATION

To my parents, brother and Chris

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CHAPTER 1

INTRODUCTION

Raising healthy replacement dairy heifers that enter the milking herd by 24 months of age is important for the success of any dairy production system. Replacement programs should provide for optimal growth so that the replacement dairy heifer reaches her lactation potential at the desired age with minimal expense (Arrayet et al., 2002). To be successful, calves should be managed in a manner that promotes consumption of nutrients to support growth and minimize the risk of disease. Replacement animals that become sick do not grow well or produce up to their genetic potential. Treating sick replacement calves is expensive and time consuming. Consequently, sickness and death among replacement heifers can represent huge losses in the dairy industry.

Benefits from the addition of antibiotics to the liquid diet of dairy replacement heifers include increased weight gains and improved overall health. However, due to emerging public health concerns about antibiotic resistance, farmers and researchers are testing the effectiveness of feed additives such as probiotics as a substitute for antibiotics. Probiotics are microbial feed supplements, which benefit the host animal by improving its intestinal microflora. Probiotics have been shown to increase body weight gain and feed efficiency and decrease the incidence of scours and mortality when fed to calves (Donovan et al., 2002). The objective of this study was to investigate the potential effects of feeding a commercially available *Lactobacillus acidophilus* on feed intake, feed efficiency, weight gain and overall health of dairy calves.

CHAPTER 2

LITERATURE REVIEW

Nutrient requirements of the young calf

Throughout the first two to three weeks of life, the digestive system of the newborn calf is developing very rapidly. Liquid feed bypasses the reticulo-rumen to the omasum and abomasum via the reticular groove where digestion is similar to that of the monogastric animal (Ruckebusch, 1988). Nutrient requirements are best met using high quality liquid feeds formulated from highly digestible sources of carbohydrates, proteins and fats. Rumen development in the calf is stimulated by consumption of dry feed and its fermentation in the immature rumen. Development of the ruminal epithelial tissue, the major site of volatile fatty acid (VFA) absorption, is dependent on the presence of VFA, particularly butyrate. Dry feed or starter should be high in readily fermentable carbohydrates, and have an adequate amount of digestible fiber. In the immature rumen, cellulose digestibility is limited. Long hay should not be fed before weaning because it is not effective in developing the rumen and limits metabolizable energy intake; however, adequate particle size is recommended to prevent abnormal ruminal papillae development or keratinization (NRC, 2001).

There are three phases in the development of digestive function in calves. The first phase is the liquid feeding phase in which all or most nutrient requirements are met by milk or milk replacer. In this stage the reticular groove functions to shunt liquid feeds directly to the omasum and abomasum, avoiding microbial breakdown in the rumen. The next phase is the transition

phase during which nutrient requirements of the calf are met by the combination of both liquid diet and starter. The ruminant phase is the last stage in which calves consume solid feed only and derive most of their nutrients from rumen microbial fermentation (NRC, 2001).

Energy requirements of calves

In the most recent edition of the National Research Council (NRC, 2001), the energy requirements of calves are derived on the basis of metabolizable energy. In young replacements fed milk or milk replacer only and weighing between 25 and 50 kg, daily NE_M ranges from 0.96 to 1.62 Mcal, where $NE_M = 0.086 LW^{0.75}$. The efficiency of use of metabolizable energy (ME) from milk or milk replacer to meet maintenance requirements is set at 86 percent. Maintenance ME is defined as $0.100 \text{ Mcal/kg}^{0.75}$ daily. Requirements for ME are calculated with the equation:

$$\text{ME requirement (Mcal/d)} = 0.100 \text{ BW}^{0.75} + (0.84 \text{ BW}^{0.355})(\text{BWG}^{1.2})$$

where BW and BWG are measured in kilograms. Because of the high and variable metabolic rate of calves in the first week of life, these ME requirements may be underestimated (NRC, 2001).

By the second week of life, calves should be consuming a substantial amount of nutrients from starter. Starter consumption can be encouraged by providing free access to water and by using a highly palatable nutritious starter from first week of life until weaning. Starter consumption is critical to the development of an active functioning rumen. The maintenance requirements and efficiency of ME use do not differ much between an all milk diet and a diet consisting of milk and dry feed. Therefore, regardless of diet, the NE requirements for maintenance and gain should not change. Efficiencies of utilization of ME for maintenance and gain will be a little lower for starter than for milk or milk replacer. The efficiencies of ME use

from dry feeds for maintenance and gain were fixed at 75 and 50 percent respectively. The efficiency of ME use from the total diet is then calculated as the average of individual efficiencies for milk and starter weighted according to their contribution to the total ME in the diet. The NRC predicts that intake of DM from starter increases from about 0.8 to 0.1 percent of body weight at 3 weeks of age to 2.8 to 3.0 percent of body weight at 8 weeks of age (NRC, 2001).

Effects of environmental temperature on energy requirements of young calves

Newborn calves have enough energy reserves to last only one day under cold conditions. The energy standards that have been discussed are based on the premise that the calf is in a thermoneutral zone. The thermoneutral zone is 15 to 25 degrees Celsius for calves 0 to 3 weeks of age. This zone may shift due to age, amount of feed intake, amount of subcutaneous fat, and the length and thickness of the haircoat. If the temperature is lower than 15 degrees Celsius, the maintenance requirement will increase, so extra energy should be fed. There are three ways to increase the energy intake of calves. One way is to increase the amount of liquid diet they are consuming. Another way would be to increase the percentage of milk solids in the liquid diet. However, the NRC states that DM should not exceed 20% in the milk replacer. Thirdly, one could increase the fat content of a liquid diet. The disadvantage of adding fat to the liquid diet is that intake will decrease (NRC, 2001). In a study by Kuehn et al. (1994), no benefit was observed in calf growth or performance when supplemental fat was added to the milk replacer or starter in thermoneutral conditions. Fat in the milk replacer depressed DMI and DE intake of starter through weaning. However, these authors stated that high fat concentrations in milk replacer or starter may benefit calves in cold environments or under continuous stress.

Protein requirements of calves

Protein requirements for dairy calves are divided into two components: maintenance and gain. Maintenance constitutes obligatory nitrogen losses in urine and feces and gain pertains to nitrogen stored in tissues. The protein requirement is expressed in apparent digestible protein (ADP). The equation for ADP is as follows in the NRC:

$$\text{ADP (g/d)} = 6.25 [1/\text{BV} (\text{E} + \text{G} + \text{M} \times \text{D}) - \text{M} \times \text{D}]$$

where BV is biological value (the efficiency of nitrogen use for growth above maintenance, equal to a value of 0.80), E is endogenous urinary nitrogen, G is the amount of nitrogen in gain, M is metabolic fecal nitrogen, and D is the amount of dry matter consumed. Loss of nitrogen in hair and skin is ignored in the present edition of the NRC (2001).

Other aspects of calf nutrition

Colostrum

Due to a thick epitheliochorial placentation in cattle, placental transfer of immunoglobulin (Ig) is minimal. Calves are essentially born with no immunity; therefore, they are highly dependent on the passive transfer of Ig from the colostrum of the dam. The immunoglobulins in colostrum are derived from plasma proteins and are transported through mammary secretory cells into the colostrum. Upon ingestion of the colostrum, Ig bind to receptors in the microvilli of the intestine and are absorbed by nonspecific endocytosis into the epithelial cells of the jejunum and ileum. The Ig is enclosed in a vacuole, which moves to the cell membrane and expels its contents by exocytosis into the lamina propria. From there, the Ig

passes into the systemic circulation via lymphatics and venous capillaries. This transfer of the proteins ceases 24 hours after the calf is born (Pedersen et al., 2000).

Colostrum is essential for newborn calves due to its disease protection by passive immunity and its supply of energy. Intake of high quality colostrum soon after birth is critical to the survival of calves. Low blood IgG concentrations are directly related to calf morbidity and mortality as well as long term calf performance. There is a positive linear relationship between colostrum IgG concentrations and IgG absorption (Arthington et al., 2000). The immunoglobulin content of colostrum is highly variable. Therefore, in order for a calf to receive at least 100 grams of IgG, at least 3 liters of colostrum must be ingested from multiparous cows within an hour after birth (NRC, 2001). Intestinal absorption decreases rapidly within the first 24 hours after birth affecting the absorption efficiencies of a wide range of compounds. In a study by Keller et al. (2001), intestinal absorption was monitored with the digestion marker, chromic oxide. Serum IgG levels indicated that calves given colostrum after one hour, had absorbed 20 to 37% of ingested IgG. In contrast, calves that were given colostrum 65 hours after birth absorbed less than 2% of ingested IgG. Absorption efficiencies were calculated using the assumption that serum volume of a newborn calf was 6.5% body weight.

Colostrum is also an excellent and very important source of vitamins and minerals. Another unique characteristic of colostrum is that, unlike colostrum replacements, it contains several hormones and growth factors that stimulate growth and development of the digestive tract and other organ systems.

Water and electrolytes

Water is the most important and most overlooked nutrient. It is essential for optimum growth and consumption of dry feed. Water in milk replacer does not satisfy the needs of the calf and therefore should be offered in addition to a liquid diet. Since water constitutes 70-75% of the weight of a calf, it is vital to thermoregulation and osmoregulation. Young calves with scours experience many problems with regulation of body water. Scours in calves can result in a 10-12% reduction in body weight as water, loss of electrolytes and death. Recent evidence shows that electrolyte imbalances are more important than dehydration itself in causing death from scours. At the first signs of diarrhea, electrolytes should be added to a calf's liquid diet to rehydrate the animal before severe dehydration becomes fatal (NRC, 2001). Water is also important for welfare purposes. Provision of adequate drinking water can prevent nonnutritive oral behavior and can decrease episodes of feed refusal (Gottardo et al., 2002).

Milk replacers

The majority of dairy farms in the United States use milk replacers as the liquid diet for calves. The dairy industry has almost completely replaced dried skim milk with dried whey products in milk replacer for calves. Whey products are digested just as well as dried skim milk in calves and are less expensive. Milk replacers are classified as either all-milk protein or alternative protein. Milk replacers consisting of all-milk proteins include whey protein concentrate, dried whey and delactosed whey. Often times, half of the milk proteins are replaced with lower cost ingredients such as soy protein concentrate, animal plasma, whole-blood proteins and modified wheat gluten. However, since the amino acid profile of some non-milk proteins differs from milk proteins, young calves with an immature proteolytic digestive system may not

be able to adequately digest them. As a result, only all-milk protein milk replacer should be fed to calves less than 3 weeks of age for optimal growth during this stage of life (NRC, 2001).

Probiotics

Antibiotics are commonly used as feed additives for calves. Subtherapeutical use of antibiotics has been shown to increase feed consumption, body weight gains and phagocytic efficiency, and decrease the incidence of scours, mortality and protein requirement in calves (Abu-Tarboush et al., 1996). However, the use of antibiotics in production animal feed can have serious consequences. Public health concerns have been raised due to the ability of antibiotic resistant populations of bacteria to develop in animals and consumers (Donovan et al., 2002). Also, residual antibiotics in dairy foods, meat, eggs, and milk are unacceptable (Abe et al., 1995). The benefits of antibiotics will most likely diminish over time. As a result, they will be reduced and even eliminated in the future. Alternatives for antibiotics have been explored. One of the more promising alternatives to antibiotics are probiotics.

A probiotic is defined as a live microbial feed supplement, which beneficially affects the host animal by improving its intestinal microbial balance (Oh et al., 2000). The effectiveness of probiotics are based on claims that “ingestion of these organisms results in colonization of the digestive tract, prevention of pathogen proliferation, neutralization of enterotoxins produced in situ, modulation of certain bacterial enzyme activity, enhancement of the small intestine digestive capacity, and the exertion of adjuvant effects on the immune system” (Cruywagen et al., 1996). Probiotics have been used in both animals and humans. Studies in dairy calves have shown mixed results with probiotic use. Some have demonstrated improvement in calf performance and overall health, whereas other studies fail to show a significant difference

between the control and treatment. For instance, in a study where Holstein calves were fed milk replacers containing either antibiotics or Enteroguard (Pharmax Biologicals, Des Moines, IA) (a blend of fructooligosaccharides, allicin and gut active microbes), overall body weight gain, severity of scours, and starter intake did not differ between the two treatments, indicating similar calf performance can be obtained by utilization of a probiotic without the undesirable long-term effects of an antibiotic (Donovan et al., 2002). Also, according to Davidson et al. (2000), the health benefits in humans from frozen yogurt supplemented with bacterial probiotics include improved lactose utilization, anticarcinogenic activity, and control of intestinal infections.

An effective probiotic must have certain properties. First of all, it must have the ability to become part of the normal microflora in the intestine. It should survive passage through the gastrointestinal tract and be able to adhere and colonize the intestinal tract. Organisms that can produce a substance that can inhibit growth or kill existing organisms in the intestine have a distinct advantage because there are so many other organisms in the intestine (Oh et al., 2000). In humans, probiotics are utilized to improve lactose digestion. Some of the characteristics that are important in selection of cultures for use in products are β -galactosidase production, bile resistance, and acid tolerance. β -galactosidase is an enzyme that hydrolyzes lactose to glucose and galactose (Ibrahim and O'Sullivan, 2000). Not only are probiotics used to treat lactose malabsorption, but also viral, bacterial, and radiotherapy induced diarrhea, constipation, inflammatory bowel disease, and food allergy (Cesena et al., 2001). However, careful selection of bacterial strains is important. Intra-specific differentiation is necessary to separate probiotic from pathogenic strains (Klein et al., 1998).

The genera of *Lactobacilli* is one of the most commonly used probiotics in food products. Some species of lactic acid bacteria that have been used as probiotics include *L. acidophilus*, *L.*

delbrueckii subsp. *bulgaricus*, *L. casei*, *L. fermentum*, *L. plantarum*, and *L. reuteri*, and *Bifidobacterium* species. *Lactobacillus acidophilus* strains are widely used as probiotic cultures in dairy and pharmaceutical products because the strain is one of the dominant lactobacilli in the human intestine (Oh et al., 2000). *L. acidophilus* is a homofermenter, which means it ferments lactose efficiently to lactic acid (Cruywagen et al., 1996). Again, a probiotic strain has a distinct advantage if it is a good competitive inhibitor. Lactic acid bacteria produce a number of antimicrobial substances such as organic acids, hydrogen peroxide, and bacteriocins. This property makes *L. acidophilus* an excellent probiotic. According to a study by Oh et al. (2000), *L. acidophilus* strains exhibit more acid and bile tolerance than other lactic acid bacteria. In particular, *L. acidophilus* 30SC was able to survive in bile because it was able to deconjugate bile acids. It was also active over a wide pH range and stable in heat treatments. *L. acidophilus* and *Bifidobacterium* can tolerate a pH of 3 and 2 to 8% concentrations of bile acid (Tejada-Simon et al., 1999). *L. acidophilus* showed antimicrobial effects towards *Listeria* and *Bacillus* species, spore forming bacteria, *B. cereus* and *B. subtilis*. The antimicrobial component secreted was found to be proteinaceous because when treated with proteinase K and pronase E there was a loss of antimicrobial activity (Oh et al., 2000).

Because the only carbohydrate that young calves can digest is lactose, and *L. acidophilus* is a homofermenter that ferments lactose efficiently to lactic acid as an end product, this microbial strain is often used in calf studies. In the study by Cruywagen et al. (1996), there were no effects by *L. acidophilus* on diarrhea, feed efficiency, or overall health of calves, but the calves treated with *L. acidophilus* had higher average daily gain in the second week of life than the control. Calves on milk replacer alone lost 4% of initial body weight in the first two weeks of life, but calves fed the *L. acidophilus* treatment lost only 0.8% of initial body weight during

the first two weeks. According to Cruywagen et al. (1996), response to *L. acidophilus* is best seen when calves are stressed. In a study by Ruppert et al. (1994), when calves were kept under stressful conditions, feed intake was higher when the diet was supplemented with a probiotic than for controls. According to Fuller (1989), probiotics are only effective when animals are stressed by the presence of a microbial population that depresses growth. Since calves are quite susceptible to disease in the first two weeks of life, this would support a recommendation to use probiotics in calves less than two weeks of age. In addition, the number of leukocytes in the blood of piglets was increased by feeding *L. acidophilus* (Pollmann et al., 1980). Therefore, it is possible that the reason for decreased incidence of diarrhea and mortality of piglets was due to an elevated immunity in host animals supplemented with this probiotic (Abe et al., 1995). In another study, administration of yogurt supplemented with *L. acidophilus* and *Bifidobacterium* enhanced mucosal and systemic IgA responses to the cholera toxin immunogen in mice (Tejada-Simon et al., 1999).

Bacillus subtilis is another probiotic that has exhibited beneficial results. Like *L. acidophilus*, this species has been tested to see if it improves body weight gain and overall health. *B. subtilis* spores added to the feed of horses, dogs and cats improved intestinal function. Improved intestinal health often has a positive effect on animal growth. However, in a field trial in which *B. subtilis* was included as one of the components of a microbial product, little improvement was seen in calf growth or morbidity. This led Jenny et al. (1991) to perform a study in which diets were supplemented with only *B. subtilis*. Calves fed *B. subtilis* had higher body weight gain from 0 to 6 weeks of age compared with calves fed the control or another treatment of a mixed microbial concentrate (Jenny et al., 1991).

Several other bacterial strains have been tested for use as feed additives. *Bifidobacterium pseudolongum* has been isolated from many animals including calves, piglets, chickens and dogs and therefore has a wide host specificity (Abe et al., 1995). *B. pseudolongum* added to the diet of pre-weaned calves supported increased body weight gain and decreased incidence of scours (Donovan et al., 2002). A study by Abe et al. (1995) reported that *B. pseudolongum* had a beneficial effect on calf body weight gain and feed conversion. They proposed that this was a result of improved intestinal environment based on lower fecal scores. Administration of this probiotic was as effective as antibiotics in protecting against diarrhea. In piglets supplemented with *B. pseudolongum*, body weight gain was greater than controls. Body weight gain was greatest during the suckling period suggesting that probiotics should be administered soon after birth to be most effective. At birth there are no bacteria in the intestine, so the probiotic administered at this time will likely have a better opportunity to colonize in the intestine over the pathogenic bacteria. Like *L. acidophilus*, *Bifidobacterium* has been reported to stimulate immunity in animals (Abe et al., 1995). This same microbe added to frozen yogurt has provided various health benefits in humans as well (Davidson et al., 2000).

Streptococcus faecium and *Streptococcus cervisiae*, combined with other microbial supplements, were both shown to improve feed efficiency in calves (Cruywagen et al., 1996). In other studies, feeding the *Lactobacillus bulgaricus* fermentation product to calves tended to have a positive effect on starter intake and gain during the pre-weaning period. *L. casei* and *L. bifidus* have also been used as feed supplements in calves. In a study by Kyriakis et al. (1999), spores of *Bacillus licheniformis* and *Bacillus toyoi* were used as probiotics in piglet feed. Two different concentrations of *B. licheniformis* and one concentration of *B. toyoi* were used with varying results. The 10^6 concentration of *B. licheniformis* and *B. toyoi* resulted in significantly less

diarrhea than the control, but the 10^7 concentration of *B. licheniformis*, resulted in significantly less diarrhea than the other two groups and the control.

Yeast are another form of probiotics commonly fed to cattle. One such rumen specific yeast is *Saccharomyces cerevisiae*, also called Levucell SC. *S. cerevisiae* mainly affects the functioning of the rumen (Agarwal et al., 2002). Studies with this yeast have shown that the inclusion of Levucell SC in the diet decreases lactic acid concentrations and improves rumen pH which stabilizes the rumen microflora populations supporting greater fiber fermentation. Yeast also stimulates the rumen microbe *Megasphaera elsdenii* by providing it with amino acids and vitamins that it needs to flourish. This limits the production of *Streptococcus bovis*, which often rapidly acidifies the rumen contents by producing lactic acid from starch. Limiting acidosis is important because it maintains an efficient microflora which can increase feed intake.

In a study by Agarwal et al. (2000), various strains of *S. cerevisiae* were tested for their tolerance to certain environmental constraints which are encountered in the gastrointestinal tract. Such constraints included lysozyme, pancreatic enzymes, low pH, organic acids and bile salts. Based on the results of this experiment, *S. cerevisiae* NCDC 49 is considered the best strain, which can tolerate the adverse conditions of the gastrointestinal tract when used as a probiotic in the diet of animals.

Conclusion

In conclusion, though some studies report minimal improvements in animal health or rate of growth, several studies demonstrate that probiotics are beneficial to the calf, especially in the early stages of its life. Several strains of bacteria and yeast have been tested, with the most

beneficial being *Lactobacillus* and *Bifidobacterium*. These probiotics may minimize or eliminate the use of antibiotics in feed and; thus, are extremely promising for the dairy industry.

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CHAPTER 3**EFFECT OF SUPPLEMENTAL *LACTOBACILLUS ACIDOPHILUS* ON THE GROWTH
AND PERFORMANCE OF DAIRY CALVES¹**

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Abstract

Eighty Holstein calves were used in a 56 day trial to determine the effect of the probiotic LA 51, a *Lactobacillus acidophilus* product, added to milk replacer at increasing concentrations on the overall health and growth rate of Holstein replacement calves. Treatments consisted of a control, 5×10^4 cfu LA 51, 5×10^6 cfu LA 51, and 5×10^8 cfu LA 51. Intake of milk replacer and calf starter were similar for all treatments, but the control and 5×10^8 LA 51 groups tended to have higher starter intake than the other two treatments, both before and after weaning. Total body weight gain and average daily gain was significantly higher for the control and 5×10^8 LA 51 than the other two treatments. Excluding the control, the concentration of probiotic had a positive linear relationship with all variables measured at the end of six weeks. No differences were observed in scour or respiratory illness among the treatments. The results of this preliminary study suggest that supplementation of 5×10^8 LA 51 has the potential to support higher rates of gain, which persist after weaning and supplementation ceases.

Introduction

Raising healthy replacement dairy heifers that enter the milking herd by 24 months of age is important for the success of any dairy production system. Researchers have been looking for ways to improve growth and minimize disease in order to produce a profitable cow. One promising method is the use of probiotics in the liquid diets of these calves.

Probiotics are microbial feed additives that colonize in the digestive tract of an animal and improve animal growth and health by its interactions with the microflora in the intestine. A good probiotic will colonize the digestive tract, have the ability to withstand acid and bile, prevent pathogen proliferation by competitive inhibition, neutralize enterotoxins produced in situ, modulate certain bacterial enzyme activity, enhance the small intestine digestive capacity, and exert of adjuvant effects on the immune system (Cruywagen et al., 1996). There are several different strains of both bacteria and yeast that have been utilized in this manner. For example, research has been conducted with the bacterial strains *Bifidobacterium longum*, *Bactillus subtilis*, *Streptococcus thermophilus*, and *Lactobacillus acidophilus*, and yeasts such as *Sacchromyces cerevisiae* with mixed results.

Lactobacillus acidophilus strains have been used frequently in the liquid diet of replacement calves. Because *L. acidophilus* ferments lactose efficiently to lactic acid, it is beneficial to calves, which can only digest lactose (Cruywagen et al., 1996). This strain is a good competitive inhibitor, exhibits good acid and bile tolerance, stable in heat and is active over a wide pH range (Oh et al., 2000). In a study by Cruywagen et al. (1996), when *L. acidophilus* was added to milk replacer, there were no effects on diarrhea, feed efficiency, or overall health of calves, but the calves treated with *L. acidophilus* had higher average daily gain in the second week of life than the control. Calves on milk replacer alone lost 4% of initial body weight in the

first two weeks of life, but calves fed the *L. acidophilus* treatment lost only 0.8% of initial body weight during the first two weeks. Other studies report improvements in overall calf health. Studies by Pollmann et al. (1980), Abe et al. (1995), and Tejada-Simon et al. (1999), report elevated immune responses in animals supplemented with this bacterial strain.

The objective of this research is to determine the effect of different concentrations of a commercially available *Lactobacillus acidophilus* supplement in milk replacer fed to calves on feed intake, feed efficiency, weight gain and overall health of dairy calves.

Materials and Methods

Eighty Holstein calves were used in an eight week randomized block design trial. Calves were assigned randomly to one of four experimental treatments. Twenty-three of the calves were offspring of cows located at the University of Georgia Dairy Research Center (Coastal Plain Experiment Station, Tifton, GA) and 57 calves were purchased from commercial dairy farms in Dothan, AL or Bushnell, FL. Purchased calves ranged from 2 days to 2 weeks of age.

Calves were housed in individual stalls bedded with peanut hulls with woven wire dividers. Stalls were under roof without siding. Fresh bedding was provided as needed.

Calves born at the Dairy Research Center received colostrum immediately after birth and for the following three days. Upon arrival to Tifton, purchased calves were supplemented with electrolytes to minimize the effects of stress associated with transport. Blood samples were collected from each calf at four days of age or one day after arrival for analysis of blood protein concentrations using a refractometer. Nasal swabs were collected from each calf and sent to the University of Georgia Veterinary Diagnostic Laboratory for analysis of persistently infected

bovine viral diarrhea (BVD). One calf tested positive for BVD, and was immediately removed from the study and subsequently euthanized.

A commercial milk replacer (20:20 Calf Milk Dairy Partners, Manchester, TN) was fed twice daily at 0730 and 1500 h each day at the rate of 1.37 kg/1.89 L of water. Treatments consisted of a control and three different concentrations of lactobacillus 51 inoculant provided by the Nutritional Physiology Corporation (Indianapolis, IN). All treatments were prepackaged in amounts for 20 calves. Concentrations were 0 (control, carrier only), 5.0×10^4 cfu, 5.0×10^6 cfu, and 5.0×10^8 cfu. Each packet of inoculant was mixed with 1000 mL of luke warm water, before adding it to the milk replacer. When fewer than 20 calves were on a treatment, 50 mL of the mixed inoculant in water was added to the milk replacer for each calf on the treatment. Each treatment-was mixed separately to prevent cross contamination. Calves were individually fed in buckets which were labeled with the treatment and calf number. Milk replacer intake for each calf was recorded at each feeding and was corrected for any refusal. All feeding buckets and mixing containers were sanitized daily.

Fresh water was provided for each calf for ad libitum consumption. A commercial calf starter was offered after one week on treatments. However, older calves were offered calf starter immediately. The amount of starter consumed was recorded daily and increased or decreased daily for each calf according to intake.

Fecal scores and respiratory scores were recorded daily for each calf to monitor their health throughout the trial. Fecal scores were assigned on a 1 to 4 scale as described by Larson et al. (1977) where 1 was normal and 4 was liquid. Respiratory scores were assigned on a 1 to 3 scale as described by Bascom et al. (2002) where 1 was normal, 2 was a runny nose/eyes, and 3 was mucus discharge from nose/eyes and fever.

Calves were weighed once each week during the eight-week trial, immediately before the afternoon feeding. Calves were vaccinated with Bovi-Shield with Leptospirosis (Pfizer Animal Health, Exton, PA) according to a routine calf vaccination schedule. Calves were dehorned between one and three weeks of age during the trial with an electric dehorner. If a calf became ill or injured it was treated according to standard protocols. Respiratory illness was treated with Excenel (Pharmacia &Upjohn Company, Kalamazoo, MI), or Micotil (Elanco Animal Health, Greenfield, IN) depending on severity. Calves with scours were given an electrolyte solution to prevent dehydration.

At the end of the week six, calves were abruptly weaned. For the following two weeks, the calves were kept in their individual pens and fed only calf starter.

Samples of milk replacer and calf starter were collected weekly for analysis of DM, ash (AOAC, 1990), CP (Leco FP-528 Nitrogen Analyzer, St. Joseph, MO), ADF (AOAC, 1990), and NDF (Van Soest et al., 1991) and minerals (AOAC, 1990).

Data were subjected to analysis of variance using PROC MIX procedures of SAS (2003). Data from 10 calves were not included in the final analysis due to chronic respiratory problems, injury, or death. Several tropical storms occurred during the course of the trial resulting in periods of high humidity and high temperatures resulting in several cases of chronic respiratory problems. The model included treatment, week, and the appropriate interactions. Initial body weight and blood protein concentrations were included as covariates. Calf within treatment was included as a random variable and week was considered a repeated measure. When significance ($P < 0.10$) was detected, the PDIFF option was used to separate the means. To evaluate the effect of treatment excluding the control, data were subjected to organized contrast.

Results

Feed Composition

The chemical composition of the milk replacer and calf starter is presented in Table 3.1. The composition of both was consistent through each week of the trial. The standard deviation indicated that there was little variation in the ingredients of both milk replacer and starter throughout the trial. The nutrients that compose the liquid and dry feed were within normal ranges described by the NRC.

Body Weight

The average initial body weight and age of the calves is presented in Table 3.2. The number of calves on each treatment differed due to the exclusion of certain calves that were ill, injured, or died. Initially, the trial consisted of 20 calves on each of the four treatments. The average age of the calves at initiation of treatment ranged from 8.7 days for the 5×10^8 LA 51 to 9.9 days for the 5×10^4 concentration of LA 51. Calves averaged 9.5 ± 0.5 days of age. The initial body weight was consistent and averaged 39.5 ± 1.6 kg body weight. Average weights ranged from 37.3 kg for 5×10^6 LA 51 to 40.8 kg for 5×10^8 LA 51.

Total body weight gain and average daily gain during the first six weeks of the trial, before the calves were weaned, was higher ($P < 0.01$) for calves on the control treatment and the 5×10^8 concentration LA 51 than for the other two treatments. Though not significantly different from the control, the 5×10^8 LA 51 calves had the highest total gain over the first six weeks. In addition, a trend was observed as the control and 5×10^8 LA 51 again had the highest total body weight gain and average daily gain ($P < 0.08$) for the first six weeks and over the total eight weeks of the trial.

Table 3.1. Chemical composition of milk replacer and calf starter fed to calves.

Item	Milk Replacer		Calf Starter	
	Average	SD	Average	SD
DM, %	95.0	0.5	84.7	1.2
	----- % of DM -----			
CP	21.2	0.2	19.8	2.3
ADF	1.2	0.3	13.6	0.3
NDF	1.2	0.3	21.7	0.2
Fat	21.9	0.2	4.0	0.2
Ash	10.5	0.3	5.4	0.2
NFC	45.2	0.4	49.1	2.6
Ca	0.86	0.03	0.45	0.11
P	0.75	0.05	0.48	0.01
Mg	0.15	0.01	0.20	0.01
K	2.49	0.11	1.03	0.07
Na	0.83	0.05	0.24	0.02

In an effort to determine the effect of dose level, data from the calves fed the control group were excluded and the statistical analysis was repeated. Figure 3.1 demonstrates that the results indicated a positive linear response ($P < 0.01$). As the concentration of the probiotic increased, so did the body weight of the calves. At the end of the eighth week, it appears that the average body weight for calves offered 5×10^6 LA 51 starts to level off, while the 5×10^4 LA 51 treatment's body weight surpasses the 5×10^6 LA 51.

Calf Health

Overall calf health was good as demonstrated by the scour and respiratory scores seen in Table 3.2. These scores did not significantly differ among the four treatments; however, the 5×10^8 LA 51 treatment had the lowest average scour score of 1.40 out of 4. The average total blood protein for the calves was 5.67 g/100mL. Calves that were deleted from the trial had low

total blood protein concentrations (usually < 5.0 g/100mL) and tended to have chronic respiratory problems, scours and reduced feed intake.

Intake

Milk replacer intake averaged 3.9 ± 0.02 kg/d and was similar for all calves consistent with the experimental design as shown in Figure 3.2. Calves also consumed an average of 1.38 ± 0.15 kg/d of calf starter throughout the entire trial. Figure 3.3 demonstrates that the calf starter intake was similar for all treatments. However, calves fed the 5×10^4 LA 51 and 5×10^6 LA 51 treatments tended to have lower starter intake than the control and the 5×10^8 LA 51. This slight decrease in starter intake may partially explain the lower body weight gains and rate of gain seen in these treatments compared with the control and 5×10^8 LA 51 treatments. As the weeks progressed, calf starter intake for all groups increased gradually and then dramatically increased after weaning at six weeks. There was no significant difference between treatments.

Table 3.2. Body weight (BW) and gain of Holstein calves fed increasing amounts of a probiotic in their milk replacer.

	Concentration of LA 51 fed, cfu				SE	<i>P</i> <
	0	5×10^4	5×10^6	5×10^8		
N	18	19	17	16		
Initial age, days	9.8	9.9	9.4	8.7		
Initial BW, kg	39.5	40.5	37.3	40.8		
Final BW, kg	78.5	75.9	71.3	82.8		
Gain, kg						
0 - 6 wk	26.7 ^a	21.9 ^b	23.7 ^b	27.0 ^a	2.8	0.01
0 - 8 wk	39.0 ^a	35.4 ^b	34.0 ^b	42.0 ^a	4.0	0.08
Average daily gain, kg/d						
0 - 6 wk	0.64 ^a	0.52 ^b	0.56 ^b	0.64 ^a	0.07	0.01
0 - 8 wk	0.70 ^a	0.63 ^b	0.60 ^b	0.75 ^a	0.07	0.08
Scour score	1.45	1.53	1.47	1.40	0.07	0.39
Respiratory score	1.01	1.02	1.01	1.02	0.01	0.75

Discussion

The milk replacer used in the study would be expected to provide enough metabolizable energy (ME) and protein (MP) to support gains of 0.45 kg/d according to the 2001 NRC calculations. Additional ME and MP in support of additional gain would need to be provided from calf starter intake or from improved digestion of milk replacer. Intake of calf starter was slightly higher for the control and 5×10^8 LA 51 throughout the trial, which increased the amount of ME and MP. This accounts for the higher gains in calves offered those treatments. Also, calf starter intake continued to increase for the calves fed 5×10^8 LA 51 during weeks seven and eight, whereas smaller increases were observed for the control calves. This suggests that the probiotic fed at the 5×10^8 concentration stimulated intake and the effect continued after feeding had ceased. This trial was designed to test the potential for the product. It is reasonable to expect that a greater response would have been observed if treatments had continued after weaning when intake of dry feed was increasing.

The reason for a lack of response from calves fed either the 5×10^4 LA 51 or the 5×10^6 LA 51 is not readily apparent. The control group aside, perhaps a concentration of no less than 5×10^8 LA 51 is needed to stimulate early rumen growth, and to successfully colonize enough *L. acidophilus* to have a beneficial effect. In general, strains of *L. acidophilus* exhibit more acid and bile resistance than other lactic acid bacteria (Oh et al., 2000). At the lower concentrations of LA 51, the amount of bacteria entering the gastrointestinal tract may not have been great enough to resist the bile and acid that it encountered. The 5×10^8 LA 51 possibly provided enough bacteria to withstand the bile and acid and colonize in the intestine. According to Oh et al. (2000), bile resistance and the ability to inhabit the intestinal tract appear to be correlated.

Colonization of a probiotic strain in an already existing microbial ecosystem requires more than adherence and acid and bile resistance alone. If LA 51 has the ability to compete against other microorganisms, by the production of antimicrobial substances for instance, it greatly increases its chances of colonizing the gastrointestinal ecosystem. These antimicrobial substances are proteinaceous in nature and called bacteriocins (Oh et al., 2000). The bacteriocins produced by this *Lactobacillus* strain may only be strong enough to inhibit pathogenic strains in the intestine at the highest concentration (5×10^8 LA 51) that was administered.

Increased body weight gains and calf starter intake observed with 5×10^8 LA 51 could be related to VFA concentrations in the blood. An increased appetite can result from favorable concentrations of VFA in the blood. Oh et al. (2000), suggested that probiotics which secrete lactic acid added to feed will increase the amount of VFA which in turn inhibits growth and fecal shedding of *Escherichia coli* 0157:H7. This study indicates that direct or indirect microbial interactions provide a useful means to reduce or eliminate enteric pathogens, which would improve calf health and weight gains. Favorable VFA concentrations may also be indirectly linked to weight gains. If the probiotic were to produce high levels of butyric acid, there would be an increase in stimulation of rumen development in calves. Mentschel et al. (2001) noted that butyric acid greatly increased papillar length by inhibiting ruminal apoptosis in vivo. This increased surface area would lead to greater absorption of nutrients and improved growth rate.

Since many of the calves had repeated injections of intramuscular antibiotics, this could have affected the microbial flora of the gastrointestinal tract of these calves, affecting the response of the calves to the LA 51. In a study by Stanek and Kofler (1998), the use of sodium ceftiofur (Excenel) was administered intramuscularly to cattle with complicated septic diseases. As a side effect, five cows out of 14 suffered from slight diarrhea, which disappeared without

additional treatment. This leads one to speculate that this antibiotic has an effect on the intestinal microflora of these cattle, leading to the gastrointestinal upset. However, other studies with sodium ceftriaxone or tilmicosin (Micotil) did not mention any gastrointestinal disturbances.

Similar to studies by Cruywagen et al. (1996) and Donovan et al. (2002), incidence of scours and respiratory illness did not differ among any of the four treatments. Calves that had the lower total blood protein levels tended to have chronic respiratory problems. These problems were most likely related to higher temperatures and humidity levels associated with tropical storms that occurred during the course of the trial. Another possible cause of the respiratory illness could have been the bedding with peanut hulls, which may have contained aflatoxins. Since the calves tended to nibble on their bedding, ingestion of these toxins may have decreased immunity and therefore led to sickness. In a study by Neathery et al. (1980), calves with aflatoxicosis exhibited signs which included reduced feed intake, weight gains, nitrogen balance, pulse rate and respiration rate. According to Brucato et al. (1986), clinical signs of aflatoxicosis in calves included anorexia, dehydration and nasal discharge.

Conclusions

In conclusion, though milk replacer intake was consistent for all treatments, calf starter intake was higher for the control and 5x10⁸ LA 51. After weaning and supplementation had ceased, 5x10⁸ LA 51 treated calves had higher intake, indicating this concentration stimulated appetite. Increased body weight gains and calf starter intake may be related to VFA concentrations. Treatment with the LA 51 appeared not to affect fecal or respiratory scores.

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CHAPTER 4

CONCLUSIONS

The results of this study indicate that inclusion of 5×10^8 LA 51 in the milk replacer fed to Holstein calves appears to have the potential to support higher rates of gain which persist after supplementation ceases. The mechanism for higher gains appears to be related to slightly higher intake of calf starter. Although gains for calves fed the control diet were similar, they were greater than normally expected which may have been due to slightly higher intake of calf starter. When only data from calves fed LA 51 was considered, a linear increase in gain was observed with increasing concentrations of LA 51. Furthermore, treatment with the LA 51 did not appear to have an effect on fecal or respiratory health.

Additional studies are recommended to compare supplementation of LA 51 with a control diet. In addition to body weight gain and rate of gain, gain in wither and hip height and body length are recommended to account for potential gains in frame growth that may not be reflected in weight gain. Another study to test the effect of the LA 51 added to calf starter after weaning may give more evidence of a significant difference between the control and 5×10^8 LA 51.

Figure 3.1

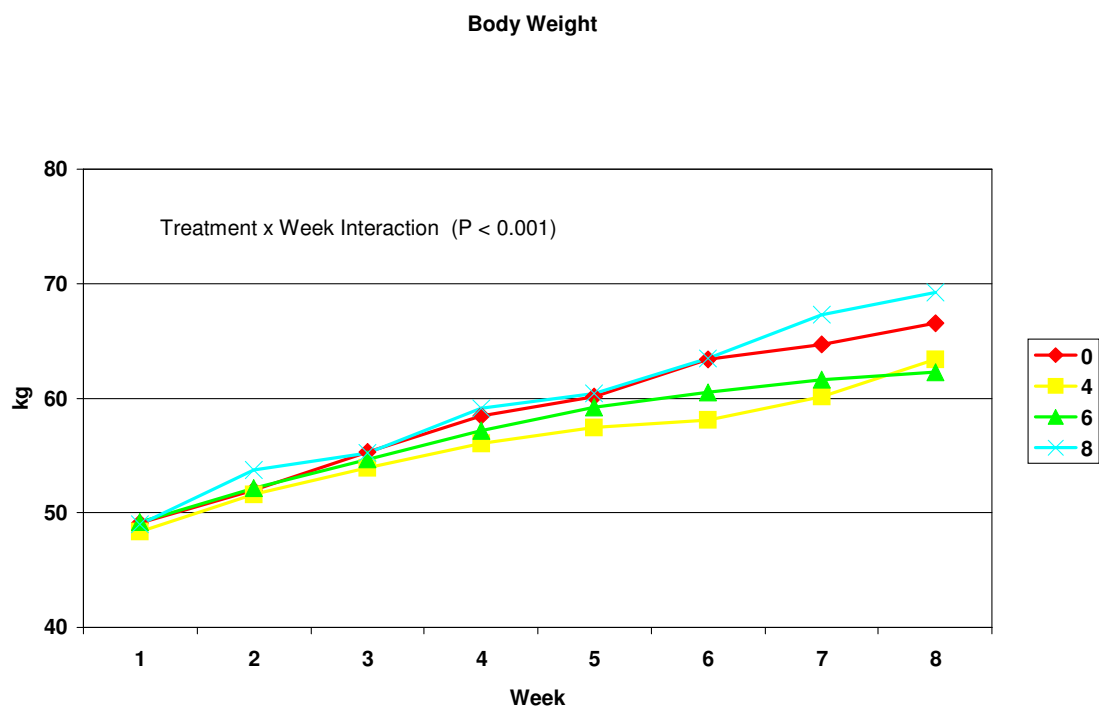


Figure 3.2

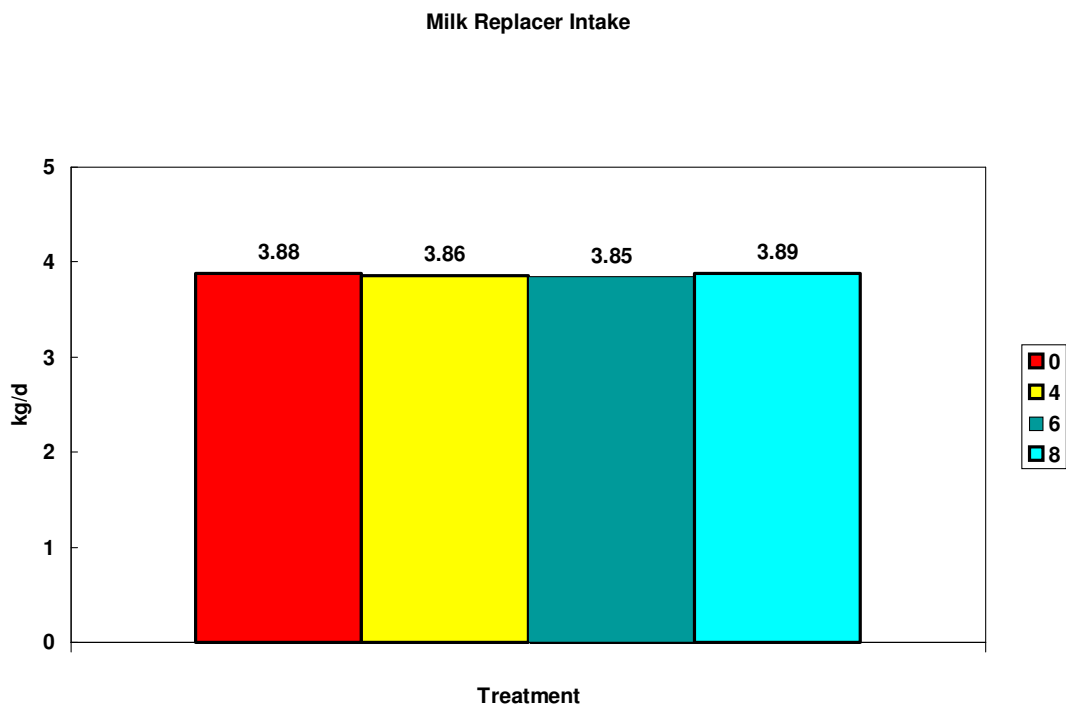


Figure 3.3

