MEASUREMENT OF ADAPTIVE AND INNATE IMMUNE FUNCTION

IN CALVES RAISED UNDER

TRADITIONAL AND ACCELERATED GROWTH REGIMENS

by

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ABSTRACT

This study compared conventional and accelerated milk replacer feeding regimens on growth, respiratory and digestive health, vaccination response, and neutrophil mRNA levels. Holstein calves (10 male and 5 female) were randomly assigned to a 10-week study on their second day of life. Treatments were control (CON; n = 8) and accelerated (ACC; n = 7) milk replacer feeding programs. CON calves were fed a 20% crude protein (CP) and 20% fat milk replacer (Advance Calvita Supreme; Milk Specialities Global, Carpentersville, IL) at 1.25% birth body weight daily from weeks 1 through 6 of life and 0.625% birth body weight daily during week 7. A 28.5% CP and 15% fat milk replacer (Advance Excelerate; Milk Specialities Global, Carpentersville, IL) was fed to ACC calves at 1.5% birth body weight daily during week 1, 2% current body weight daily from weeks 2 through 6, and 1% current body weight daily during week 7. All calves were given milk replacer twice daily during weeks 1 though 6, once daily during week 7, and were completely weaned during week 8. Calf starter intake was measured daily through week 8. Body weight and wither height were measured weekly. Fecal scores (1 = firm to 4 = liquid) and respiratory scores (1 = normal; 2 = abnormal) were recorded twice daily. Neutrophils were isolated from blood at weeks 1, 3, 5, and 8. Quantitative PCR was used to measure neutrophil mRNA levels of 9 functionality genes: *L-selectin*, *BPI*, *IL-1R1*, *TNF-αR*, *TLR-2*, *TLR-4*, *SOD1*, *MPO*, and *NCF1*. Adaptive immune function was measured by vaccinating calves against ovalbumin at weeks 1, 3, and 5 and measuring serum anti-ovalbumin IgG concentration at weeks 1, 3, 5, and 8. There was no

treatment effect on wither height, respiratory score, or serum anti-ovalbumin IgG concentration. Body weight during weeks 4 through 10 was greater for ACC than CON calves ($P < 0.01$). Calf starter intake was greater for CON than ACC calves during weeks 4 through 7 ($P < 0.01$), with no difference during week 8. CON calves had firmer feces than ACC calves (fecal score 1.4 vs. 1.7, $P = 0.02$). Neutrophil *L-selectin* mRNA levels were 51% greater in ACC than CON calves ($P = 0.03$). Feeding calves a 28.5% CP and 15% fat milk replacer in an accelerated feeding regimen increased growth and may enhance innate immune function, as indicated by the increased neutrophil mRNA levels of *L-selectin*.

INTRODUCTION

1.1   General Dairy Calf Rearing

Dairy calves are born with immature immune systems, so they are at an increased risk for infection and mortality during their first few weeks of life. Therefore, calves are given colostrum soon after birth to receive passive immunity (VandeHaar, 2005). Colostrum is the first milk secreted by the cow after parturition and contains immunoglobulins (antibodies) and important nutrients. The immunoglobulins are absorbed into the calf's bloodstream, providing the calf with some immunity against pathogens. In addition, calves are usually physically separated from their dam and one another in individual pens to prevent disease transmission.

Because calf digestive systems are not fully developed at birth, they are functionally similar to those of nonruminants. They cannot digest roughages and must obtain their nutrients from milk (VandeHaar, 2005). Calves are often fed milk replacer, a powdered milk product reconstituted in water, as a substitute for milk and given ad libitum access to water. After several days of life, they are also given ad libitum starter, concentrate that calves consume which provides energy and helps to stimulate rumen development.

Starter consumption is often used as a guide for determining when calves are ready to be weaned, which is when milk replacer is removed from the diet. Calves are typically weaned when they are 4 to 8 weeks old. After weaning they obtain all

their nutrients from concentrate and forages (VandeHaar, 2005). Therefore, calves must be consuming consistent amounts of concentrate during the preweaning period before removing milk replacer. After weaning, calves are typically moved from their individual pens and placed in group housing.

1.2 Dairy Calf Feeding Programs

1.2.1 Traditional Feeding Program

Traditional feeding programs have been used extensively in the past and utilize a conventional milk replacer with a 20% crude protein (CP) and 15 to 22% fat concentration (Cowles et al., 2006). The milk replacer is limit-fed to calves at 1 to 1.5% body weight per day on a dry matter basis and reconstituted with water to 12% solids divided into 2 feedings (VandeHaar, 2005). The rationale for limit feeding milk replacer with traditional feeding programs is to promote starter consumption for earlier weaning. Early weaning saves both time and money associated with purchasing and feeding milk replacer to calves twice daily.

1.2.2. Accelerated Feeding Program

Accelerated feeding programs utilize milk replacers with greater CP concentrations fed in greater amounts to calves compared to conventional milk replacers. The primary advantage for choosing an accelerated feeding program is greater growth rates, to which produce larger calves in shorter periods of time. Typically, heifers are bred based on weight and size and not age, so heifers can become part of the milking herd earlier if they can be bred sooner (Serjsen et al., 1982). Therefore, calves will mature earlier and dairy farmers can earn quicker returns on their investments.

1.2.3   Accelerated vs. Traditional Feeding Programs

1.2.3.1   Greater Body Growth

Feeding greater levels of milk replacer has consistently been shown to enhance calf growth. In a study conducted by Abdelsamei et al. (2005), Holstein steer calves were fed a 23% CP and 20% fat milk replacer at 5 different rates: 0.88, 1.67, 2.41, 3.38, and 3.73% birth body weight. Calves fed milk replacer at the greater rates had greater average daily gains (ADG) and were heavier at weaning. Bartlett et al. (2006) found that male Holstein calves fed milk replacer at 1.75% body weight had greater ADG, final heart girths, and body weights compared to those fed milk replacer at 1.25% body weight. In a study conducted by Cowles et al. (2006), Holstein heifers were fed milk replacer for 6 weeks with weaning during week 7. Treatments were conventional milk replacer (20% CP and 20% fat) fed at 562 g/d during weeks 1 through 6 or accelerated milk replacer (28% CP and 20% fat) with amounts increasing from 740 g/d week 1 to 1,358 g/d week 6. Although there was no difference for wither height, calves fed the accelerated milk replacer had larger hip widths and heights during the weaning and postweaning periods and had greater heart girths overall. During preweaning and weaning, these calves also had greater ADG, and they tended to have greater body weights during preweaning. Likewise, Foote et al. (2005) found that calves fed an accelerated milk replacer (28% CP and 20% fat) at 1.14 kg/d were heavier from the second through seventh week of life and had greater ADG than calves fed a standard milk replacer (20% CP and 20% fat) at 0.45 kg/d. In a study conducted by Hill et al. (2008), Holstein heifer calves were assigned to 1 of 4 dietary treatments: control milk replacer (20% CP and 21% fat) fed at 1.09% birth body weight, high protein and low fat milk replacer (28% CP and 20% fat) fed at 2.35% birth body

weight, high protein and high fat milk replacer (27% CP and 28% fat) fed at 2.35% birth body weight, or high protein and high fat milk replacer (27% CP and 28% fat) fed at a higher rate of 3.54% birth body weight. Calves fed the control milk replacer weighed the least and had the smallest wither height, body length, hip height, and hip width compared to calves fed the greater amounts of milk replacer. Furthermore, increasing the feeding rate from 2.35% body weight to 3.54% body weight increased body weight and ADG but did not affect wither height, body length, hip height, or hip width. Raeth-Knight et al. (2009) also found that calves fed an intensive high solids milk replacer (28% CP and 16% fat) at 1.9% birth body weight were heavier and had greater hip heights than calves fed intensive milk replacer at 1.5% birth body weight or calves fed conventional milk replacer at 1.3% birth body weight. Altogether, these data indicated feeding accelerated milk replacers increased body weight, ADG, and heart girth and sometimes increased hip height and width, wither height, and body length.

1.2.3.2   CP, Fat, and Energy Content

Body weight gain is influenced by milk replacer CP concentration and feeding rate. In a study conducted by Blome et al. (2003), male Holstein calves were assigned to milk replacers with varying CP concentrations (16.1, 18.5, 22.9, or 25.8%) fed at 1.5% birth body weight. As CP concentrations increased, calves were heavier and had greater ADG and final heart girths. In a study conducted by Bartlett et al. (2006), calves were fed varying CP concentrations: 14, 18, 22, or 26%. As CP concentrations increased, body weight, ADG, and final heart girth also increased. Therefore, increasing CP concentrations in milk replacer results in heavier calves with larger builds. However, body weight gain is most efficient when both CP

concentrations and feeding rates are increased because this supplies the calf with high protein and additional energy (Bartlett et al., 2006). Decreased calf performance can occur when CP concentrations are not properly balanced with energy needs.

The ratio between CP and fat concentrations in milk replacer also impacts calf growth. Typically, fat concentration is not increased as protein concentration increases because it increases fat deposition without increasing weight or height. In other words, greater fat concentrations result in fatter calves. This increased fat deposition during prepuberty is undesirable because it results in a subsequent decrease in milk production (Serjsen and Purup, 1997).

1.2.3.3   Starter Intake

Though calves gain body weight more quickly as a result of consuming accelerated milk replacers, starter intake is consistently decreased prior to weaning compared to control calves (Hill et al., 2008). Calves in the study conducted by Abdelsamei et al. (2005) consumed less alfalfa hay dry matter when they consumed the highest levels of milk replacer compared to control calves. However, it is rare to feed dairy calves alfalfa hay instead of starter, but these calves were being used as models for beef calves. Cowles et al. (2006) found that calves fed accelerated milk replacer had decreased starter intake compared to control calves prior to weaning. However, 2 weeks following weaning, starter intake for these calves did not recover to match that of the control calves. Therefore, Cowles et al. (2006) noted that calves fed the accelerated milk replacer were numerically, but no longer significantly, heavier than the control calves 2 weeks after weaning. However, calves fed the accelerated milk replacer in that study were medicated more often than control calves, and illness negatively impacts appetite and growth.

1.2.3.4 Heath Indices

Dairy farmers not only want to raise larger calves in a shorter amount of time, but they also want them to be healthy. Therefore, feeding milk replacer with enhanced nutrition might decrease calf morbidity and mortality (Foote et al., 2007a). Scours and pneumonia are two common calf diseases (Roy, 1980), so health indices are often used to monitor digestive and respiratory health. Calves fed accelerated milk replacers are more likely to have softer feces than calves fed conventional milk replacers (Nonnecke et al., 2003; Quigley et al., 2006; Raeth-Knight et al., 2009). However, softer feces do not necessarily imply impaired health because simply feeding milk replacer can cause loose feces (Kuhne et al., 2000). Furthermore, Nonnecke et al. (2003) found that overall health was the same for calves fed accelerated and conventional milk replacers, and medical costs in a study conducted by Raeth-Knight et al. (2009) were not different for any treatment. On the other hand, some studies have shown that calves fed accelerated milk replacers were medicated more often for health disorders than control calves (Cowles et al., 2006; Quigley et al., 2006). In a study conducted by Foote et al. (2007b), Holstein bull calves were fed to achieve three daily rates of gain: no growth (0.0 kg/d), low growth (0.55 kg/d), and high growth (1.2 kg/d). Normally, though, calves are not fed to achieve no or low growth. No growth calves had zero incidence of scours, whereas no growth and low growth calves had zero incidence of respiratory illness. However, both scours and respiratory illness occurred for the high growth treatment. Due to the small sample size, though, statistical inferences could not be made. Overall, studies have shown either no difference or worse short-term health for calves fed accelerated compared to control milk replacer. However, these indices are only indicators of current health, and there is

increasing interest in the impact of accelerated feeding programs on dairy calf immune system development and long-term health.

1.3   Immune System

The immune system is the body's defense mechanism against invading pathogens and is comprised of the innate and adaptive immune responses.

1.3.1   Innate Immune System

The innate immune response is activated within minutes to hours after tissue damage or infection and targets pathogens non-specifically. However, it does not have memory, so the immune response will not increase with future exposure to the pathogen. Important cells of the innate immune response include macrophages, dendritic cells, neutrophils, and natural killer cells (Tizard, 2009). The main innate immune response is inflammation, which increases the blood flow to the site of trauma, thus bringing cells to attack and destroy pathogens. As phagocytic cells, macrophages and neutrophils are important in the destruction of pathogens. Macrophages migrate towards the site of infection after neutrophils but are also involved in other functions, such as initiating healing and stimulating the adaptive immune response. Like macrophages, dendritic cells serve as antigen-presenting cells and stimulate the adaptive immune response (Tizard, 2009). On the other hand, natural killer cells are quickly activated lymphocytes that attack tumors and cells infected with viruses. However, neutrophils serve an essential function in the initial response of the innate immune system because they are the first cells to arrive during inflammation with the sole purpose to destroy pathogens.

1.3.1.1 Neutrophils

Neutrophils, derived from myeloid stem cells, are the first cells to arrive at damaged tissues (Burton et al., 2005). They constitute 20 to 30% of blood leukocytes in cattle and have a granular cytoplasm. Neutrophils move from the blood towards the site of infection via a process known as chemotaxis. P-selectin is expressed on the surface of capillary endothelial cells when these cells are stimulated by bacterial components, such as lipopolysaccharide, or molecules released by damaged tissues. This glycoprotein then binds to L-selectin, which is expressed on the surface of passing neutrophils (Tizard, 2009). Once the neutrophils arrive at the site of infection, toll-like receptors expressed on neutrophil surfaces help to recognize and bind to the pathogens. In particular, toll-like receptor 2 (TLR-2) binds primarily to gram-positive bacteria, whereas toll-like receptor 4 (TLR-4) helps neutrophils to recognize gram-negative bacteria (Kuijpers and Roos, 2004).

Neutrophils use phagocytosis to engulf the pathogen and then proceed to destroy it with the respiratory (oxidative) burst and antimicrobial peptides. The respiratory burst generates oxidants to destroy pathogens. When neutrophils bind to bacteria, they consume more oxygen, and the NADPH oxidase enzyme complex is activated (Tizard, 2009). The NADPH oxidase enzyme complex is comprised of 5 subunits with one of them called the neutrophil cytosolic factor 1 (NCF1; El-Benna et al., 2009). In the process of respiratory burst, the NADPH oxidase enzyme complex produces a superoxide anion. This superoxide anion is then converted into hydrogen peroxide via the enzyme superoxide dismutase (SOD1). Myeloperoxidase (MPO) then converts the hydrogen peroxide to bactericidal compounds, such as hypohalides, using intracellular halide ions. In addition to the respiratory burst process, neutrophils also produce antimicrobial peptides to destroy pathogens. One example is

bactericidal/permeability-increasing protein (BPI), which destroys gram-negative bacteria by damaging the inner membrane after binding to their lipopolysacharride (Tizard, 2009).

Cytokines also serve important functions in regulating neutrophil activity. Two important cytokines are IL-1 and TNF-α. TNF-α is involved with neutrophil apoptosis, chemotaxis, and respiratory burst. IL-1 is important primarily in chemotaxis but also functions in phagocytosis and respiratory burst (Tizard, 2009). Cytokines must bind to their respective receptors on the surface of neutrophils to be effective. Therefore, the neutrophil receptors tumor necrosis factor alpha receptor type 1 (TNF-αR) and interleukin-1 type 1 receptor (IL-1R1) are important for the functions of TNF-α and IL-1. In an ideal situation, the neutrophils use all of these tools to destroy invading pathogens quickly, and the calf shows no sign of illness.

1.3.3   Adaptive Immune System

When the innate immune system cannot rid the body of infection, the adaptive immune system is activated. Unlike the innate immune response, the adaptive immune response has memory and is specific, so it improves with repeated exposure to a pathogen. It has a slower onset compared to the innate immune response, and the two main cell types involved are the T and B lymphocytes (Tizard, 2009). The adaptive immune system is further divided into two main branches: cell-mediated and humoral immune responses. The cell-mediated immune response utilizes specialized cells, such as cytotoxic T cells, to destroy intracellular pathogens, such as viruses. On the other hand, the humoral immune response involves antibodies and is involved with extracellular pathogens, such as bacteria (Tizard, 2009).

1.3.3.1   Humoral Immune Response

The humoral immune response involves the antibody-antigen complex. Antibodies are proteins that bind to antigens, which are foreign substances that produce an immune response. There are 5 classes of antibodies: IgG, IgM, IgA, IgD, and IgE. IgG has the greatest concentration in serum (Tizard, 2009). Because antibodies are specific, they will only bind to the antigens that caused their production. A primary response occurs during the first exposure when only a small quantity of antibodies is produced. A secondary response occurs with repeated exposure and serum antibody concentrations greatly increase (Tizard, 2009).

1.3.3.1.1   Vaccination Response

Vaccination enhances the humoral immune response. Vaccines can activate the humoral or cellular immune response. Most killed bacterial or viral vaccine preparations activate only the humoral response. Live vaccines activate both the cellular and humoral immune response. Typically, vaccinations are used to provide immunity to dairy calves against certain diseases, but vaccinations have also been used in experiments to measure the adaptive immune response. To evaluate the adaptive immune response, calves can be vaccinated against a pathogen, such as *Mannheimia haemolytica*, or with a foreign protein, such as ovalbumin. *Mannheimia haemolytica* is a bacterial serotype associated with bovine pneumonia (Hodgins and Shewen, 1998). Ovalbumin is a protein from chicken egg white that is often used in conjunction with an adjuvant (a substance added to elicit a greater immune response) to stimulate the antibody production. Hodgins and Shewen (1998) vaccinated calves against capsular polysaccharide *Pasteurella haemolytica* A1 and found that older calves had higher antibody responses. Foote et al. (1997a) vaccinated calves with ovalbumin at 3 and 5

weeks of age. Results showed that the dairy calves generated antigen-specific IgG_1 and IgG_2 antibody responses, which were increased with the second vaccination. In addition, the primary vaccination response took 2 weeks after the first vaccination, whereas the secondary vaccination response took only 1 week after the second vaccination.

1.4 Nutrition and Immune Function

1.4.1 Effect of Malnutrition on Immune Function

Malnutrition has been shown to negatively impact both cell-mediated and humoral immune responses of the adaptive immune system. Griebel et al. (1987) fed Holstein bull calves for either maximal growth or protein energy malnutrition. The malnourished calves weighed less and had reduced lymphocyte interleukin-2 activity, lymphocyte proliferation, and delayed vaccination response to K99 antigen compared to calves fed for maximal growth.

1.4.2 Effect of Accelerated Feeding Programs on Immune Function

Studies have shown that accelerated feeding programs tend to suppress adaptive immune response. In a study conducted by Foote et al. (2005), Holstein calves fed accelerated milk replacer (28% CP and 20% fat) at 1.14 kg/d had decreased proliferation of T helper cells, cytotoxic T cells, and gamma-delta T cells compared to calves fed a conventional milk replacer (20% CP and 20% fat) at 0.45 kg/d. Foote et al. (2007b) fed Holstein bull calves to achieve three daily rates of gain: no growth (0.0 kg/d), low growth (0.55 kg/d), and high growth (1.2 kg/d). Although the high growth calves were heavier than the other calves, they had reduced lymphocyte viability. However, growth rate did not affect antigen-specific IgG_1 and IgG_2 responses to

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ovalbumin. Nonnecke et al. (2003) found that lymphocytes from calves fed an accelerated milk replacer (30% CP and 20% fat) at 2.5% body weight produced more inducible nitric oxide, a bactericidal compound, and less interferon-γ, a cytokine involved with the cell-mediated immune response. However, in the study conducted by Foote et al. (2007b), only non-stimulated lymphocytes from high growth calves showed increased nitric oxide production. In addition, the decreased interferon-γ production observed by Nonnecke et al. (2003) was not reproduced by lymphocytes stimulated with ovalbumin or *M. bovis*-derived purified protein derivative. Furthermore, Pollock et al. (2004) fed calves milk replacer at a high or low plane of nutrition and vaccinated with Keyhole limpet haemocyanin (KLH) at 3 weeks of age and with horse erythrocytes (HRBC) 1 day after weaning. The calves fed at a higher plane of nutrition had deceased humoral response as indicated by lower IgG_2 and IgA responses to KLH and lower anti-HRBC titers.

These studies have evaluated the impact of accelerated feeding programs on the adaptive immune system but have not considered the innate immune system, which is the calf's first defense against pathogens. Furthermore, these studies have varied widely in CP and fat concentration, as well as feeding levels. Despite the overwhelming negative effects of accelerated feeding regimens on adaptive immune function, health indices were not as one-sided with some studies reporting no difference in health indices. Because the immune system is comprised of both the innate and adaptive immune responses, the effect of accelerated growth regimens on innate immune responses is worthy of study.

1.5   Objectives

   The objectives of this study were to evaluate performance and the innate and adaptive immune responses in dairy calves assigned to one of two feeding regimens: conventional milk replacer and accelerated milk replacer.

Chapter 2

MATERIALS AND METHODS

2.1   Animals and Housing

All procedures were approved by the University of Delaware College of Agriculture and Natural Resources Agricultural Animal Care and Use Committee. Fifteen Holstein calves (10 male and 5 female) from the University of Delaware dairy herd were used in this study, which was conducted from January to May 2009. A visual overview of the sampling protocol is depicted in Figure 1. At birth, calves were removed from their dams and moved to outdoor individual calf hutches bedded with straw and wood shavings at the University of Delaware dairy farm. Navels were dipped in iodine, and calves were weighed to obtain their birth body weight. Calves were given a total of 3.8 L of good-quality colostrum (> 50 g/L IgG as measured by a colostrometer) divided into two feedings within the first 6 and 18 h after birth. Usually the calf was given its dam's colostrum, but when IgG concentrations were less than 50 g/L, colostrum previously collected and stored at -20°C was used. Calves remained housed in their calf hutches until 56 days of age, when they were moved to group housing.

2.2   Dietary Treatments and Feeding

Calves were blocked by sex and date of birth and were randomly assigned to the 10-week study on their second day of life. To be included in the study, calves

had to weigh at least 31.7 kg and appear to be in good general health. Twin calves of the same sex were assigned to the same block and administered opposite treatments whereas twin calves of the opposite sex were not included. Calves assigned to the control (CON) treatment (n = 8) were fed a 20% crude protein (CP) and 20% fat conventional milk replacer (97.7% DM; Advance Calvita Supreme; Milk Specialities Global, Carpentersville, IL) at 1.25% birth body weight per day divided into two daily feedings from weeks 1 through 6 of life and at 0.625% birth body weight per day fed once daily during week 7. Calves assigned to the accelerated (ACC) treatment (n = 7) were fed a 28.5% CP and 15% fat accelerated milk replacer (98.1% DM; Advance Excelerate; Milk Specialities Global, Carpentersville, IL) at 1.5% birth body weight per day during week 1 and 2% current body weight per day from weeks 2 through 6 of life divided into two daily feedings. During week 7, ACC calves were fed 1% current body weight per day once daily. The milk replacer was reconstituted in water to achieve 12.5% and 15% total solids for CON and ACC treatments, respectively. The amount of milk replacer fed to ACC calves was adjusted weekly based on body weight. For both treatments twice daily feedings occurred at 0700 and 1500 h whereas once daily feedings occurred only at 0700 h. All calves were completely weaned at the beginning of week 8 (50 days of age).

Since part of the study was conducted during the winter, supplemental energy (Advance Milk Energizer; Milk Specialities Global, Carpentersville, IL) was added to both milk replacers when temperatures were lower than 10°C. 28 g of the supplemental energy source were added when the temperature was between -1 and 10°C, and 56 g of the supplemental energy source were added when the temperature was between -18 and -1°C.

Each calf had ad libitum access to water, and water was replaced at each feeding time. Calves were offered starter ad libitum starting on their third day of life. Calf starter contained 40% steam flaked corn, 24% soybean meal, 11% barley, 5% molasses, 4.9% protected soybean meal, 2.8% cottonseed meal, 2% distillers concentrates, 1% soybean oil, 0.7% wheat middlings, 0.7% corn gluten meal, and 7.7% supplemental minerals, vitamins, and binders. Calves were initially offered 0.23 kg of starter daily with amounts offered increasing by 0.23 kg as starter intake increased. Remaining starter was weighed and discarded once daily after the 1500 h feeding. Starter intake was recorded daily from day 3 of life to the end of week 8 (56 day of age) when calves were moved to group housing. Starter samples were collected weekly, compiled together by month, and sent to Cumberland Valley Analytical Services (Hagerstown, MD) for wet chemistry analysis. On average, starter contained 87.3% DM and was 20.6% CP and 3.9% crude fat on a dry matter basis.

2.3 Growth and Health Measurements

Body weight and wither height were measured once weekly through week 10 of the study. Body weight was measured using a portable scale (Nasco, CO4974N, Fort Atkinson, WI). Wither height was measured using a measuring stick with a sliding crossbar (Nasco, C11503N, For Atkinson, WI). Calf health was monitored daily, and fecal and respiratory scores were recorded twice daily. Fecal score was monitored as an index of digestive health using the following score: 1 = firm, well-formed, 2 = soft, pudding-like, 3 = runny, pancake batter, and 4 = liquid, splatters. Respiratory score was monitored as an index of respiratory health using the following score: 1 = normal, 2 = runny nose, 3 = heavy breathing, 4 = cough-moist, 5 = cough-dry, and 6 = fever. All health disorders were treated according to herd protocol and recorded.

2.4 Blood Collection and Ovalbumin Vaccination

In order to measure the adaptive immune response, calves were vaccinated against ovalbumin and serum concentrations of anti-ovalbumin IgG were quantified. On days 7 ± 4 (week 1), 21 ± 4 (week 3), and 35 ± 4 (week 5) of life, calves were given a 1-mL intramuscular injection that contained 0.5 mg ovalbumin (Sigma-Aldrich, Saint Louis, MO) and 0.5 mg of the adjuvant Quil-A (Accurate Chemical & Scientific Corp., Westbury, NY).

Jugular venipuncture was used to collect blood from each calf once weekly on days 7 ± 4 (week 1), 21 ± 4 (week 3), 35 ± 4 (week 5), and 56 ± 4 (week 8) of life. On weeks 1, 3, and 5, blood was collected immediately before the ovalbumin vaccination. Blood was collected in one 10-mL serum tube (BD Vacutainer Systems, Franklin Lakes, NJ) for measurement of anti-ovalbumin IgG production and two 10-mL K_2 EDTA tubes (BD Vacutainer Systems, Franklin Lakes, NJ) for neutrophil isolation and measurement of white blood cell (WBC) concentration. After blood collection, EDTA tubes were inverted and placed immediately on ice.

Blood collected in the serum tubes was allowed to clot at room temperature for approximately 4 h before being centrifuged at 1000 x g for 30 min at 21°C. Serum was transferred to two 1.5-mL microcentrifuge tubes and stored at -20°C until analysis of anti-ovalbumin IgG production.

2.5 White Blood Cell Count and Neutrophil Isolation

Blood collected in the EDTA tubes was combined into one 50-mL conical tube for each calf. 10 µL of blood from the conical tube was pipetted into 10 mL of diluent (Isoton II Diluent, Beckman Coulter, Inc., Fullerton, CA), and 4 drops of a commercial lysing reagent (Zap-Oglobin II Lytic Reagent, Beckman Coulter, Inc.,

Fullerton, CA) were added to lyse the red blood cells. WBC count was measured using a Z2 Coulter Particle Count and Size Analyzer (Beckman Coulter, Inc., Fullerton, CA).

Remaining blood in the conical tubes was then centrifuged at 1000 x g for 45 min at 4°C. A sterile transfer pipette was used to remove the plasma, buffy coat, and 2/3 of the red blood cell pack from the conical tube. The remaining red blood cells in the red blood cell pack were lysed by adding 12 mL of 4°C hypotonic lysing solution (10.6 mM Na$_2$HPO$_4$, 2.7 mM NaH$_2$PO$_4$) and inverting for 90 seconds. Then 6 mL of 4°C hypertonic restoring solution (10.6 mM Na$_2$HPO$_4$, 2.7 mM NaH$_2$PO$_4$, 430 mM NaCl) was added, and tubes were inverted to restore isotonicity. Samples were centrifuged at 800 x g for 5 min at 4°C. The supernatant was poured off, 10 mL of Hank's Balanced Salt Solution (HBSS; Mediatech, Inc., 21-020-CV, Manassas, VA) were added, and the sample was vortexed to re-suspend the pellet. Centrifugation and re-suspension of the pellets were repeated once. Samples were once again centrifuged at 800 x g for 5 min at 4°C. The supernatant was poured off, and 1 mL of HBSS was added to each conical tube. A sterilized Pasteur pipette was used to break up the neutrophil pellet and transfer the sample into two RNase-free 1.5-mL microcentrifuge tubes. The tubes were centrifuged at 9,600 x g for 15 min at 4°C. The supernatant was pipetted off, and 500 µL of TRIzol reagent (Invitrogen, Carlsbad, CA) were added to the sample after breaking up the pellet with the pipette tip. Samples were stored at -80°C until RNA isolation. Neutrophil viability was determined by Trypan blue exclusion, and neutrophil differential was determined microscopically using a hematology staining kit (Protocol HEMA 3® Stain Set, Fischer Scientific Company, Kalamazoo, MI). Neutrophil viability and percentage of neutrophils were calculated to be 97.5% and 87.5% respectively.

2.6   Serum Anti-Ovalbumin IgG Production

Serum levels of anti-ovalbumin IgG were measured using an enzyme-linked immunosorbent assay as described by Amaral (2008). Briefly, a 96-well plate was coated with 100 μL of coating solution (1.4 mg ovalbumin per mL of carbonate-bicarbonate buffer at pH 9.6) and incubated for 48 hours at 4°C. The plate was washed 4 times with 200 μL of wash solution (137.0 mM NaCl, 8.1 mM Na$_2$HPO$_4$, 1.1 mM KH$_2$PO$_4$, 2.7 mM KCl, 0.5 mL/L tween at pH 7.4). After adding 200 μL of blocking solution (137.0 mM NaCl, 8.1 mM Na$_2$HPO$_4$, 1.1 mM KH$_2$PO$_4$, 2.7 mM KCl, 30 mL/L tween, 10 g/L bovine serum albumin at pH 7.4) to each well, the plate was incubated for 1 h at room temperature. After incubation, the plate was washed again 4 times. Positive and negative controls and samples were diluted 1/50 and 1/200 with wash solution, and 100 μL of each dilution was added to duplicate wells. Positive and negative controls were obtained by pooling serum samples from all calves for week 8 and week 1, respectively. After 2 h of incubation at room temperature, the plate was washed 4 times, and 100 μL of conjugated antibody (Anti-Bovine IgG Alkaline Phosphatase, antibody produced in rabbit; Sigma-Aldrich, Saint Louis, MO) diluted 1/50 with Tris-HCl (414.4 mM Tris-HCl, 563.5 mM NaCl, 11.5 mM KCl, 0.5 mL/L at pH 7.4) was added to each well. The plate was incubated for 1 h at room temperature and washed 4 times. 80 μL of p-nitrophenyl phosphate liquid substrate system (Sigma-Aldrich, N7653, Saint Louis, MO) was added to each well, and the plate was incubated at room temperature for 30 min before being read at 405 nm.

Relative ovalbumin IgG concentration is presented as optical density after a correction factor was applied for each plate. The mean positive control optical density for the 1/50 and 1/200 dilutions were averaged together for each plate. The correction factor was calculated for each individual plate by dividing the largest average by that

plate's average. For each sample, the 1/50 dilution and 1/200 dilution optical densities were averaged, and the mean was multiplied by the correction factor to obtain the final corrected mean, which was used for data analysis.

2.7   Neutrophil mRNA Isolation and Quantification

RNA was isolated from neutrophils using the PureLink™ Micro to Midi Total RNA Purification System (Invitrogen, Carlsbad, CA). 1 µg of RNA isolated from each sample was DNase treated (New England Biolabs, Ipswitch, MA) and used to create 160 µL of cDNA using the Promega Reverse Transcriptase System (A3500, Promega Corporation, Madison, WI).

Primers for *β-actin*, *RPS9*, and *L-selectin* were from Nebzydoski (2009). Primers for *SOD1* and *TLR2* were from Moyes et al. (2010). Primers for the remaining neutrophil functionality genes were chosen using Primer Express software and published bovine sequences from NCBI (Table 1). Neutrophil mRNA levels of *BPI*, *IL-1R1*, *MPO*, *NCF1*, *TLR-4*, *TNF-αR*, *L-selectin*, *SOD1*, *TLR-2*, *RPS9*, and *β-actin* were quantified from cDNA using quantitative real time RT-PCR. 10 µL reaction mixes contained 5 µL SYBR green master mix (Applied Biosystems, Foster City, CA), 4 µL cDNA, 0.4 µL of each of the left and right primers (10 m*M*), and 0.2 µL of water. mRNA levels were analyzed in triplicate wells. Each sample was quantified using a relative standard curve obtained by serial dilution of pooled calf neutrophil cDNA. The relative mRNA level for each neutrophil functionality gene was divided by the geometric mean mRNA level of the two housekeeping genes, *RPS9* and *β-actin*.

2.8   Statistical Analysis

The MIXED procedure of SAS was used for data analysis (SAS, 1999). Starter intake, fecal scores, and respiratory scores were averaged for each week prior to statistical analysis. Because respiratory scores were categorical, respiratory scores at each time point were converted to 1 = normal (original score 1 or 2) and 2 = abnormal (original score 3, 4, 5, or 6) prior to calculation of weekly means. Fixed effects were treatment, week, sex of the calf, and the two and three-way interactions. For growth measurements, initial values were included as covariates. Calf was included as a random effect. The Kenwardrodger adjustment for denominator degrees of freedom was used. Week was included as a repeated measurement using the autoregressive (1) covariance structure and the subject was calf nested within treatment. mRNA data were log transformed prior to analysis to obtain normal distribution of residuals. For ease of presentation, relative mRNA levels were multiplied by 10 prior to log transformation. Significance was declared at $P < 0.05$ while trends were declared at $P < 0.10$.

Chapter 3

RESULTS

3.2   Health Disorders and Treatments

During the study, calf health disorders were treated according to herd protocol and recorded. One male ACC calf (1 day old) was given a 1-cc intramuscular injection of flunixin meglumine (Banamine, Schering-Plough Animal Health, Kenilworth, NJ) and received physical therapy to stretch contracted tendons in the front legs. The same calf (12 days old) was also given a 5-cc intramuscular injection of ceftifur sodium (Naxcel, Pharmacia & Upjohn, Kalamazoo, MI) for respiratory illness for 2 days. A female ACC calf (15 days old) had discharge in one eye that resolved itself on its own within a day. Two ACC heifer calves (26 and 30 days old) and one CON heifer calf (22 days old) were treated for coughing and diarrhea with 5-cc intramuscular injections of ceftifur sodium (Naxcel, Pharmacia & Upjohn, Kalamazoo, MI) for 5 days. Another ACC bull calf (40 days old) was treated for diarrhea with 5-cc intramuscular injections of ceftifur sodium (Naxcel, Pharmacia & Upjohn, Kalamazoo, MI) for 5 days.

3.2   Intake and Growth

Growth and starter intake results for CON and ACC calves are listed in Table 2 and shown in Figure 2. Treatment affected body weight ($P = 0.007$) and starter dry matter intake ($P = 0.006$). On average ACC calves were heavier and

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consumed less starter compared to CON calves. Treatment had no effect on wither height ($P = 0.31$). A week $\times$ treatment interaction existed for body weight and starter dry matter intake ($P < 0.0001$). Body weight and starter DM intake increased over time for both treatments. CON calves weighed less than ACC calves during weeks 4 through 10. ACC calves had decreased starter DM intake compared to CON calves during weeks 4 through 7, but there was no difference following weaning during week 8. Week affected body weight ($P < 0.0001$), wither height ($P < 0.0001$), and starter dry matter intake ($P < 0.0001$). Wither height increased over time for both treatments.

3.3   Respiratory and Fecal Scores

Treatment affected fecal score ($P = 0.02$) but had no effect on respiratory score ($P = 0.18$; Table 2). On average ACC calves had softer feces compared to CON calves. However, there was an interaction of sex $\times$ week ($P = 0.005$) and sex $\times$ week $\times$ treatment ($P = 0.03$) for respiratory score (Figure 3). Bull calves had the highest respiratory scores during weeks 1, 2, 7, and 8 whereas heifer calves had highest respiratory scores during weeks 3 through 6. ACC heifer calves had the highest respiratory scores during weeks 3 and 5 compared to the other calves. Week also affected fecal score ($P = 0.04$). Fecal scores decreased over time for both treatments.

3.4   Ovalbumin Response and Neutrophil mRNA

Treatment had no effect on serum anti-ovalbumin IgG concentration ($P = 0.45$) or WBC count ($P = 0.39$; Table 2; Figure 4). Week affected anti-ovalbumin IgG concentration ($P < 0.0001$). Anti-ovalbumin IgG concentration increased over time for both treatments.

Neutrophil mRNA levels from CON and ACC calves are listed in Table 3 and presented in Figures 5 through 9. Least square means of the log of the mRNA levels are presented. Week affected the mRNA levels of *L-selectin* (P = 0.03), *BPI* (P = 0.004), and *TNF-αR* (P = 0.02). Expression of *BPI* and *TNFα-R* decreased over time, whereas mRNA levels of *L-selectin* increased over time. Treatment also influenced expression of *L-selectin* (P = 0.03). ACC calves had increased expression of *L-selectin* compared to CON calves. By comparing the inverse log of the least squares means of *L-selectin* for CON (56) and ACC (84), *L-selectin* was increased 51% for ACC compared to CON. It is also noteworthy to mention that for each of the genes except *TLR-2*, *SOD1,* and *BPI*, week 8 mRNA levels were numerically higher for ACC than for CON calves. At week 8, ACC mRNA levels were 125% greater for *L-selectin*, 89% greater *for TLR-4*, 107% greater for *NCF1*, 142% greater for *MPO*, 40% greater for *TNF-αR*, and 71% greater for *IL-1R1*.

Chapter 4

DISCUSSION

4.1   Growth

4.1.1   Body Weight

ACC calves were heavier than CON calves starting week 4 and remained heavier until the end of the study at week 10. The observed increase in weight is likely due to the increases in both milk replacer CP concentration and feeding level. For example, several studies have shown that increasing CP concentrations while maintaining the same feeding level increases preweaning body weight gain (Blome et al., 2003; Bartlett et al., 2006). Increasing the feeding level regardless of CP concentration also increases gain until weaning (Abdelsamei et al., 2005). However, few studies have measured persistency of CP concentration or feeding level effect on postweaning body weight. During weaning ACC calves weighed 10.5 kg more than CON calves, and most of this difference was maintained through 3 weeks after weaning when ACC calves were 7.6 kg heavier than CON calves. However, Cowles et al. (2006) found that calves fed accelerated milk replacer were not significantly heavier than control calves 2 weeks after weaning although their body weights were numerically greater than those of control calves. The reason for the difference in results between the present study and that of Cowles et al. (2006) could be due to differences in starter intake or illness, as will be discussed later.

4.1.2 Growth Measures

In this study, the two indices of growth evaluated were body weight and wither height. There was no treatment effect on wither height. Other studies have also seen no effects on wither height. For example, Cowles et al. (2006) also found no difference in wither height for calves fed accelerated or control milk replacer. Furthermore, Hill et al. (2008) found that increasing the feeding rate of the high protein and high fat milk replacer from 2.35% to 3.54% did not increase final wither height, body length, hip height, or hip width. However, some studies have found effects of accelerated feeding regimens on different growth measures. In the study conducted by Cowles et al. (2006), calves that were fed accelerated milk replacer were taller at the hips during preweaning and weaning, had larger hip widths during weaning and postweaning, and had larger heart girths during the entire time. Raeth-Knight et al. (2009) reported increased hip heights in calves fed accelerated milk replacer. We attribute our lack of difference in wither height to choosing the wrong growth measure to monitor and to small sample size. Numerically, ACC calves had greater wither heights, so in a larger study, these results might be significant.

4.2 Starter DM intake

CON calves consumed more starter DM compared to ACC calves during weeks 4 through 7 in accord with results of others (Abdelsamei et al., 2005; Cowles et al., 2006; Hill et al., 2008). Few studies have measured starter intake postweaning. In our study, starter intake for ACC calves did not differ from that of CON calves after one week postweaning. During weaning at week 7, CON calves consumed 0.5 kg/d of starter DM more than ACC calves. During week 8, the first week after complete weaning, CON calves consumed 0.04 kg/d of starter DM more than ACC calves, and

the difference was not significant. Cowles et al. (2006) continued to measure starter intake for 2 weeks postweaning, and starter intake for calves fed accelerated milk replacer never rose to that of the control calves. Because lowered starter DM intake of accelerated calves failed to increase postweaning, preweaned growth advantages were lost in the accelerated groups during the postweaning period.

4.3 Health Indices

There was also no treatment effect on white blood cell count. To our knowledge, there have not been any other studies conducted that evaluated the effect of accelerated feeding regimen on white blood cell count. However, Foote et al. (2007b) found that growth rate did not affect neutrophil or lymphocyte percentages.

ACC calves had numerically, but not significantly, higher respiratory scores compared to CON calves (1.3 vs. 1.2). Nonnecke et al. (2003) also measured respiratory score and found that calves fed accelerated milk replacer had increased scores.

A treatment effect existed for fecal scores. ACC calves had softer feces than CON calves (fecal score 1.7 vs. 1.4) concordant with results of Nonnecke et al. (2003) and Raeth-Knight et al. (2009). However, softer feces do not necessarily imply impaired health because feeding milk replacer in general can cause softer feces (Kuhne et al. 2000). Daniels et al. (2008) found no treatment effect on fecal score, and Cowles et al. (2006) reported that calves fed accelerated milk replacer actually had firmer feces than control calves during weaning. Therefore, the effect on fecal consistency appears to be inconsistent.

During the study, 1 ACC calf was treated for respiratory illness, 2 ACC and 1 CON calves were treated for diarrhea and respiratory illness, and 1 ACC calf was

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treated for diarrhea. Although, there were too few animals to perform statistical analysis, there were 4 treatments for ACC calves compared to 1 treatment for CON calves. These differences may have contributed to the numeric and significant differences in respiratory and fecal scores, respectively. Some studies have shown that accelerated feeding programs negatively impact calf health. In the study conducted by Foote et al. (2007b), Holstein bull calves were assigned to dietary treatments to achieve 1 of 3 daily rates of gain: no growth (0.0 kg/d), low growth (0.55 kg/d), or high growth (1.2 kg/d). Calves fed milk replacer to achieve high growth rates had incidence of both respiratory illness and scours. Quigley et al. (2006) found that feeding accelerated milk replacer increased the number of days with diarrhea and number of days treated with antibiotics. Cowles et al. (2006) also found that control calves were medicated for fewer days, and that likely contributed to the failure of accelerated calves to maintain their weight advantage postweaning. On the other hand, other studies have found that accelerated feeding regimens have had no effect on health. Medical costs did not vary across treatments in a study conducted by Raeth-Knight et al. (2009), and Nonnecke et al. (2003) remarked that overall health was the same for calves fed accelerated and conventional milk replacer. Overall, our study supports others who have found that accelerated milk replacer feeding programs seem to increase incidence of digestive and respiratory disorders. Further studies utilizing larger numbers of animals would be beneficial to evaluate the impacts of these two programs on calf morbidity, mortality, and long-term health.

4.4 Vaccination Response

There was no treatment effect on serum anti-ovalbumin IgG concentration. Similar results were reported by Foote et al. (2007b). In that study, calves were

vaccinated subcutaneously in the midcervical region with 4 mg ovalbumin combined with an adjuvant at 3 and 5 weeks of age. In the present study, calves were given intramuscular vaccinations in the hindquarters with 0.5 mg ovalbumin in adjuvant. Furthermore, calves in this study were vaccinated at 1, 3, and 5 weeks of age. However, the quantity of ovalbumin and the method of vaccination appear to have no effect on results because a vaccination response was obtained in the study conducted by Foote et al. (2007b) and the present one. Relative concentrations of IgG increased each week. On the other hand, results from a study conducted by Pollock et al. (1994) showed that feeding level affected vaccination response. Calves fed milk replacer at a greater level had decreased anti-KLH IgG_2 and IgA responses and decreased anti-HRBC titers compared to the calves fed milk replacer at a lower level. The milk replacer used in Pollock et al.'s (1994) study contained 25% CP but did not report fat concentrations. However, the milk replacer used in Foote et al.'s (2007b) study contained 30% CP and 20% fat. Therefore, the nutrient content of the milk replacer may affect vaccination response.

4.5   Neutrophil Functionality Genes

A variety of neutrophil functionality genes were evaluated to assess different aspects of neutrophil function. We measured neutrophil mRNA levels of *L-selectin* as a function of chemotaxis and *TLR-2* and *TLR-4* for pathogen recognition. *NCF1*, *SOD1*, and *MPO* were measured to evaluate respiratory burst, and BPI was measured as a function of antimicrobial activity. We measured *IL-1R1* and *TNF-αR* as indicators of cytokine receptor expression and cellular responsiveness to the appropriate ligand. ACC calves had increased mRNA levels of *L-selectin* compared to CON calves. *L-selectin* is also expressed on lymphocytes to help with chemotaxis

towards lymph nodes (Tizard, 2009). Foote et al. (2005), however, found decreased *L-selectin* expression on lymphocytes in non-stimulated cultures from calves fed accelerated milk replacer compared to those fed control milk replacer. The treatment effect on mRNA levels of *L-selectin* suggests that an accelerated feeding regimen may benefit the innate immune response by improving the neutrophil's migration from the bloodstream towards the site of inflammation. Because mRNA levels for each gene except *BPI*, *TLR-2*, and *SOD1* were numerically greater for ACC than CON calves at week 8, other neutrophil functions may be enhanced and should be investigated further.

Chapter 5

CONCLUSION

Calves raised under an accelerated feeding regimen were heavier than calves raised under a traditional feeding regimen, and this weight advantage was maintained postweaning. Despite decreased starter intake during the preweaning period, ACC calves matched the intake of CON calves one week after weaning. ACC calves had softer feces compared to CON calves, which was likely caused by the increased consumption of milk replacer. There was no treatment effect on ovalbumin vaccination response, and there was a numeric increase in the number of times ACC calves were treated for respiratory and digestive illnesses compared to CON calves. However, ACC calves had increased neutrophil mRNA levels of *L-selectin* compared to CON calves. Therefore, an accelerated feeding regimen may benefit the innate, rather than the adaptive, immune response and contribute to a better initial immune response. Future studies could measure neutrophil functions with in vitro assays or investigate possible roles of other cells important in the innate immune response, such as macrophages, natural killer cells, or dendritic cells. Overall, accelerated feeding regimens promote increased body growth and may enhance the innate immune system, as indicated by increased mRNA levels of *L-selectin*. However, effects of accelerated feeding regimens on long-term health should be evaluated.

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Appendix

TABLES AND FIGURES

Table 1. Primers used in real-time RT-PCR analysis to determine mRNA levels of neutrophil functionality genes.

Gene[1]	Primers	Product Length (bp)	NCBI Accession Number
BPI	5'- AGCCTTATTTCCAGACGCTGC	104	NM_173895.2
	3'- ATCCAGGTTATTGGCTGTGGC		
IL-1R1	5'- GTTTACGGCATTAGCGCCTT	106	XM_593695
	3'- TTGTTGATCTTCTCTGAACCGC		
MPO	5'- GCCGCTCAGCAGAGTCTTTT	116	BC149472.1
	3'- CGATTTGGTTCTGGCGGTT		
NCF1	5'- TCCTCAACTTCTTCAAGGTGCG	108	NM_174119.3
	3'- CAGCGTTGTTCTTGCCATCTTT		
TNFα-R	5'- GTGCAGTGCGTGTGTTTGTGTC	110	NM_174674.2
	3'- ATCTTCGCAACCACTGCCTTG		
TLR-4	5'- TCAGAAACCTCCGCTACCTTG	118	NM_174198.6
	3'- TTCTGAAAAGAGTTGCCTGCC		

[1]*BPI* = bactericidal/permeability-increasing protein; *IL-1R1* = interleukin-1 type 1 receptor; *MPO* = myeloperoxidase; *NCF1* = neutrophil cytosolic factor 1; *TNFα-R* = tumor necrosis factor alpha receptor; *TLR-4* = toll-like receptor 4.

Table 2. Effect of conventional and accelerated milk replacer on growth and health parameters in Holstein calves.
Data are shown as least square means. SEM = standard error of the mean.

	Treatment			P-values		
Item	CON[1]	ACC[2]	SEM	Treatment	Week	Week x Treatment
Body weight, kg	59.8	66.5	1.38	0.007	<0.0001	<0.0001
Wither height, cm	83.1	84.4	0.83	0.31	<0.0001	0.17
Starter DM intake, kg/d	0.75	0.48	0.06	0.006	<0.0001	<0.0001
Ovalbumin IgG, optical density (OD)	0.27	0.30	0.03	0.45	<0.0001	0.68
log(white blood cells, #/mL)	7.10	7.14	0.03	0.39	0.18	0.60
Respiratory score[3,4]	1.2	1.3	0.08	0.18	0.54	0.44
Fecal score[5]	1.4	1.7	0.07	0.02	0.04	0.12

[1]CON = Conventional milk replacer feeding program (20% CP/20% fat milk replacer).
[2]ACC = Accelerated milk replacer feeding program (28.5% CP/15% fat milk replacer).
[3]Interaction of sex × week ($P = 0.005$) and sex × week × treatment ($P = 0.03$) was also significant for respiratory score.
[4]Respiratory score was monitored as an index of respiratory health using the following score: 1 = normal and 2 = abnormal.
[5]Fecal score was monitored as an index of digestive health using the following score: 1 = firm, well-formed, 2 = soft, pudding-like, 3 = runny, pancake batter, and 4 = liquid, splatters.
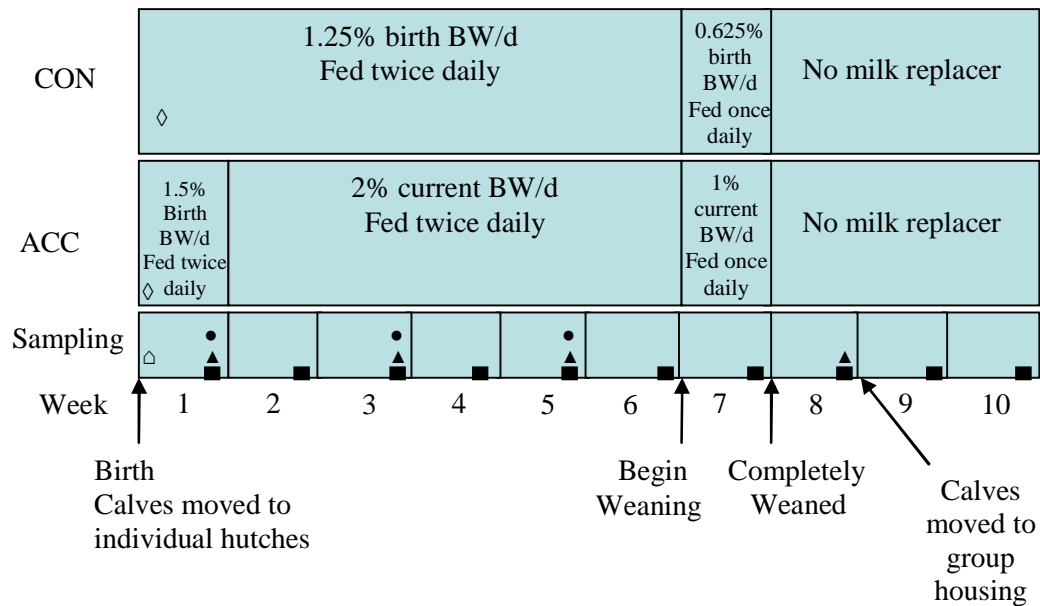
Table 3. Effect of milk replacer on neutrophil mRNA levels of functionality genes.
Neutrophils were isolated from blood samples at wk 1, 3, 5, and 8 from Holstein calves fed conventional (CON) or accelerated (ACC) milk replacer. Neutrophil mRNA levels were determined relative to the housekeeping genes *RPS9* and *β-actin*. Data were log transformed prior to analysis. SEM = standard error of the mean.

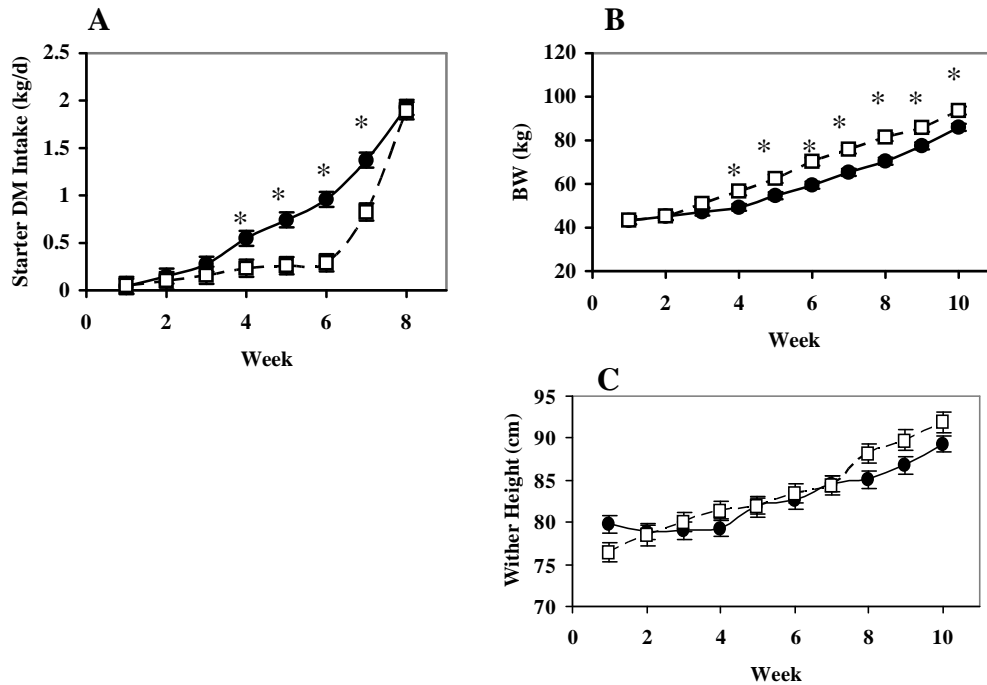| | log(mRNA Levels) | | | *P*-values | | |
Gene	CON[1]	ACC[2]	SEM	Treatment	Week	Week x Treatment
L-selectin	0.75	0.92	0.06	0.03	0.03	0.53
TLR-2	1.03	1.04	0.04	0.87	0.10	0.79
TLR-4	1.01	1.09	0.04	0.14	0.12	0.27
NCF1	0.93	1.01	0.05	0.25	0.25	0.17
SOD1	1.01	0.92	0.04	0.13	0.09	0.39
MPO	0.91	1.08	0.1	0.34	0.43	0.55
BPI	0.54	0.64	0.09	0.45	0.004	0.93
TNF-αR	0.91	0.92	0.05	0.89	0.02	0.28
IL-1R1	0.70	0.78	0.08	0.49	0.20	0.34

[1]Control = Conventional milk replacer feeding program (20% CP/20% fat milk replacer).
[2]Accelerated = Accelerated milk replacer feeding program (28.5% CP/15% fat milk replacer).
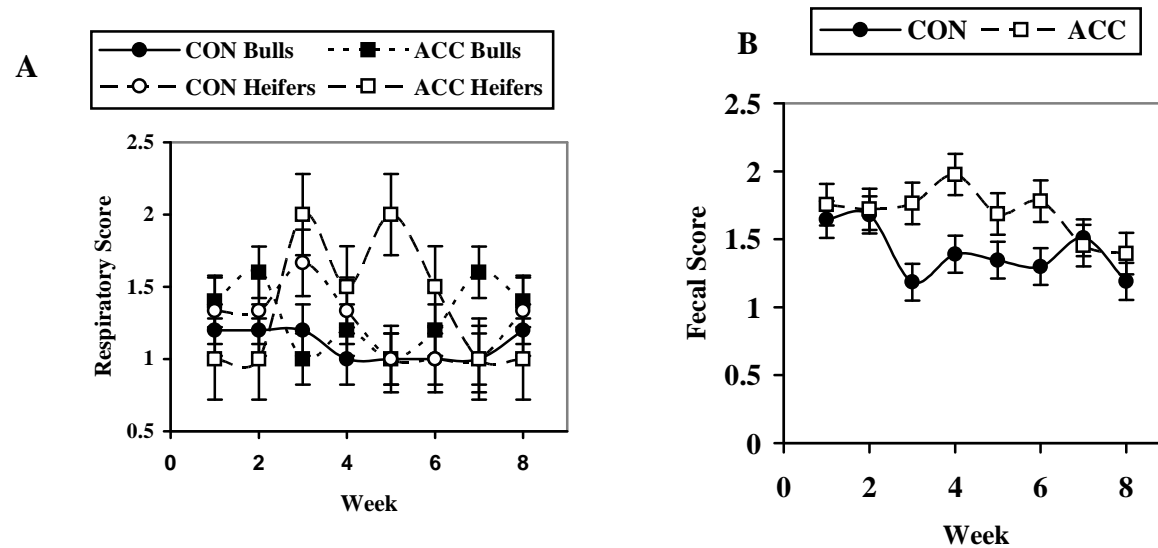
Figure 1. Diagram of experimental protocol. After two feedings of colostrum, Holstein calves were randomly assigned to one of two dietary treatments on day 2 of life. Treatments were control (CON; n=8) and accelerated (ACC; n=7) milk replacer feeding programs. CON calves were fed milk replacer with 20% CP and 20% fat at 1.25% birth body weight (BW) from wk 1 to 6 and 0.625% birth BW during wk 7. ACC calves were fed milk replacer with 28.5% CP and 15% fat at 1.5% birth BW during wk 1, 2% current body weight (BW) from wk 2 to 6, and 1% current BW during wk 7. All calves were given milk replacer twice daily from wk 1 to 6, once daily during wk 7, and were weaned completely during wk 8. BW and wither height were measured weekly. Fecal scores (1=firm to 4=liquid) and respiratory scores (1=normal; 2=abnormal) were recorded twice daily. Neutrophils were isolated from blood at wk 1, 3, 5 and 8. Quantitative PCR was used to measure neutrophil mRNA levels of 9 functionality genes. Adaptive immune function was measured by vaccinating calves against ovalbumin at wk 1, 3 and 5 and measuring anti-ovalbumin IgG production at wk 1, 3, 5 and 8. ◊ = Calves had two colostrum feedings before given milk replacer. △ = Birth BW measured. ■ = BW and wither height measured. ▲ = Blood collection. ● = Ovalbumin vaccination.
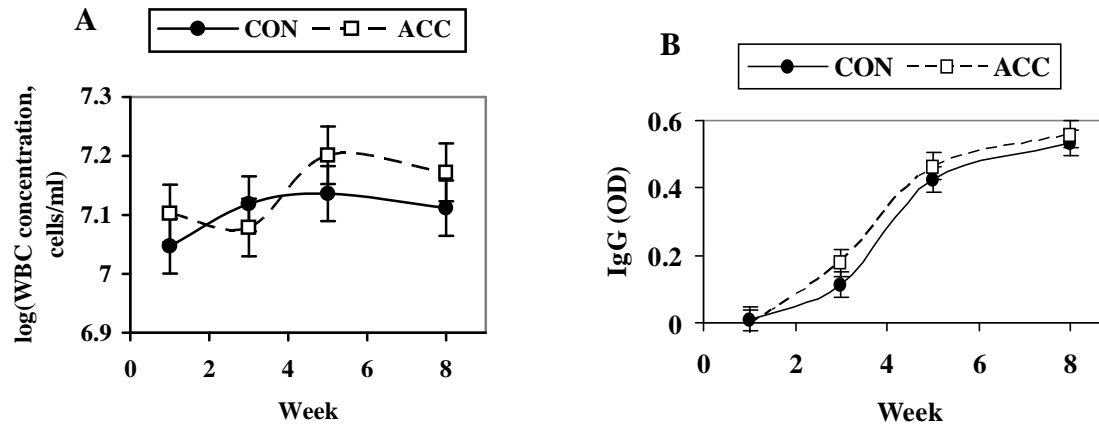
Figure 2. Starter DM intake (A), body weight (BW; B), and wither height (C) of Holstein calves fed a conventional (–●–; CON) or accelerated (--□--; ACC) milk replacer. Calves were assigned to one of two dietary treatments on day 2 of life. CON calves (n=8) were fed milk replacer with 20% CP and 20% fat at 1.25% birth BW from wk 1 to 6 and 0.625% birth BW during wk 7. ACC calves (n=7) were fed milk replacer with 28.5% CP and 15% fat at 1.5% birth BW during wk 1, 2% current BW from wk 2 to 6, and 1% current BW during wk 7. All calves were given milk replacer twice daily from wk 1 to 6, once daily during wk 7, and were weaned completely during wk 8. Starter was offered ad libitum beginning on day 3 of life, and intake was measured daily through wk 8 and averaged by week. BW and wither height were measured weekly through wk 10. ∗ = ACC different from CON ($P \leq 0.05$).
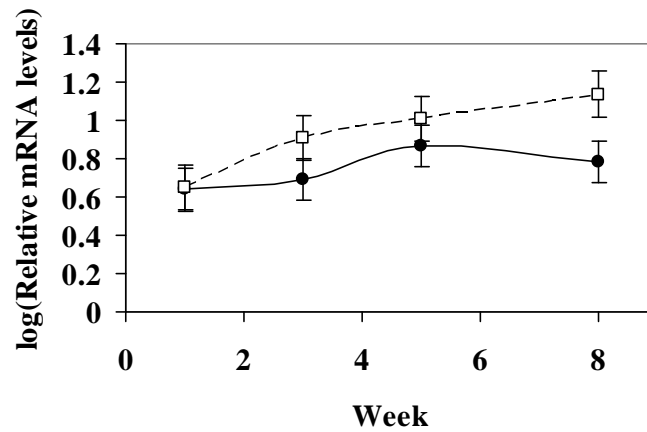
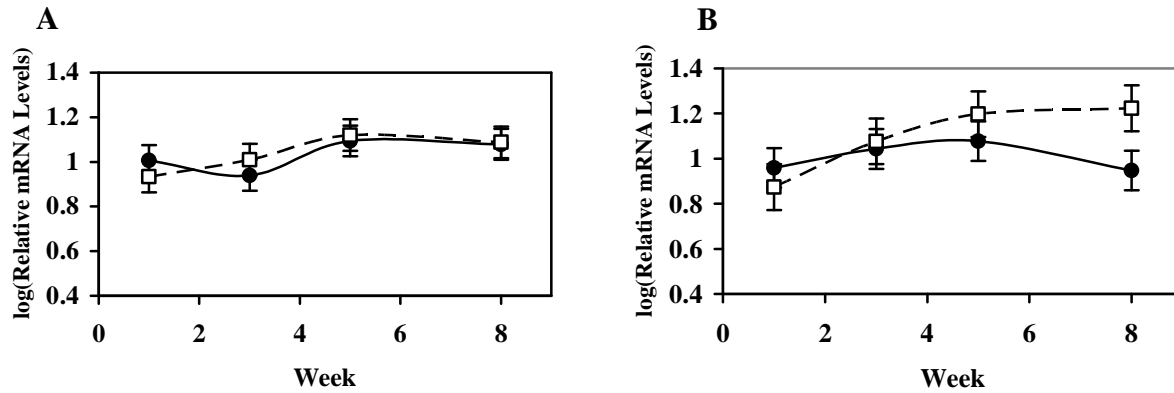**Figure 3. Effect of conventional (CON) and accelerated (ACC) milk replacer on health indices in Holstein calves.** Calves were randomly assigned to one of two dietary treatments on day 2 of life. CON calves (n=8) were fed milk replacer with 20% CP and 20% fat at 1.25% birth BW from wk 1 to 6 and 0.625% birth BW during wk 7. ACC calves (n=7) were fed milk replacer with 28.5% CP and 15% fat at 1.5% birth BW during wk 1, 2% current BW from wk 2 to 6, and 1% current BW during wk 7. All calves were given milk replacer twice daily from wk 1 to 6, once daily during wk 7, and were weaned completely during wk 8. Respiratory (A) and fecal (B) scores were assigned twice daily. Respiratory score was monitored using the following score: 1 = normal and 2 = abnormal. Fecal score was monitored using the following score: 1 = firm, well-formed, 2 = soft, pudding-like, 3 = runny, pancake batter, and 4 = liquid, splatters. Weekly average respiratory and fecal scores were used for statistical analysis.
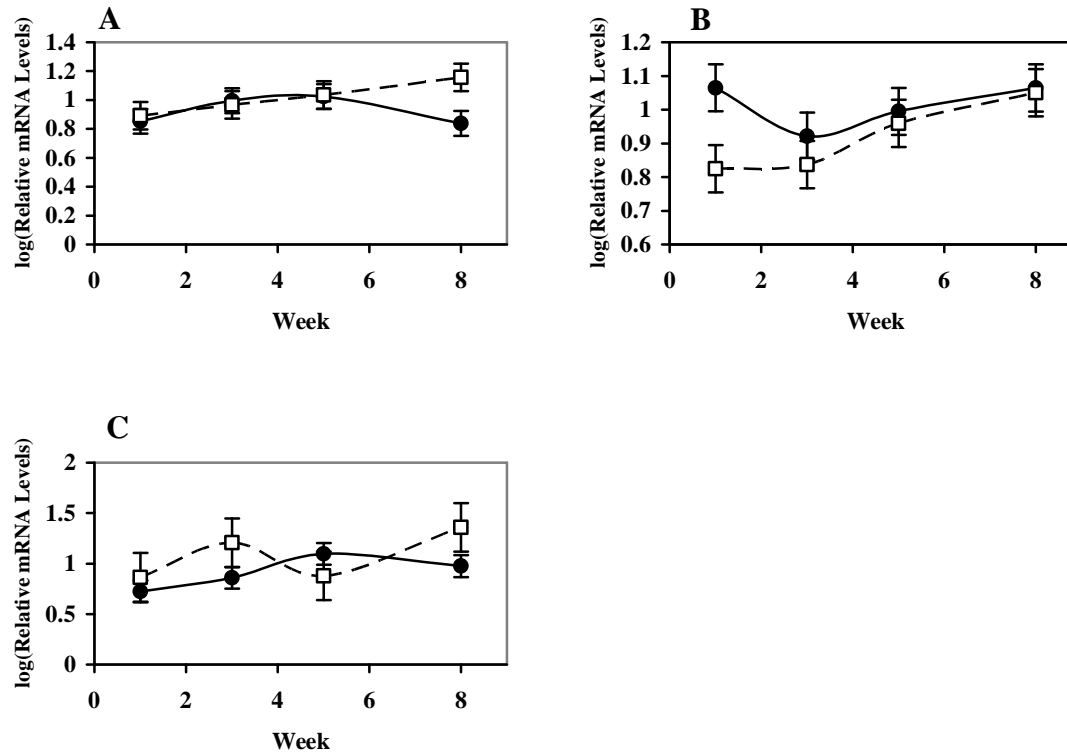
Figure 4. Effect of conventional (CON) and accelerated (ACC) milk replacer on white blood cell count and ovalbumin response in Holstein calves. Calves were randomly assigned to one of two dietary treatments on day 2 of life. CON calves (n=8) were fed milk replacer with 20% CP and 20% fat at 1.25% birth BW from wk 1 to 6 and 0.625% birth BW during wk 7. ACC calves (n=7) were fed milk replacer with 28.5% CP and 15% fat at 1.5% birth BW during wk 1, 2% current BW from wk 2 to 6, and 1% current BW during wk 7. All calves were given milk replacer twice daily from wk 1 to 6, once daily during wk 7, and were weaned completely during wk 8. White blood cell (WBC) count (cells/ml; A) was measured at wk 1, 3, 5, and 8. Adaptive immune function was measured by vaccinating calves against ovalbumin at wk 1, 3 and 5 and measuring anti-ovalbumin IgG production via optical density (OD; B).
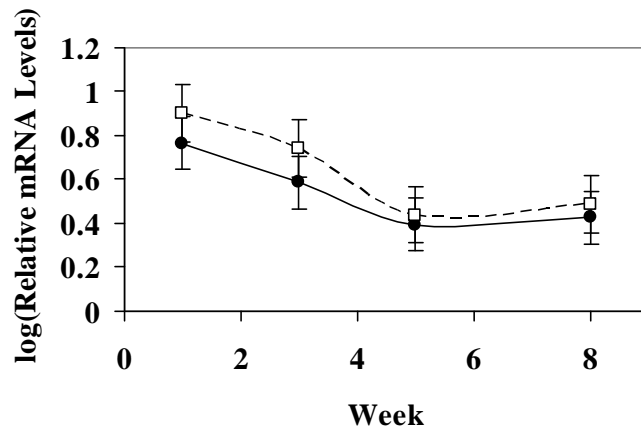
Figure 5. Neutrophil mRNA levels of *L-selectin* in Holstein calves fed conventional (–●–; CON) or accelerated (--□--; ACC) milk replacer. mRNA levels were determined relative to the geometric mean of mRNA levels of *β-actin* and *RPS9*. Relative mRNA levels were multiplied by 10 and log transformed prior to analysis.
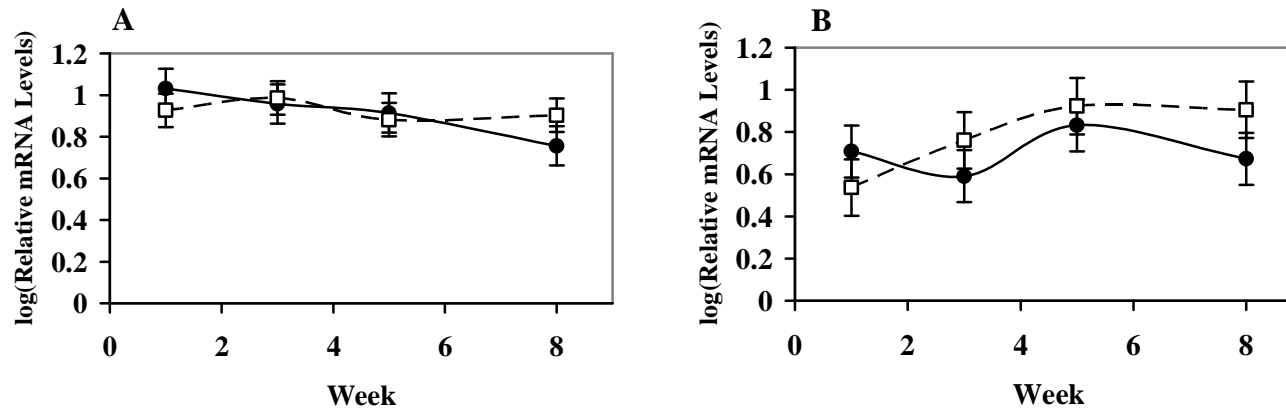
A

B

Figure 6. Neutrophil mRNA levels of *TLR-2* (A) and *TLR-4* (B) in Holstein calves fed conventional (–●–; CON) or accelerated (--□--; ACC) milk replacer. mRNA levels were determined relative to the geometric mean of mRNA levels of *β-actin* and *RPS9*. Relative mRNA levels were multiplied by 10 and log transformed prior to analysis.

Figure 7. Neutrophil mRNA levels of *NCF1* (A), *SOD1* (B), and *MPO* (C) in Holstein calves fed conventional (–●–; CON) or accelerated (--□--; ACC) milk replacer. mRNA levels were determined relative to the geometric mean of mRNA levels of *β-actin* and *RPS9*. Relative mRNA levels were multiplied by 10 and log transformed prior to analysis.

Figure 8. Neutrophil mRNA levels of *BPI* in Holstein calves fed conventional (–●– ; CON) or accelerated (--□--; ACC) milk replacer. mRNA levels were determined relative to the geometric mean of mRNA levels of *β-actin* and *RPS9*. Relative mRNA levels were multiplied by 10 and log transformed prior to analysis.

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Figure 9. Neutrophil mRNA levels of *TNF-αR* (A) and *IL-1R1* (B) in Holstein calves fed conventional (–●–; CON) or accelerated (--□--; ACC) milk replacer. mRNA levels were determined relative to the geometric mean of mRNA levels of *β-actin* and *RPS9*. Relative mRNA levels were multiplied by 10 and log transformed prior to analysis.