The influence of milk replacer Plane of nutrition on the performance, innate immune responses and pathophysiological response to a sub-clinical *Salmonella typhimurium* challenge

by

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ABSTRACT

The objectives were to determine the influence of plane of nutrition during the pre- and post-weaning periods on performance and innate immune activity of Jersey calves. Forty-six (3 ± 1 d of age) calves were randomly assigned to 2 treatments. Treatments were a low (LP; n=23) and high Plane of nutrition (HP; n=23). Calves in LP treatment were fed 409 g/d DM of a 20% protein and 20% fat milk replacer; whereas calves in HP treatment were fed 610 and 735 g/d DM of a 28% protein and 25% fat milk replacer during the 1st wk and wk 2-6, respectively. Weaning was initiated on d 42 by removing the PM feeding and calves were completely weaned when they were consuming 600 g of a calf starter ration after d 49. Calves on the HP on nutrition were fed a calf starter with 20% CP and 18% ADF while the LP nutrition calves were fed a calf starter with 18% CP and 14.5% ADF. Calves were fed their respective calf starter diets through the entire study. Peripheral blood samples were collected on d 0, 7, 21, 28, 42 and 77 for biochemical analyses. Blood samples collected on d 7, 21, 42, and 77 were also analyzed for *ex vivo* innate immune responses. Twenty bull calves (HP n=9 and LP n=11) on d 77 were orally challenged with 1.5 x 10^7 colony forming units of *Salmonella typhimurium (*ATCC14028). Indwelling rectal thermometers collected a measurement every 5 min. and peripheral blood samples were collected daily at 0800 throughout the study and . plasma analytes and innate immune responses were determined. As expected, metabolizable energy intake, crude protein intake, and average daily gain were greater (P<0.001) for HP calves when compared to LP calves. There were treatment x time interactions

($P<0.001$) for plasma concentrations of glucose and urea nitrogen. Glucose concentrations were greater ($P<0.01$) on d 21, 28, 42 and tended to be greater ($P<0.10$) on d 77 among HP calves, when compared to LP calves. Urea nitrogen concentrations tended to be greater ($P<0.10$) on d 7 among HP calves, when compared to LP calves, but were less ($P<0.01$) than LP calves on d 42 and 77. Secretion of TNF-α from diluted whole blood when co-cultured with lipopolysaccharide was higher ($P<0.05$) among HP calves on d 7, when compared to LP calves. In contrast, neutrophil expression of L-selectin was greater ($P<0.05$) among LP calves on d 7, 21, and 42, when compared to HP calves. No treatment or treatment x time differences ($P=0.798$) were observed for neutrophil oxidative burst capacities during the study. Following the *Salmonella typhi.* challenge, the percentage of neutrophils producing an oxidative burst was greater ($P<0.05$) among HP calves from d 1 – 5 after the challenge. Similarly, the intensity of the oxidative burst tended to be greater ($P<0.10$) among HP calves on d 2 and 3 after the challenge. In addition, the secretion of tumor necrosis factor-α tended ($P<0.10$) to be greater on d 1 and was greater ($P<0.05$) on d 5 and 6 after the challenge among HP calves. Median ranks of haptoglobin concentrations were lower ($P<0.05$) among HP calves throughout the challenge; however, there was no difference ($P=0.99$) between LP and HP calves on rectal temperatures. These data indicate that LP calves have activated innate immune responses during the pre-weaning period, but HP calves have a more aggressive innate immune response to an oral *Salmonella typhimurium* challenge, which may improve resistance to disease.

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CHAPTER I

REVIEW OF LITERATURE

1.1 Introduction

The dairy industry is growing rapidly in the southwest region of the United States, in both size of milking operations and number of cows. In 2001, 600 thousand dairy cows occupied Texas and New Mexico, and by 2009, cow numbers increased by 150 thousand. In order for these 750 thousand cows to produce milk, they must conceive and give birth to an offspring once every 12-18 months. The calving interval length depends on fresh cow health, nutrition, and reproductive efficiency. Approximately half of the calves born each year are heifer calves. Healthy heifer programs assure the successful future of the dairy industry in years to come.

The current method of stimulating starter intake by restricting fluid milk consumption has been practiced for several decades. Contrary to popular belief, restricting fluid milk is significantly different than starving growing calves. Although calves are not currently fed to their maximized daily rate of gain potential when consuming restricted volumes of fluid milk, they still gain considerable amounts of weight over the 7-10 week pre-weaning period. This conventional method of raising heifers takes approximately 13-15 months until heifers reach breeding size.

The resurgent interest in feeding dairy calves higher planes of nutrition during the pre-weaning period has been evaluated over the past decade. Increasing the plane of nutrition is primarily accomplished by increasing the total quantity of milk solids fed to

calves. In order to prevent excessive lipid deposition and increased lean tissue accretion, feeding an increased concentration of protein, more similar to whole milk is required than when restrictive feeding calves. Increasing the plane of nutrition improves average daily gain (ADG) and feed efficiency, decreases age at first breeding, and may improve health and future lactational performance. More research is needed in this area before sound conclusions can be drawn regarding how increasing the plane of nutrition of pre-weaned calves influences health and future performance.

1.2 The Immune System

The immune system development begins early in gestation (Halliwell and Gorman, 1989). As the fetus grows the immune system begins to mature through the development of immune cells. There is a steady increase in the peripheral lymphocytes, primarily T lymphocytes, throughout gestation, while other white blood cell populations expand (Cortese, 2009). Within the first 15 d of life, the largest concentration of white blood cells in the blood is immediately after parturition (Mao et al., 1994).

At birth neonates are removed from a sterile *in utero* environment and introduced to a pathogen filled *ex utero* environment. Physiological immaturity of the neonatal immune system is thought to render the newborn more susceptible to infectious diseases than mature cattle (Burgio et al.,1989). The susceptibility of the neonate to microbes is not due to any inherent inability to mount an immune response, but rather is due to their unprimed immune system (Tizard, 1992). Thus, the immunonaive neonate is dependent

on passively acquired maternal immunoglobulins, immune cells, and other substances from colostrum for protection during the first wk of life (Barrington and Parish, 2001). This is essential considering serum during the first wk of life contains elevated cortisol levels that suppress lymphoblastogenesis of calf lymphocytes from the spleen, thymus, and lymph nodes (Manak, 1986).

Measuring the immunocompetence of calves is primarily completed by measuring specific immune variables and comparing those to levels observed in mature cattle. Comparing the animal's capability of producing inducible nitric oxide and interferon-γ, Rajaraman et al., (1997) concluded that peripheral blood mononuclear cells (PBMC) from wk old calves fed colostrum and milk are functionally hyporesponsive when compared to PBMC from mature cattle. Other functional differences that suggest calves are immunonaive when compared to mature cattle include decreased antibody production (Nagahata et al., 1991), reduced neutrophil function (Dore et al., 1991; Kampen et al., 2006) , and reduced tumor necrosis factor-α (TNF-α) secretion (Nonnecke et al., 2003B) However, it has been proposed that judging the immunocompetency of the neonate based on the composition and function of adult PBMC population may need to be reevaluated (Nonnecke et al., 2003A).

The maturation of the immune system within the first wk of life is dependent on multiple variables including cortisol levels, adequate nutrition, and exposure to pathogens. When measured over time serum cortisol levels declined from 30 ± 4.6 ng/ml in calves 6 h of age to 5.5 ± 1.1 ng/ml in 10 d old calves (Manak, 1986). This alleviation

of immunosuppression is reflected by both the changes in composition and functional capacity of the calf's mononuclear leukocyte population within the first wk of life (Rajaraman et al., 1997 and Nonnecke et al., 2003A). Kampen et al. (2006) reported neutrophils are functional and able to mount an effective phagocytic response as early as one wk of age, however respiratory burst activity levels were sub-mature during the first 2 mo of life. Percentage of T and B lymphocytes, major histocompatibility complex II cells and interleukin-2r cells all increased over time, displaying a negative correlation with cortisol (Mao et al., 1994 and Nonnecke et al., 2003A). At 5 mo of age, the number of B cells and the calf's ability to produce immunoglobulins gradually increase to levels observed in mature cattle (Rajaraman et al., 1997 and Kampen et al., 2006). In cattle, a mature immune system is often seen at 6-8 mo of age (Cortese, 2009).

1.3 Passive Transfer

The unique physiology of the bovine placenta does not allow maternal serum antibodies to be passed to the fetus, therefore, the neonatal calf is born without any immunoglobulins. However, the gastrointestinal tract of the newborn is able to absorb intact immunoglobulins during the first 12 to 24 hours of life. This is known as passive immunity, because the immunoglobulins are passively derived from the dam's colostrum, which is the liquid secreted from the mammary tissue within the first 48 hours post-parturition.

While there are many different isotypes of immunoglobulins (e.g., IgG, IgA, IgM), IgG is the predominant immunoglobulin found in colostrum (Butler, 1983 and

NAHMS, 2007). Colostrum quality depends directly on lactation of cow, nutrition, health, and length of dry period. The ability of the neonate to absorb IgG starts to decline progressively after 4 to 6 hours and ceases after 24 hours after birth (Stott et al., 1979). The inability to provide sufficient amounts of colostrum during this brief window may result in failure of passive transfer of immunoglobulins.

Failure of passive transfer results in increased risk for neonatal disease and mortality and a negative effect on future health, longevity and performance in the herd (DeNise et al., 1989; Davis and Drackley, 1998; Faber et al., 2005). Almost one in five heifer calves (19.2 percent) have failure of passive transfer of maternal immunity, this is a result of either insufficient amounts of colostrum or low quality colostrum (NAHMS, 2007). Total serum protein greater than or equal to 5.0 to 5.2 g/dL is correlated with successful passive transfer of immunity (Tyler et al., 1996). Previous studies noted that passive immune transfer through colostrum was more beneficial than the use of sub-therapeutic antimicrobial agents in feed when evaluating morbidity and mortality (Berge et al., 2005).

Research has shown conflicting data regarding the optimal quantity of colostrum to feed neonatal calves. The amount depends strongly on the quality of the colostrum, if the concentration of immunoglobulins is below the optimal 50 g IgG/L (NAHMS, 2007) the calf will require a greater volume. Colostrum from multiparous cows has a higher antibody concentration than that of first calf heifers, and it is most often the colostrum producers use to feed neonates. Freezing antibody rich colostrum from multiparous cows

in individual bottles is a method used in industry to prevent from having to feed low-quality colostrum from first calf heifers.

1.4 Enteric Problems

Calf scours is the single most important cause of death in milk fed calves (NAHMS, 2007). In 2006, the average mortality rate in dairy calves born alive across the United States was 7.8 ± 0.2% (NAHMS, 2007), with 56% of those deaths being enteric related. Calf health is adversely affected by weather, stress, nutrition, exposure to infectious agents, and the calf's successful passive transfer of antibodies (Berge et al., 2005; Hulbert et al., 2011; Hulbert and Ballou, 2012). Dehydration is a leading cause of mortality for calves suffering from scours. Calves can quickly lose 10 to 12% of their body weight as water during scours (NRC, 2001).

Scours are a non-specific symptom of disease and can be classified as either nutritional or pathogen related. Nutritional scours are primarily caused by stress to the calf or its environment. These scours are the result of excess lactose in the intestinal tract when milk is not allowed sufficient time to digest. Pathogens utilize this excess lactose as an energy source for growth, and calves that present with nutritional scours often develop infectious or pathogen associated scours. A large number of infectious agents, both bacterial and viral, can cause diarrhea in neonatal food animals. *Escherichia coli* (E. coli) and *Salmonella* have the largest economic impact and are the most commonly found, but *Clostridium perfringens*, *Bacteriodes fragilis*, *Campylobacter*, and *Yersinia*

enterocolitica have all been identified as bacterial agents causing enteric disease (R.E. Holland, 1990). Coronavirus and rotavirus are the most commonly identified viral agents that cause scours. Generally, enteropathogens interact in the body as intestinal lesions and are capable secreting enterotoxins (R.E. Holland, 1990). Despite the availability of preventative vaccines against enterotoxigenic E. coli, rotavirus, and coronavirus, improved treatment protocols for calf diarrhea are required (Constable, 2004). Improved protocols would aid in the rapid recovery of infected animals and decrease the risk of animal to animal transmission.

1.4.1 Enteric Disease Signs and Symptoms

Many clinical signs may be seen in calves infected with enteric disease. In a state of clinical disease, calves will often have elevated rectal temperature, nasal discharge, dry muzzle and decreased suckle reflex. A slight decrease in starter ration and water consumption may be an indicator of enteric disease. Early onset of enteric disease is often difficult to identify since the signs are not solely indicative of enteric disease. As the severity increases calves will have sunken eyes, lethargy, no suckle reflex and extreme dehydration. Maintaining hydration is the key component to keep scouring calves alive, diseased animals will have decreased water intake, which further disrupts hydration status. Once hydration homeostasis is disrupted cells will begin to crenate as water moves into extracellular space, cell membranes will become less permeable, and the animal will have a decrease in metabolic break down of adenosine triphosphate (ATP), resulting in

lethargy and depressed appetite. Scouring is the mechanism the body utilizes to shed pathogens from the gastrointestinal tract and maintaining hydration decreases the risk of the calves permanently damaging the intestinal tract. Untreated scouring often result in the death of calves due to dehydration.

Septicemia is a result of a mass systemic infection in the body. In scouring calves the infection would establish in the gastrointestinal tract and if untreated, pathogens could potentially migrate into the blood supply. The capability of migrating from the lumen of the gastrointestinal tract is pathogen specific. Distinguishing between the early stages of septicemia and localized infection can be difficult, as early signs of sepsis are vague and may be similar to clinical signs of enteric disease (White,1985; Quie, 1976; Ballou et al., 2011). The abundance of bacteria in the bloodstream and at the site of infection leads to a large systemic pro-inflammatory response by the body. This inflammatory response is the body defending itself against the bacteria, but if excessive or uncontrolled, it often results in multiple organ dysfunction syndrome, septic shock and death (Ballou et al., 2008; Fecteau et al., 2009)

1.4.2 Diagnoses and Treatments

Diagnosing enteric disease is difficult, intense observation of calves is among the best diagnostic tool. Fecal plate counting, on selective growth media allows producers to diagnose pathogens, although this is not a widely adopted method due to cost, equipment, and time requirements. When treating infected calves, the two main goals are (1)

replenish electrolytes the calf lost from scouring (maintain hydration) and (2) eliminate pathogens. Oral electrolytes are offered between feedings as a separate bottle or powdered electrolytes are added to the bottle/bucket of milk in the calf's routine diet. When calves are unwilling to suckle, an esophageal feeder can be used to manually administer fluids. In severe cases, animals are often treated for multiple days with a combination of electrolytes, antibiotics, and anti-inflammatory drugs, this cocktail maintains hydration, kills foreign bacteria, and decreases the inflammatory response that may cause septic shock, respectively.

Non-antibiotic remedies are also capable of treating enteric disease. Bismuth salts, kaolin-pectin, probiotic bacteria, egg protein from hyperimmunized laying hens, enzymes and charcoal are often used to treat calf diarrhea (Berge et al., 2005 and Ballou, 2011). Kaolin-pectin and charcoal are both used in the prevention of absorption of pathogens or toxic material. Probiotics are used to improve intestinal microbial balance by acting as a competitive inhibitor, and bismuth salts inhibit bacterial growth in the intestines and gut. These methods are often used as a personal or farm preference, organic farms are not allowed to administer antibiotics, thus, they rely on these treatments to improve the health of infected calves.

1.5 Milk Composition and Quality

There are multiple brands of milk replacers, each with a unique composition. The largest variables within available milk replacers are the protein and fat content and

medicated versus non-medicated. A very common formulation of milk replacer fed at restricted quantities consists of a 20:20% ratio of protein to fat, on a dry matter (DM) basis. When feeding higher volumes of milk replacer to achieve a greater rate of gain the protein concentration needs to be increased, 28:25%, 25:25%, and 25:18% of protein and fat are ratios commonly used in these higher planes of nutrition feeding programs (Van Amburgh et al., 1998). Medicated milk replacer forces animals to consume sub-therapeutic levels of antibiotics over a portion of the pre-weaned period and are often used by producers as prophylactic measures. Under FDA regulations healthy calves may only consume milk replacer supplemented with 10 to 20 g per ton of antibiotics, however calves that require medication can be fed a therapeutic milk replacer that contains 1600 g per ton of these antibiotics for up to 14 d.

Raw milk typically has a 28:27% protein to fat ratio. The nutritional value of raw (non-saleable) milk makes it appealing to feed when compared to purchasing high component milk replacer. Research supports that non-saleable raw milk can be cost effective to feed when compared to purchasing milk replacer. Godden et al. (2005) estimated savings at $0.69 / calf per day when pasteurized, non-saleable raw milk was fed instead of milk replacer.

To decrease the risk of enteric disease in pre-weaned calves, waste milk should be pasteurized; reducing the number of viable pathogens. Milk is pasteurized by multiple methods: high temperature short time, ultra high temperature, and conventional pasteurization. Unlike sterilization, pasteurization does not kill all the micro-organisms in

the milk. Due to pasteurized milk lacking complex B vitamins, a common practice is to supplement with milk fortifiers to increase the nutritional content. This is an ideal opportunity to feed sub-therapeutic levels of antibiotics without feeding milk replacer.

A popular method of feeding milk to pre-weaned animals is designed to stimulate calf starter feed ration intake. To do so, the quantity of milk or milk replacer offered is restricted below *ad libitum* intakes during the pre-weaning period (Hulbert et al., 2011). Calves are predominantly fed liquid milk for the first 6-8 wks of life. Calf starter feed is often introduced to calves as early as 3 days of life. The conventional method of feeding calves is to provide 3.8L at 12% solids DM of fluid milk per day using individual bottles or buckets, which provides 454 g DM of milk solids / day.

Over the past decade, there has been a resurgence in the interest of feeding dairy calves higher planes of nutrition during the pre-weaning period (Van Amburgh et al., 1998; Brown et al., 2005; Drackley, 2008). Calf health, performance, and future milk production are all variables that have shown to be influenced by feeding a higher plane of nutrition. Calves fed *ad libitum* amounts of milk consumed 1.1 kg of DM per day in the pre-weaned period (Jasper et al., 2002). Bartlett et al. (2006) showed that by increasing the crude protein concentrations in milk replacers from 16 to 26% and feeding calves a higher plane of nutrition 675 g/day (1.75% BW) that body length, heart girth, ADG, and efficiencies of gain were increased.

Feeding calves a higher plane of nutrition may be achieved among multiple methods. A 20:20 milk replacer is conventionally fed at 454 g/DM/day diluted into 1

gallon of water, to suffice for maintenance and mild growth requirements, although increasing the amount of DM being fed would be a higher plane of nutrition. Feeding a milk replacer with a higher nutritional composition would also be considered a higher plane of nutrition, such as a 28:25% (protein:fat). This composition is similar to feeding raw milk (28:27% protein:fat), therefore, both feeding programs would enhance the growth rates of calves. Again it's important to note here that calves fed a lower plane of milk nutrition are not "starving"; there maintenance requirements are met and the calves are growing. The calves are, however, not gaining body weight at their full potential, but it remains to be understood how that influences the productivity and health of the calf. During the late 90's accelerated feeding was initially the term used for feeding higher planes of nutrition to pre-weaned calves, however milk replacer companies are now trying to coin this as "full potential" feeding.

Long-term profitability may be influenced by the rate animals grow and give birth to their first calf. Gardner et al. (1977) showed heifers gaining 1.1 kg/d compared to 0.8 kg/d calved 6.3 months earlier and yielded 48% more milk within the first 3 lactations, although other research has shown no increase in milk production when calves gain greater than 0.9 kg/d (Waldo et al., 1986; Gardner et al., 1988). A 5% decrease in milk production during first lactation was observed in heifers that were fed a high plane of nutrition as calves (Van Amburgh et al., 1998). This area of research requires more information before definitive conclusions can be drawn on the influence of pre-weaning performance and subsequent lactational performance.

1.6 Stress of Weaning

Weaning off of fluid milk is stressful to calves (Zavy et al., 1992). Timing of weaning is best determined by consumption of starter intake. A calf is ready to be weaned when it is consistently consuming adequate amounts of a calf starter feed ration to suffice for maintenance and growth requirements, which varies between breeds. Two methods of weaning calves exist in industry, progressive weaning and abrupt weaning. Progressive weaning is completed over a one-week period by removing the afternoon bottle from routine feeding before removing all fluid milk; whereas abrupt weaning is the method of removing all fluid milk at one time. Early weaning is profitable to producers as calf starter is less expensive and requires less preparation when compared to feeding milk from a bottle. A 39% decrease in daily labor was reported when calves were fed 1 bottle per day versus 2 bottles per day (Galton and Brakel, 1976). Previous research shows that calves are capable of digesting calf starter as early as 3 wks of life, although weaning this early is not commonly practiced (NAHMS, 2007, Hulbert et al., 2011).

The stress of weaning often causes a decrease in ADG over the weaning and immediate post-weaning period in calves fed the conventional plane of nutrition. Furthermore, the immediate post-weaning ADG slump was more severe among calves fed higher planes of milk nutrition (Bar-Peled et al., 1997; Jasper and Weary, 2002; Cowles et al., 2006). Decreasing the severity of the post-weaning ADG slump should be a focus of future research on intensified feeding programs.

1.7 Economics

Raising heifer calves from birth to first lactation is expensive and requires a large initial investment of facilities, feeding and labor for the heifers until they calve (around 23 months of age). Due to the elevated price of custom calf raising and the increased risk of cross-dairy pathogen transmission, 87.4% of producers choose to raise their own replacement heifers (NAHMS, 2007).

In recent years, much research has been directed at trying to increase the plane of nutrition of pre-weaned calves, as a means to decrease time to calving. In order to offset the increased cost of raising calves by feeding them higher planes of milk nutrition, the calves must have some benefit over conventional restricted-fed calves, such as improved resistance to disease, earlier age to calving, or subsequent improved lactational performance.. Previous research has shown that growth rate prior to weaning results in some form of epigenetic programming that is yet to be fully understood, but may have positive effects on lactation milk yield (Hulbert et al., 2011; Soberson et al., 2012; Ballou, *J. Dairy Sci., In Press*).

Data has been mixed when determining if increasing plane of nutrition during pre-weaning period benefits lactational performance of first calf heifers. Heifers previously fed a higher plane of nutrition during the grower phase produced 48% more milk over the first 3 lactations when compared to heifers fed a low plane of nutrition (Gardner et al., 1977). However, Van Amburgh (1998) observed a 5% decrease in milk production during the first lactation in calves previously fed a high plane of nutrition.

When comparing cost analysis of feeding different planes of nutrition multiple factors must be accounted for, these factors include price of milk replacer, price of calf starter, and consumption rates. Current milk replacer prices vary according to which plane of nutrition is fed, producers pay $88 for a 22.7 kg (00387¢/ g) bag of high plane milk replacer and $62 for a 22.7 kg (00273¢/ g) bag of conventional milk replacer. Common feeding rates for the high plane of nutrition and conventional fed calves are, 816 and 454 g/ d. Equaling the cost per day of milk replacer at $3.16 for feeding the high plane of nutrition and $1.24 for conventional. However, during the pre-weaning stage calves aren't solely consuming milk replacer, they are also consuming calf starter. A high quality pelleted calf starter cost approximately $550 per 909 kg equaling 000605¢/ g. The high levels of milk nutrition effects consumption rates and calves on the high plane of nutrition consume on average 59 g/d, whereas the conventional fed calves consume 173 g/d. When solely evaluating the cost of calf starter intake the prices are relatively low, 04¢ per day to feed calves on the high plane of nutrition and 10¢ per day for conventionally fed calves. When including both cost of milk replacer and calf starter, producers are capable of feeding calves the conventional method for $1.86, $3.20 vs $1.34/ d. However, research suggest feeding calves the high plane of nutrition allows them to be weaned 27.5 d earlier than those fed conventionally (Raeth-Knight et al., 2008). Assuming conventional fed calves are being weaned at 70 d, high plane calves would be weaned at 42.5 d. Using the prior calculated values the difference in feeding calves a high plane of nutrition and conventionally equals $42.20 difference per calf throughout the pre-weaning period, $136 vs $93.80.

If the increased cost of rearing calves eliminates the profit of increased fluid milk production, these intensified calf feeding programs will not likely be implemented into industry. The change must, in some way, be financially beneficial to the producer. Milk is a commodity that fluctuates daily and producers are constantly investigating ways to cut cost while maximizing profit. Future research needs to investigate if feeding a High Plane of nutrition affects mortality rates and breeding efficiency.

1.8 Nutritional Effects on Innate Immune System

Cellular proliferation, synthesis of immunologically active substances, cellular activation, and intracellular killing are all aspects of the immune system that are affected by nutrition (Cole, 1996). Restricted nutrition and intensified nutrition are both aspects of feeding animals that, when implemented may either benefit or damage immunocompetence. Leukocyte concentration, inflammatory cytokines, neutrophilia, and adhesion molecules are all derivatives of the innate immune system that work synergistically to eliminate infection.

Leukocyte concentration in peripheral blood is an indicator of the body's ability to resist an infection. Researchers have shown conflicting results when measuring total leukocyte concentration in peripheral blood, as well as contradicting results in calves fed varying planes of nutrition. Leukocyte concentration has been reported to be unaffected in calves on different planes of nutrition's (Griebel et al., 1987, Nonnecke et al., 2003A); whereas Ballou, (*J. Dairy Sci., In Press*) noted an increased leukocyte population in

Holstein, but not Jersey calves fed a higher plane of nutrition. A significant decrease in leukocyte count was observed in mice fed calorie restricted diets (Cano et al., 2009)

Pro-inflammatory cytokines are released by macrophages and other cell types in response to pathogens. The influence of nutrition on the secretion of pro-inflammatory cytokines also has mixed results across species. A common method to assess an animal's pro-inflammatory responsiveness is to stimulate mononuclear cells with LPS and quantify the release of pro-inflammatory cytokines in culture supernatant. Ballou, (*J. Dairy Sci., In Press*) observed that the secretion of TNF-α from isolated mononuclear cells stimulated with LPS was not affected in either Jersey or Holstein calves fed different planes of nutrition. Furthermore, in the same study when calves were challenged *in vivo* with LPS there was no difference in the pathophysiological response among calves fed the restricted and higher planes of nutrition. In contrast, data from rodents and beef steers showed that different planes of nutrition's affected the secretion of pro-inflammatory cytokines. Macrophages isolated from energy-restricted rats had reduced secretion of TNF-α when stimulated with LPS (Walrand et al., 2000). In addition, Sun et al. (2001) reported that mice on a calorie-restricted diet had a lower TNF-α response when peritoneal macrophages were stimulated with LPS *in vitro*. Recent data in beef steers fed either a restricted-diet or a low energy, high roughage diet during the receiving period had decreased concentration of TNF-α when peripheral mononuclear cells were co-cultured with LPS when compared to steers fed a high concentrate diet *ad libitum* (Schwertner et al., 2010). Lastly, a reduced secretion of TNF-α was reported when ileal

explants from pigs previously fed high protein (8.4 vs 6.0%) milk replacer were stimulated with LPS (Watson, 2011).

Neutrophil oxidative burst (OB) is an intracellular enzymatic cascade that aids in the destruction of engulfed pathogens (Parham, 2009). Studies evaluating the oxidative burst intensity from isolated neutrophils have shown similar results across species. Mice fed calorie-restricted diets had a decreased OB intensity when isolated neutrophils were stimulated with phorbol-myristate acetate/L (PMA; Walrand et al., 2000). Isolated neutrophils from Jerseys calves fed a lower plane of nutrition had a lower OB intensity when co-incubated with heat-killed *E. coli* for 10 minutes, but only during the immediate post-weaning period (Ballou, *J. Dairy Sci., In Press*). In addition to OB, Ballou (*J. Dairy Sci., In Press*) also reported an improved post-weaning whole blood bactericidal capacity against an enteropathogenic *E. coli.* Neutrophils isolated from fasting humans had a decreased OB intensity when stimulated with PMA (Walrand et al., 2001). However, the phagocytosis capacity of neutrophils was lower in prepartum dairy cows that were fed elevated energy levels during the dry period (Graugnard et al., 2012); although, levels reflected those of cows fed at maintenance after one wk of parturition.

L-selectin (CD62L) is an adhesion molecule located on neutrophils which binds to vascular addressins (CD34) located on vascular endothelial cells, this aids in the homing of neutrophils to the site of infection (Parham, 2009). Foote et al., (2005) reported T-lymphocytes from Holstein calves fed a high plane of nutrition (1140 g/d of a 20:28%, protein:fat MR) expressed less L-selectin when compared to calves fed a lower

plane of nutrition (454 g/d of a 20:20%, protein:fat MR). Among yearling Holsteins, neutrophil L-selectin expression was enhanced when fed an energy restriction diet that resulted in a negative energy balance, 60% of maintenance energy requirements (Perkins et al., 2001). However, Magné et al. (2009) examined the influence of a high fat diet (60% lipid emulsion) on the expression of L-selectin on rat neutrophils. They reported an increased expression of L-selectin among rats fed the high fat diet when compared to rats eating a standard diet.

1.9 Conclusion

Feeding a higher plane of nutrition to neonatal calves during the pre-weaning period could improve the profitability of calves over the duration of their lifespan. Decreased time to weaning, elevated immunocompetence, and subsequent lactational performance are all factors that have been enhanced by intensified feeding programs in previous research. More information on feeding varying planes of nutrition to neonatal calves is needed, along with further research on the influence that nutrition can have on the innate immune system. This information could aid in the development of alternative management systems that would maximize the economic potential of incoming youngstock in the dairy industry, and potentially alleviate the current tight margins of profitability.

CHAPTER II

THE INFLUENCE OF MILK REPLACER PLANE OF NUTRITION ON THE PERFORMANCE, INNATE IMMUNE RESPONSES AND PATHOPHYSIOLOGICAL RESPONSE TO A SUB-CLINICAL *SALMONELLA TYPHIMURIUM* CHALLENGE

2.1 Abstract

The objectives were to determine the influence of plane of nutrition during the pre- and post-weaning periods on performance and innate immune activity of Jersey calves. Forty-six (3 ± 1 d of age) calves were randomly assigned to 2 treatments. Treatments were a low (LP; n=23) and high Plane of nutrition (HP; n=23). Calves in LP treatment were fed 409 g/d DM of a 20% protein and 20% fat milk replacer; whereas calves in HP treatment were fed 610 and 735 g/d DM of a 28% protein and 25% fat milk replacer during the 1st wk and wk 2-6, respectively. Weaning was initiated on d 42 by removing the PM feeding and calves were completely weaned when they were consuming 600 g of a calf starter ration after d 49. Calves on the HP on nutrition were fed a calf starter with 20% CP and 18% ADF while the LP nutrition calves were fed a calf starter with 18% CP and 14.5% ADF. Calves were fed their respective calf starter diets through the entire study. Peripheral blood samples were collected on d 0, 7, 21, 28, 42 and 77 for biochemical analyses. Blood samples collected on d 7, 21, 42, and 77 were also analyzed for *ex vivo* innate immune responses. Twenty bull calves (HP n=9 and LP n=11) on d 77 were orally challenged with 1.5 x 10^7 colony forming units of *Salmonella*

*typhimurium (*ATCC14028). Indwelling rectal thermometers collected a measurement every 5 min. and peripheral blood samples were collected daily at 0800 throughout the study and . plasma analytes and innate immune responses were determined. As expected, metabolizable energy intake, crude protein intake, and average daily gain were greater ($P<0.001$) for HP calves when compared to LP calves. There were treatment x time interactions ($P<0.001$) for plasma concentrations of glucose and urea nitrogen. Glucose concentrations were greater ($P<0.01$) on d 21, 28, 42 and tended to be greater ($P<0.10$) on d 77 among HP calves, when compared to LP calves. Urea nitrogen concentrations tended to be greater ($P<0.10$) on d 7 among HP calves, when compared to LP calves, but were less ($P<0.01$) than LP calves on d 42 and 77. Secretion of TNF-α from diluted whole blood when co-cultured with lipopolysaccharide was higher ($P<0.05$) among HP calves on d 7, when compared to LP calves. In contrast, neutrophil expression of L-selectin was greater ($P<0.05$) among LP calves on d 7, 21, and 42, when compared to HP calves. No treatment or treatment x time differences ($P=0.798$) were observed for neutrophil oxidative burst capacities during the study. Following the *Salmonella typhi.* challenge, the percentage of neutrophils producing an oxidative burst was greater ($P<0.05$) among HP calves from d 1 – 5 after the challenge. Similarly, the intensity of the oxidative burst tended to be greater ($P<0.10$) among HP calves on d 2 and 3 after the challenge. In addition, the secretion of tumor necrosis factor-α tended ($P<0.10$) to be greater on d 1 and was greater ($P<0.05$) on d 5 and 6 after the challenge among HP calves. Median ranks of haptoglobin concentrations were lower ($P<0.05$) among HP calves throughout the challenge; however, there was no difference ($P=0.99$) between LP

and HP calves on rectal temperatures. These data indicate that LP calves have activated innate immune responses during the pre-weaning period, but HP calves have a more aggressive innate immune response to an oral *Salmonella typhimurium* challenge, which may improve resistance to disease.

2.2 Introduction

A common method of feeding dairy calves is to restrict the quantity of milk fed, which is effective in stimulating calf starter intake. The effects of this restrictive milk feeding on the immunological responses and future lactational performance are not well understood (Nonnecke et al., 2003A; Foote et al., 2005; Foote et al., 2007; Ballou, *J. Dairy Sci., In Press*). Feeding a higher plane of milk nutrition is more expensive during the pre-weaning period; therefore, calves need to reach breeding age earlier, have improved health, and/(or) have greater lactational performance to justify feeding a higher pre-weaning plane of nutrition. Raeth-Knight et al. (2008) reported that calves with elevated rates of gain during the pre-weaning period showed decreased time to first calving by 27.5 d. Moreover, Ballou (*J. Dairy Sci., In Press*) reported increased innate immune responses in post-weaned Jersey calves that were previously fed a higher plane of nutrition.

Ballou (*J. Dairy Sci., In Press*) observed an increased neutrophil oxidative burst intensity and whole blood killing capacity in post-weaned Jersey calves that were previously fed higher planes of nutrition. These data suggest that Jersey calves that were

previously fed a higher plane of milk nutrition may have an improved ability to control the growth and eliminate the body from gram negative infections. Modulating the innate immune responses of calves may reduce the number of sick calves and subsequently, the cost of raising calves. The objectives were to determine the influence of plane of nutrition during the pre- and post-weaning periods on performance and innate immune activity of Jersey calves.

2.3 Materials and Methods Experiment I

The experiment was conducted from May to July 2011. All animal procedures were reviewed and approved by the Texas Tech University Animal Care and Use Committee. Forty-six Jersey calves, 23 bulls and 23 heifers, (3±1 d of age) were transported 155 km from a commercial dairy farm to the Hilmar Cheese / Agri-Plastics Calf Research Facility at Texas Tech University (New Deal, TX). All calves were fed 3.8L of pooled colostrum at the dairy within the first 12 h of life, upon enrollment a peripheral blood sample was taken and individual total serum proteins were recorded using a hand held refractometer, which averaged 6.7 g/dL with a range of 5.5 to 7.8 g/dL. Calves were housed individually outside with straw bedding in commercial polyethylene calf hutches (Agri-Plastics, Tonawanda, NY).

2.3.1 Feeding and Weaning

Upon arrival at the research facility, each calf was weighed, shoulder height and length from the scapula to the pins were measured, and randomly assigned to either a low plane of nutrition (LP) or a high plane of nutrition (HP) treatment. Calves on the LP were fed 409 g, DM basis, of a 20% protein and 20% fat milk replacer (Herd Maker, Land O'Lakes Animal Protein Co., Shoreview, MN) in 4 L of water daily. Calves on the HP were fed 610 g, DM basis, of a 28% protein and 25% fat milk replacer (Cow's Match Jersey Blend, Land O'Lakes Animal Protein Co., Shoreview, MN) in 5 L of water daily for the first wk. Calves fed the HP were then stepped-up to 735 g of the same 28% protein and 25% fat milk replacer in 7 L of water daily during wks 2-6. Calves were fed twice daily at 0800 and 1600 h for the duration of the study. After the first wk, all calves had ad libitum access to a calf starter ration. The formulated chemical compositions of the calf starters offered to the LP and HP calves are shown in Table 1. The quantity of calf starter offered to each calf was adjusted daily for approximately a 10% refusal. No roughage was offered during the study. Weaning was initiated a d 42 by removing the 1600 milk feeding Calves were completely weaned from milk when daily consumption of calf starter exceeded 600 g, as-fed basis, for two consecutive days after d 49.

2.3.2 Observations

General attitude, appetite, and fecal scores were assessed multiple times daily by 2 independent trained observers. Attitude was classified as 1 = normal, alert, response to stimuli quick; 2 = depressed, response to stimuli decreased; 3 = lethargic, response to

stimuli greatly reduced; 4 = morbid, little or no response to stimuli (Ballou et al., 2011). Fecal scores were recorded according to the guidelines outlined by Larson et al. (1977). Scores were, 1 = firm, well-formed (not hard); 2 = soft, pudding-like; 3 = runny, pancake batter; and 4 = liquid, splatters, pulpy orange juice. Calves with fecal scores greater than 2.5 were classified as scouring.

2.3.3 Sampling and Blood Collection

Calves were weighed individually at arrival, and at d 7, 21, 42, 56, and 77 of the study. Shoulder height and length was measured at enrollment and at d 21, 42, and 77. Voluntary milk replacer refusals were recorded approximately 30 min after each feeding. Nine mL of peripheral blood samples from the jugular vein were collected at days 7, 21, 28, 42 and 77 using 3 mL and 6 mL evacuated tubes (Vacutainer®, Becton Dickinson, Rutherford, NJ) containing K_2 EDTA and heparin, respectively. The K_2EDTA tube was placed immediately on ice and the heparin tube was placed in an ice chest without ice. All blood samples were processed within 2 h of collection. Plasma was obtained from the K_2EDTA tube after centrifugation at 1,200 x g for 15 min and stored at -40°C until analyzed. Plasma was analyzed for glucose, urea nitrogen, and haptoglobin concentrations as described by Hulbert et al. (2011). All colorimetric data were measured on a SpectraMax 340PC (Molecular Devices, Sunnyvale, CA). Control serum (Randox Laboratories, Oceanside, CA) was used to calculate the intra-assay coefficients of variations of 5.4 and 6.4% for plasma glucose and urea nitrogen. Inter-assay coefficients of variation were 5.1 and 6.8% for plasma glucose and urea nitrogen, respectively. The

intra- and inter-assay coefficients of variation for plasma haptoglobin were 1.4 and 3.4 %, respectively.

2.3.4 Ex vivo Immunological Analyses

2.3.4.1 TNF-α

Peripheral blood from the heparinized tube was diluted 1:4 in RPMI medium at a final concentration of 1% antibiotic-antimycotic (Invitrogen Life Technologies, Grand Island, NY, 14072) and 1 μg/mL of lipopolysaccharide (E.coli 0111:B4; Sigma; St. Louis, MO). Duplicate cultures were incubated for 24 h at 38°C in a humidified 5% CO_2 incubator. The supernatant was removed after centrifugation for 15 min at 1,200 x g and stored at -40°C until analyzed for concentrations of tumor necrosis factor-α (TNF-α) by a commercially available ELISA kit (R&D Systems, Minneapolis, MN).

2.3.4.2 Phagocytosis and Oxidative Burst

The oxidative burst (OB) capacities of whole blood neutrophils in response to an enteropathogenic *Escherichia coli* were analyzed as described by Hulbert et al. (2011). Briefly, 200 μL of whole blood from the heparinized tube was incubated in an ice bath for 15 min. Forty microliters of a 100 μM working concentration of dihydrorhodamine (Invitrogen, Carlsbad, CA) and the *E. coli* (10^9 colony forming units / mL) were added to each sample, vortexed thoroughly, and then placed in a 38.5^{o}C water bath and incubated for 10 min. After completion of incubation, the samples were immediately placed in an ice bath for 5 min to stop the reaction at a constant rate. Erythrocytes were hypotonically

lysed and washed, and the leukocytes were analyzed using a Cell Lab Quanta SC flow cytometer (Beckman Coulter, Fullerton, CA). Using flow cytometer analysis software (QuantaSC MPL, Beckman Coulter), neutrophils were gated on the scatterplot of electric volume x side scatter. The percentage of neutrophils that were positive for OB were gated as neutrophils that had a greater fluorescence intensity than neutrophils from control cultures that were incubated without *E. coli.* Data are reported as both the percentage of neutrophils positive for OB as well as the geometric mean fluorescence intensity of the positive population.

2.3.4.3 Neutrophil Leukocyte Adhesion Molecules

Two hundred microliters of whole blood from the K_2EDTA tube was incubated at a final concentration of 5 µg/mL of anti-bovine CD62L (monoclonal antibody IgG1-isotype made in mouse; VMRD, Pullman, WA) for 1 h in an ice bath. Erythrocytes were hypotonically lysed and then rinsed once. The leukocyte pellet was resuspended in fluorescein-labeled secondary antibody at a 1:400 dilution [F(ab')2 anti-mouse IgG:FITC; AbD Serotec Raleigh, NC]. Samples were incubated on ice for an additional 1 h. Samples were then washed twice using 1x PBS and then analyzed by single-color flow cytometry. Using the flow cytometer analysis software, neutrophils were gated on the scatterplot of electric volume x side scatter. The total geometric mean fluorescence intensity (FL-1) for L-selectin was analyzed (Hulbert et al., 2011).

2.3.5 Statistical Analyses

All data were analyzed by restricted maximum-likelihood ANOVA using the MIXED procedure of SAS (v.9.2, SAS Inst. Inc., Cary, NC). Compound symmetry, auto-regressive (1), and ante-regressive (1) were the covariance structures tested for the within-subject measurements and the one with the best fit, determined from the Akaike's information criteria was used. Prior to statistical analyses, repeated data were tested for normality by evaluating the Shapiro-Wilk statistic using the UNIVARIATE procedure of SAS (v.9.2, SAS Inst. Inc.). Non-normally distributed data were log-transformed before mixed model analysis. Pairwise differences were performed at each time interval using a sliced effect multiple comparison approach with a Tukey-Kramer adjustment. Least squares means (±SEM) are reported throughout. Differences of $P \leq 0.05$ was considered significant and $0.10 \geq P > 0.05$ was considered a tendency.

2.4 Experiment II

2.4.1 Experimental design and calves

All animal care was conducted according to the Guide for the Care and Use of Agriculture Animals in Agricultural Research and Teaching and approved by the Institutional Animal Care and Use Committee of the USDA-ARS. Twenty Jersey bull calves (80 ± 1 days of age) that were previously fed either a Low Plane (LP; 409 g/d of a 20% protein and 20% fat milk replacer) or a High Plane (HP; 610 and 816 g/d of a 28%

protein and 25% fat milk replacer for wk 1 and wks 2-6, respectively) were acquired from Experiment I and transported 11.5 km to the Livestock Issue Research Unit calf facility in Liberty, TX. Calves were housed in individual stainless steel pens (1 m x 2 m) with slotted rubber mat floor in a thermo-controlled room (ranged from 20.3 to 21.9 °C). Upon arrival all calves were fitted with rectal temperature monitoring devices as described by Ballou et al. (2011). Briefly, the rectal temperature devices consisted of a DST micro-T small thermo logger (Star Oddi, Reykjavik, Iceland) enclosed in a 1 mm outside diameter x 4.5 mm long stainless steel fabricated capsule that was attached to a 0.75 mm outside diameter and 11 mm long PVC tube. The PVC tube was attached to half a fabricated piece of powder-coated aluminum pipe, 11 mm long x 5 mm internal diameter, and secured to the tail using Co-flex flexible adhesive bandage (Andover Healthcare, Andover, MA). Rectal temperatures were collected via the DST micro-T small thermo logger at 5-min intervals, and hourly averages calculated from -16 h prior to the challenge till 48 h after the challenge. After a 48 h acclamation period, calves were orally challenged with 1.5×10^7 colony forming units of *Salmonella typhimurium* (ATCC14028) in a total volume of 30 mL by oral gavage.

2.4.2 Observations and Sampling

General attitude and appetite was assessed twice daily at 0800 and 1600 h for the 10 days post-challenge. Behavior was recorded as described in Experiment 1. Appetite was assessed by observing feed consumption and measuring amount of refusals. Ten mL of peripheral blood were collected by jugular venapuncture at 0, 1, 2, 3, 4, 5, 6, 7, 8, and

9 d relative to the challenge into heparinized Vacutainer® for whole blood cultures to measure TNF-α secretion when co-cultured with LPS and to measure neutrophil oxidative burst capacities. All blood was processed within 1 h of collection. Plasma was collected from whole blood after centrifugation at 1,200 x g for 15 min and stored at -40°C. Plasma was analyzed for glucose, urea nitrogen, and haptoglobin concentrations as described in Experiment 1. The intra-assay coefficients of variations were 4.2, 4.0, and 1.7% and the inter-assay coefficient of variation were 4.4, 5.1, and 2.1% for glucose, urea nitrogen, and haptoglobin, respectively.

2.4.3 Statistical Analyses

All performance, immunological, rectal temperature, glucose, and urea nitrogen data were analyzed by restricted maximum-likelihood ANOVA using the MIXED procedure of SAS (v.9.2, SAS Inst. Inc., Cary, NC). Compound symmetry, auto-regressive (1), and ante-regressive (1) were the covariance structures tested for the within-subject measurements and the one with the best fit, determined from the Akaike's information criteria was used. Prior to statistical analyses, repeated data were tested for normality by evaluating the Shapiro-Wilk statistic using the UNIVARIATE procedure of SAS (v.9.2, SAS Inst. Inc.). Non-normally distributed data were log-transformed before mixed model analysis. Not all calves showed clinical signs of disease; therefore, morbidity data was analyzed using a Chi-Squared goodness of fit test. Haptoglobin was unable to be transformed into a normal distribution; therefore, the analysis of variance of

ranks was analyzed using the non-parametric Friedman test. Pairwise differences were performed at each time interval using a sliced effect multiple comparison approach with a Tukey-Kramer adjustment. Least squares means (±SEM) are reported throughout. Differences of $P<0.05$ was considered significant, and $0.10 \geq P > 0.05$was considered a tendency.

2.5 Results

2.5.1 Experiment I

2.5.2 Intakes and Performance

Three HP calves died within the first 14 d and were removed before data analysis. Low Plane calves consumed more ($P<0.05$, Table 3) calf starter during the duration of the study, with the exception of d 56 to 77 where no difference in intake was observed. High Plane calves had elevated ADG during the intervals from 0 to 7 d, 21 to 42 d, 0 to 42 d, 56 to 77 d, 42 to 77 d, and 0 to77 d (P<0.05, Table 3); however, LP calves tended to have elevated ADG during the immediate post-weaning period, 42 to 56 d (P<0.10, Table 2). High Plane calves mean fecal scores were greater than LP, however when comparing time scouring no difference was observed across planes of nutrition (P=0.292).

2.5.3 Efficiency

A treatment x time interaction was observed when analyzing energy efficiency across planes of nutrition (*P<0.05*, Table 2). Low Plane calves were less efficient at using metabolizable energy for BW gain during intervals 0 to 42 d, 56 to 77 d, and 0 to 77 d; however, no differences were observed during the remaining intervals (Table 2). A treatment x time interaction was observed for protein efficiency among planes of nutrition (P=0.004, Table 3), no differences were seen when comparing utilization of CP for BW gain during the pre-weaning period (Table 2); however, HP calves were more efficient during 56 to 77 d and 0 to 77 d (*P<0.05*) and tended to be elevated during 42 to 77 d interval (*P<0.10*).

2.5.4 Plasma Metabolites

There was a treatment x time interaction (*P<0.05*) for plasma glucose concentrations. No difference was observed on d 7 (*P=0.144*), but HP calves had increased plasma glucose concentrations throughout the remainder of the study (*P*<0.05) when compared to LP calves(Figure 1). There was a treatment x time interaction (P=.001) for plasma urea nitrogen concentrations. High Plane calves tended to have elevated levels on d 7 (P<0.10), but LP calves had increased concentrations during the immediately before and post-weaning (P<0.05) (Figure 1).

2.5.5 Ex Vivo Measures of Innate Immune Response

Secretion of TNF-α was greater (*P<0.05*) in HP calves on d 7 (Figure 2a), although no differences were observed on d 21, 42, and 77. Low Plane calves had greater neutrophil L-selectin protein concentrations on d 7, 21, and 42 (Figure 2b). No differences were observed between treatments in the percentage of positive neutrophils for oxidative burst response against an enteropathogenic *E.coli* or the geometric mean fluorescence intensity of oxidative burst positive neutrophils. The only difference observed for haptoglobin concentrations (*P<0.05*, Figure 3) was on d 21 and LP calves had increased concentrations.

2.6 Experiment II

2.6.1 Intakes and Disease

High Plane calves consumed increased levels of calf starter throughout the duration of the study post-challenge (*P<0.05*) compared to LP calves (2189 vs 2004 ± 46.0, g/d, respectively). No treatment difference was observed in the percentage of calves that developed clinical disease (*P=0.279*) following the *Salmonella typhimurium* challenge with 45.5 vs 22.2% of calves showing some clinical sign of disease (anorexia, decreased response to stimuli, or distended head) for LP and HP, respectively. All calves survived the 9 day observation period.

2.6.2 Plasma Metabolites

Plane of nutrition influenced plasma glucose ($P<0.05$); HP calves tended to have elevated concentrations on d 1 and 6 ($P<0.10$, Figure 4), and had increased concentrations on d 4 and 5 ($P<0.05$, Figure 4), when compared to LP calves. A treatment effect was also observed in plasma urea nitrogen concentrations ($P<0.05$, Figure 4). Low Plane calves had increased concentrations on d 0, 2, and 4 (Figure 4), when compared to HP calves.

2.6.3 Ex Vivo Measures of Innate Immune Response

High Plane calves tended to have elevated ($P<0.10$) TNF-α concentrations on d 1 and were increased ($P<0.05$) on d 5 and 6 when compared to LP calves (Figure 5a). A treatment effect ($P<0.05$) was also observed for the percentage of neutrophils positive for an oxidative burst response; whereas HP calves had a greater percentage on d 1, 2, 3, 4, and 5 (Figure 5b) compared to LP calves. When measuring geometric mean fluorescence intensity of oxidative burst positive neutrophils, HP calves tended to be elevated on d 3 and 4 ($P<0.10$, Figure 5c), when compared to LP calves. Rectal temperature in both treatments peaked at 50 h post-challenge and returned to circadian rhythm at approximately 120 h; however, a larger circadian fluctuation was observed throughout the duration of the observation period (Figure 6). There were no differences in rectal temperatures between treatments. Median ranks of plasma haptoglobin concentrations were lower ($P<0.05$) among HP calves throughout the study (Figure 7).

2.7 Discussion

Plane of nutrition positively influenced growth rates and efficiency of gain during the pre-weaning period of the current study. Similar data were reported when calves were fed a plane of nutrition that achieved an ADG higher than 0.45 kg/d (Bartlett et al., 2006; Bascom et al., 2007; Ballou, *J. Dairy Sci., In Press*). The positive growth rates portrayed by the HP calves during the pre-weaning period were halted during the immediate post-weaning period, although calves compensated for this lack of ADG during the 56 to 77 d interval. Bar-Peled et al (1997) suggest the slump in efficiency during the immediate post-weaning period is a result of calves fed a higher plane of nutrition stress more during the adaptation to solid food. The decreased starter intake may alter rumen development and disrupt growing rates during weaning (Jasper and Weary, 2002). Investigating weaning strategies that assist in eliminating this post-weaning slump should be a focus of future research. Calves fed the HP had higher fecal scores during the first 4 wks of life, which is similar to data reported by Nonnecke et al. (2003A), where Holstein calves were fed milk replacers consisting of either 30% protein and 20% fat fed at 2.5% BW or 20% protein and 20% fat fed at 1.4% BW. However, as suggested by Nonnecke et al. (2003A) the greater fecal scores of calves fed Higher Planes of milk may be because they are consuming a larger volume of water. In support of that, the incidences of scours were not greater in HP calves in the present study.

Elevated plasma glucose concentrations in the HP calves over most of the pre-weaning period is commonly reported (Quigley et al., 2006; Foote et al., 2007). The

greater plasma glucose concentrations is likely due to increased consumption of milk, which is high in lactose The reduced plasma urea nitrogen concentrations among HP calves at d 42 and 77 may be related to the greater deposition of lean tissue The HP calves had greater ADG in the periods immediately prior to the sample collections on d 42 and 77. In contrast, Ballou et al. (*Livestock Sci., In Review*) reported no difference in plasma urea nitrogen concentrations between Jersey calves fed either 454 g/ d of a 20% CP and 20% fat milk replacer or 680 g/ d of a 28% CP and 25% fat milk replacer. The difference observed between the 2 studies may be due to the quantity of the milk replacer fed because Ballou et al. (*Livestock Sci., In Review*) fed 680 g/d, which was less than the 816 g/d of the same 28% CP and 25% fat milk replacer. Therefore, it remains to be determined lean tissue accretion of calves fed 816 g/d of a 28% CP and 25% fat milk replacer is limited by metabolizable protein.

Enteric disease is common among dairy calves during the first few wks of life, as they adapt to the *ex utero* environment. Passively derived immunoglobulins and the innate immune system are important in protecting the calf from potential pathogens. The innate immune system of LP calves may have been "primed" or more active during the pre-weaning period as suggested by the elevated neutrophil L-selectin protein concentrations at d 7, 21, and 42. In agreement, Obeidat et al. (unpublished) reported that Holstein calves fed a lower plane of milk replacer had elevated neutrophil L-selectin protein concentrations during the pre-weaning period when compared to Holstein calves fed a higher plane of milk replacer. In addition, Obeidat et al. (unpublished) observed more neutrophils producing an oxidative burst and the intensity of the oxidative burst was

greater among the Holstein calves fed the lower plane of milk replacer. Obeidat et al. (unpublished) suggested that the more active neutrophil responses of Holstein calves fed a lower plane of nutrition may be due to more immunogenic stimulation because these calves had more non-nutritive suckling following milk replacer feedings (Rushen and de Passillé, 1995). In agreement, microbial immunogenic stimulation in the gastrointestinal tract increased the activity of peripheral blood neutrophils in rodent models (Clarke et al., 2010). Furthermore, Cobb et al. (unpublished) reported that peripheral blood neutrophils from Holstein calves raised outdoors in small groups during the first 3 months of life were more active than individually housed calves. Cobb et al. (unpublished) suggested that the group-housed calves were exposed to a greater load and/(or) diversity of microorganisms because of more calf-to-calf interactions. Taken together, these data suggest that Jersey calves fed LP may have more neutrophil L-selectin protein concentrations during the pre-weaning period due to decreased satiety following milk feedings, which increased non-nutritive suckling and subsequent exposure to more microorganisms. It is important to note that although plasma haptoglobin concentrations were greater at d 21 among LP calves the difference was not deemed biologically significant. In fact, the haptoglobin data indicate that among each treatment there was a low incidence of systemic inflammation throughout the pre- and immediate post-weaning periods.

The lack of a consistent effect of plane of nutrition on neutrophil oxidative burst capacities and whole blood TNF-α secretion in the present study agree with data from Ballou (*J. Dairy Sci., In Press*), where lower planes of nutrition among Holstein and

Jersey calves did not influence either of those innate immune responses during the pre-weaning period. At first glance this may appear to contradict the previous discussion that the innate immune response of LP Jersey calves was more active during the pre-weaning period; however, the intensity of the immunogenic stimulation required to activate peripheral leukocytes may be different for each immune response and/(or) cell type. For example, a greater immunogenic stimulation may be required to activate peripheral blood neutrophil oxidative burst and whole blood TNF-α secretion than L-selectin protein. This makes teleological sense, that the activation of neutrophil L-selectin would be peripheral while stimulation of neutrophil phagocytosis and oxidative burst would be influenced by the local environment of the infected tissue. In addition, it would be catastrophic for local immunogenic stimulation to result in systemic activation of inflammatory cells such as macrophages. In support of this notion, although Obeidat et al. (unpublished) observed elevated neutrophil L-selectin protein concentrations through the entire pre-weaning period, the more active neutrophil oxidative burst was only evident through d 21. Non-nutritive suckling of calves fed restricted quantities of milk will reduce as calf starter intake increases. Therefore, microbial exposure should decrease in calves fed restricted quantities of milk as the calf approaches weaning, which may explain why neutrophil L-selectin protein concentrations remained greater immediately prior to weaning in Holstein calves fed lower plane of nutrition while the difference in neutrophil oxidative burst disappeared after d 21 (Obeidat et al., unpublished). In addition, Jersey calves fed the lower plane of nutrition in the present study were fed greater quantities of milk replacer per kg of BW when compared to the Holstein calves fed the lower plane of nutrition in

the study by Obeidat et al. (unpublished). Therefore, it is conceivable that the LP Jersey calves were likely more satiated by the lower plane of milk nutrition than Holstein calves in Obeidat et al. (unpublished). Another potential explanation is that Jersey calves often display more oral behaviors than Holstein calves; therefore, it is presumable that Jersey calves, regardless of plane of nutrition, would be exposed to a higher microbial immunogenic stimulation, thus homogenizing any potential effect due to plane of nutrition on neutrophil responses. Lastly, in agreement that local immunogenic stimulation does not stimulate peripheral blood secretion of TNF-α when co-cultured with lipopolysaccharide both Obeidat et al. (unpublished) and Cobb et al. (unpublished) reported activated neutrophil responses of pre-weaned Holstein calves fed a low plane of nutrition and group housed, respectively without any differences in the secretion of TNF-α when stimulated with lipopolysaccharide.

In addition to increased immunogenic stimulation among calves fed a lower plane of nutrition, Obeidat et al. (unpublished) offered another possible explanation, suggesting that feeding higher planes of nutrition during the pre-weaning period may directly suppress neutrophil responsiveness. In support of this hypothesis, Graugnard et al. (2012) reported that neutrophil phagocytosis was reduced in dry cows that were fed higher energy diets when compared to calves fed lower energy diets. Saiepour et al. (2006) reported that neutrophil phagocytosis was decreased when physiological concentrations of insulin were applied to neutrophils *ex vivo*. Indirectly supporting this hypothesis is that anorexia is a commonly observed during disease among vertebrates. Force-feeding mice that were infected with *Listeria monocytogenes*, similar to concentrations in healthy

mice, increased mortality when compared to mice that were offered feed ad libitum, but not force-fed (Murray and Murray, 1979). Further, there was a positive relationship between weight loss and survival of the mice that were not force-fed. In addition, mice that are acutely starved and infected with *L. monocytogenes* had improved survival rates compared to mice offered food ad libitum (Wing and Young, 1980). Neither serum nor lymphocytes from the starved mice conferred protective immunity to *L. monocytogenes* in non-starved mice; therefore the resistance of starved mice is most likely associated with innate immune responses. Although the underlying mechanism is unknown, this data taken with that of Obeidat et al. (unpublished) indicate that neutrophil responses are reduced in calves fed higher planes of nutrition during the pre-weaning period. Future research should determine the health implications of this altered immune response phenotype during the pre-weaning period.

The greater neutrophil L-selectin protein concentrations observed among the LP calves during the pre-weaning period was not observed during the immediate post-weaning period, at d 77. In fact, no differences in any of the innate immune responses were observed at d 77, which contrasts with data reported by Ballou (*J. Dairy Sci., In Press*), where neutrophil oxidative burst and whole blood bactericidal capacity of Jersey calves previously fed a lower plane of nutrition were reduced during the immediate post-weaning period relative to Jersey calves previously fed a higher plane of nutrition. These differences in innate immune responses reported by Ballou (*J. Dairy Sci. In Press*) occurred when both the intakes of metabolizable energy and CP per kg of metabolic BW were not different between treatments; therefore, neither the supply of metabolizable

energy nor CP could explain the observed response. In fact, in the present study the ME and CP per metabolic BW were actually lower during the immediate post-weaning period among HP Jersey's. The data reported by Obeidat et al. (unpublished), also indicated that previous plane of nutrition during the pre-weaning period did not have any carryover effect during the immediate post-weaning period. Furthermore, Obeidat et al. (unpublished) also observed that both the intake of crude protein and metabolizable energy when expressed per kg of metabolic BW were lower during the immediate post-weaning period among calves that were previously fed a higher plane of nutrition during the pre-weaning period.

As mentioned in the previous paragraph, Ballou (*J. Dairy Sci., In Press*) reported that Jersey calves that were previously fed a higher plane of nutrition during the pre-weaning period had improved neutrophil oxidative burst and whole blood bactericidal capacities during the immediate post-weaning period. Experiment 2 was designed to test the hypothesis that Jersey calves fed a higher plane of nutrition would have improved resistance to an oral *Salmonella typhimurium* challenge during the immediate post-weaning period. If an infection evades the physical barriers of the immune system and cannot be controlled by humoral factors an ideal cellular innate immune response is a rapid increase in the activity of phagocytes in order to clear the infection, followed by a rapid return to homeostasis. A delay in the response of phagocytes increased the risk and severity of disease (Heyneman et al., 1990). The infection model used in the present study was a mild challenge with an expected clinical disease $dose_{50}$ and was chosen because the objective was to test the hypothesis that feeding a higher plane of nutrition

would improve disease resistance and was not designed to evaluate the response to disease. Data from the current study indicate that calves that were fed a higher plane of nutrition had a more rapid increase in many cellular innate immune responses, including the secretion of TNF-α when whole blood was stimulated with LPS as well as neutrophil oxidative burst to an *E. coli*. It cannot be completely ruled out that the more rapid response among the HP calves was due to greater immunogenic stimulation caused by an impaired physical barrier of the gastrointestinal mucosa or humoral factors among those calves. However, the attenuated plasma concentrations of the acute phase protein, haptoglobin, and the numerical decrease in the frequency of calves classified as having clinical signs of disease among the HP calves suggests that HP calves actually had an improved innate immune response to the oral *Salmonella typhimurium* challenge. Additional research is needed to confirm that Jersey calves fed a higher plane of nutrition have a more rapid up regulation in cellular innate immune responses and improves the resistance to enteric disease during the immediate post-weaning period.

2.8 Conclusion

Implementing milk replacer feeding programs at higher planes of nutrition in neonatal Jersey calf nutrition enhances their ability to resist disease during the immediate post-weaning period. High Plane calves had a larger ADG over the duration of the study, furthermore the excess milk replacer offered during the pre-weaning period to HP calves allowed for greater efficiency. Glucose levels were higher in HP calves in both studies

when compared to LP, this is related to the increased milk consumption during the pre-challenge period and the increased starter intake during the challenge period. Considering the decreased intensity of L-selectin positive neutrophil expression in Low Plane calves and the lower levels of neutrophils positive for oxidative burst in Low Plane calves shown by Obeidat et al. (unpublished) it appears that feeding the higher plane of nutrition during the pre-weaning period reduces neutrophil responses. More research is needed to understand the underlying mechanisms by which plane of nutrition influences the innate immune responses of pre- and post-weaned calves. Furthermore, the biological significance of the reduced neutrophil responses observed during the pre-weaning period in calves fed the HP with respect to resistance to infectious disease warrants further research.

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APPENDIX

TABLES AND FIGURES

Table 1.0. The formulated nutrient content of the milk replacer and calf starter fed to Jersey calves

	Milk replacer		Calf starter	
Nutrient	LPN[1]	HPN[1]	LPN	HPN
Dry matter, %				
Crude protein(min[2]), %	20.0	28.8	18.0	20.0
Ether extract (min), %	20.0	25.8	2.2	2.2
Crude fiber (max[2]), %	0.15	0.15	12.5	16.0
Acid detergent fiber (max), %	-	-	14.5	18.0
Calcium (min), %	0.75	0.75	0.80	0.80
Calcium (max), %	1.25	1.25	1.20	1.20
Phosphorus (min), %	0.70	0.70	0.45	0.50
Selenium (min), ppm	-	-	0.30	0.30
Vitamin A (min), IU/kg	44,000	44,000	6,380	7,700
Vitamin D3 (min), IU/kg	11,000	11,000	-	-
Vitamin E (min), IU/kg	220	275	-	-

[1]LPN = low plane of nutrition; HPN = high plane of nutrition.
[2]min = minimum; max = maximum.

Table 2.0. Initial BW, total serum protein concentrations, and the pre- and post-weaning performance and feed efficiencies of Jersey calves fed different planes of milk replacer nutrition.

Item	[1]LPN	[2]HPN	[3]SEM	P-Value
Initial BW, kg	24.2	25.3	0.71	0.2900
Total serum protein, g/dL	5.5	5.6	0.004	0.9241
Pre-weaning				
ADG, kg/d	0.292	0.501	0.019	0.0001
DM intake, kg/d	0.581	0.719	0.019	0.0001
DM intake per metabolic BW, g / kgBW$^{0.75}$	44.813	49.599	1.012	0.0001
ME intake, Mcal/d	2.39	3.542	0.065	0.0001
ME intake per metabolic BW, Mcal / kgBW$^{0.75}$	0.149	0.178	0.002	0.0001
ME efficiency, g ME consumed:kgBWgain	8.54	7.36	0.388	0.0343
CP intake, kg/d	0.118	0.197	0.004	0.0001
CP intake per metabolic BW, g / kgBW$^{0.75}$	9.106	13.606	0.233	0.0001
CP efficiency, g CP consumed:kgBWgain	415.26	410.98	19.52	0.8747
Post-weaning				
ADG, kg/d	0.382	0.419	0.028	0.0001
DM intake, kg/d	1.15	1.04	0.057	0.0001
DM intake per metabolic BW, g / kgBW$^{0.75}$	67.376	52.278	2.374	0.0001
ME intake, Mcal/d	3.045	3.126	0.127	0.0001
ME intake per metabolic BW, Mcal / kgBW$^{0.75}$	0.179	0.158	0.005	0.0001
ME efficiency, g ME consumed:kgBWgain	8.42	7.6	0.258	0.0269
CP intake, kg/d	0.242	0.237	0.011	0.0001
CP intake per metabolic BW, g / kgBW$^{0.75}$	14.183	11.961	0.489	0.0001
CP efficiency, g CP consumed:kgBWgain	543	487.89	15.327	0.0129

Starter intake is shown as the average over the specific time period. Energy efficiency is reported as Mcal:kgBWgain and Protein efficiency is reported as g CP:kgBWgain.

[1]LPN = Low Plane of Nutrition; 409 g/DM per day, weeks 1-6

[2]HPN = High Plane of Nutrition; 610 and 735 g/ DM per day, weeks 1 and 2-6, respectively

[3]SEM = Largest Standard Error of the Mean for both treatments

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Table 3.0. Intake efficiencies in calves challenged orally with *Salmonella typhimurium*
(Exp. 2)

Item	[1]LPN	[2]HPN	SEM	P-Value
CP intake, kg/d	360.7	437.8	8.67	<.0001
ME intake, Mcal/d	5.11	5.82	0.119	<.0001
ME efficiency, ME consumed/kgBW$^{0.75}$	0.261	0.257	0.006	0.658
CP efficiency, CP consumed/kgBW$^{0.75}$	18.4	19.33	0.443	0.119

[1]LPN = Low Plane of Nutrition
[2]HPN = High Plane of Nutrition

Figure 1

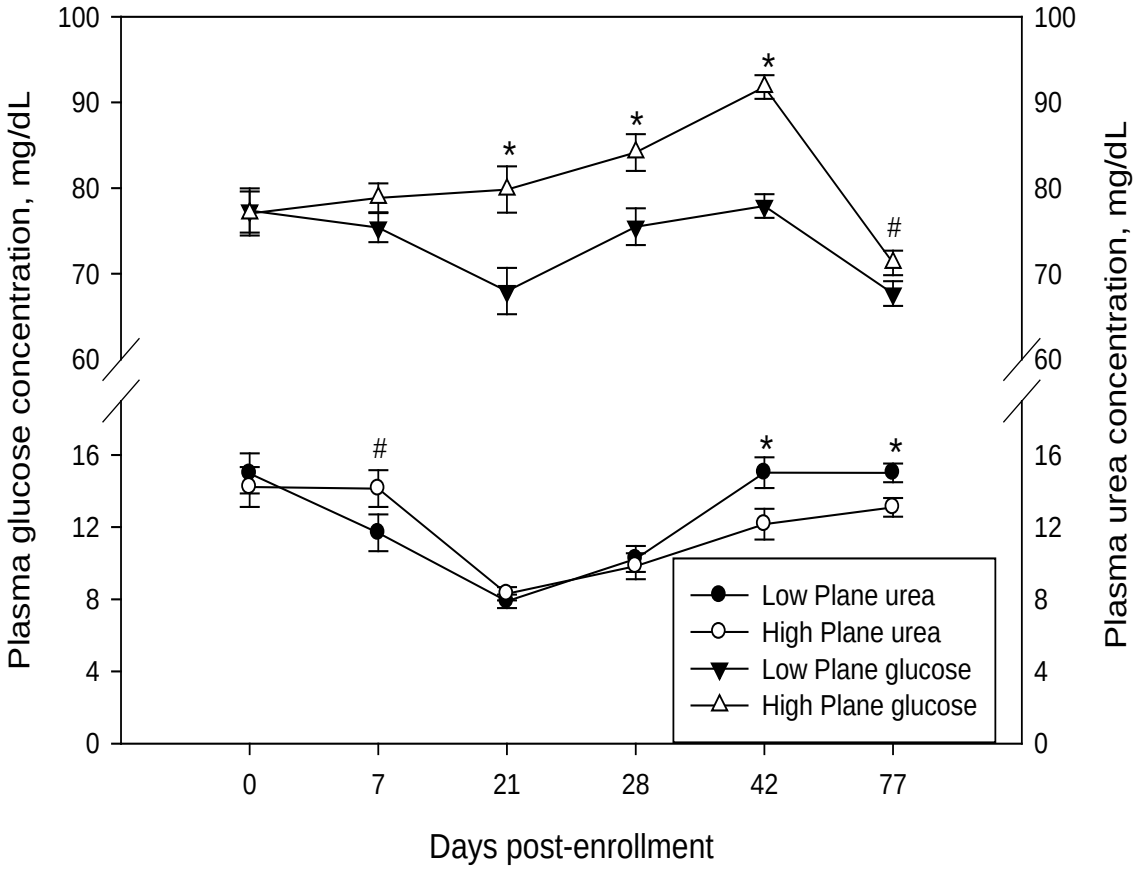

Figure 1. Plasma concentrations of glucose and urea nitrogen in calves provided milk and a starter ration at either a low or high Plane of nutrition. There was a treatment x time interaction ($P<0.05$) on plasma glucose and urea nitrogen levels during pre- and post-weaned period. Dietary treatments were Low Plane calves consuming 409 g/DM per day and High Plane calves consuming 610 and 735 g/DM per day for weeks 1 and 2-6, respectively. Sliced time effects reported as * $P<0.05$; # $P<0.10$. Data are reported as LSmean ± SEM.

Figure 2a

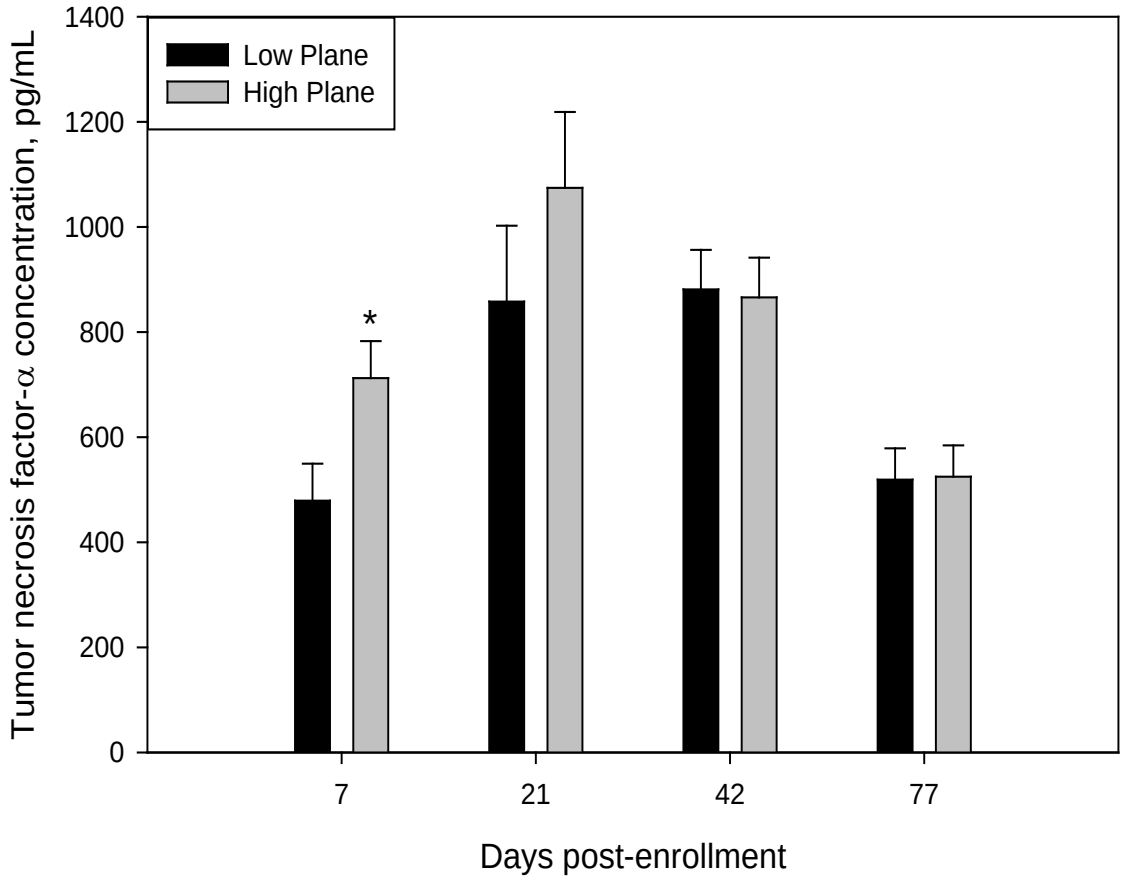

Figure 2a. Tumor necrosis factor-α concentrations in calves provided milk and a starter ration at either a low or high Plane of nutrition. There was a time effect (P<0.05) on tumor necrosis factor-α concentrations. Dietary treatments were Low Plane calves consuming 409 g/DM per day and High Plane calves consuming 610 and 735 g/DM per day for weeks 1 and 2-6, respectively. Sliced time effects reported as * P<0.05. Data are reported as LSmean ± SEM.

Figure 2b

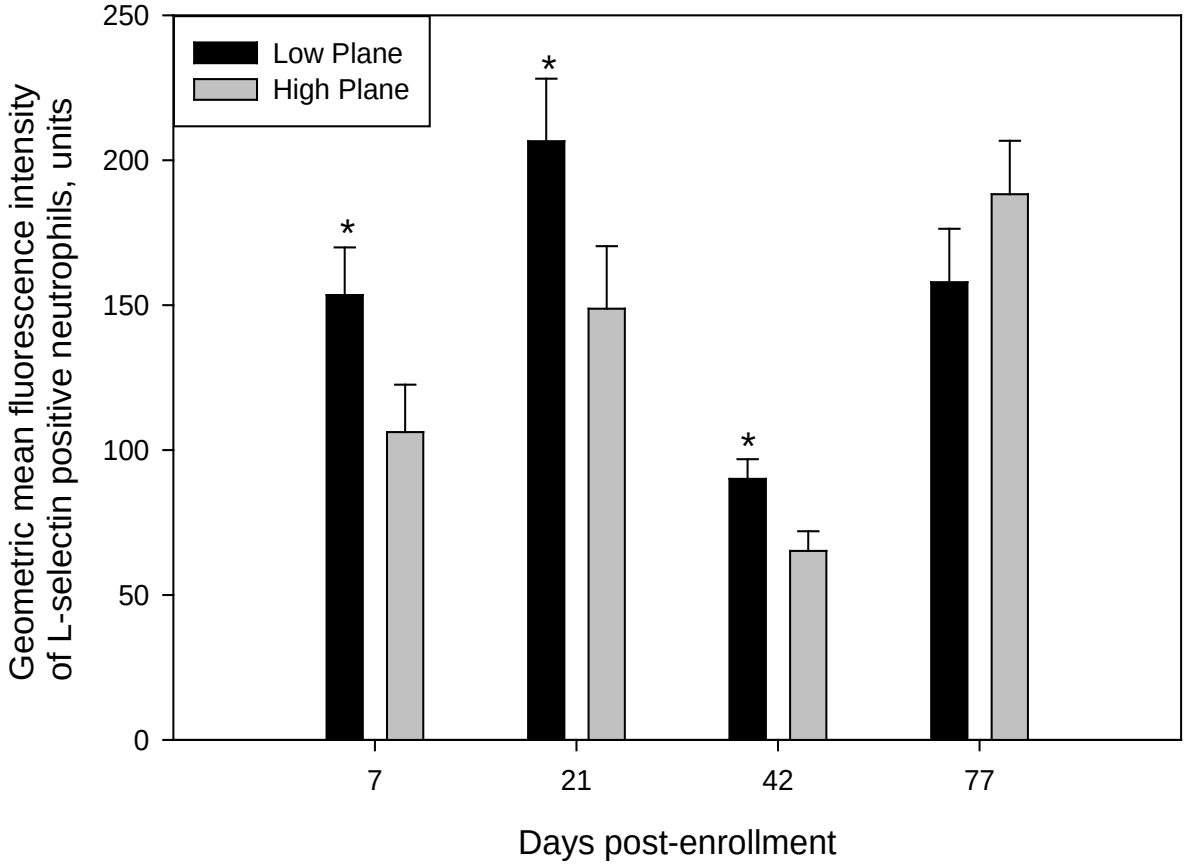

Figure 2b. Geometric mean fluorescence intensity of L-selectin positive neutrophils in calves provided milk and a starter ration at either a low or high Plane of nutrition. There was a time effect ($P<0.05$) on L-selectin positive neutrophils. Dietary treatments were Low Plane calves consuming 409 g/DM per day and High Plane calves consuming 610 and 735 g/DM per day for weeks 1 and 2-6, respectively. Sliced time effects reported as * $P<0.05$. Data are reported as LSmean ± SEM.

Figure 3

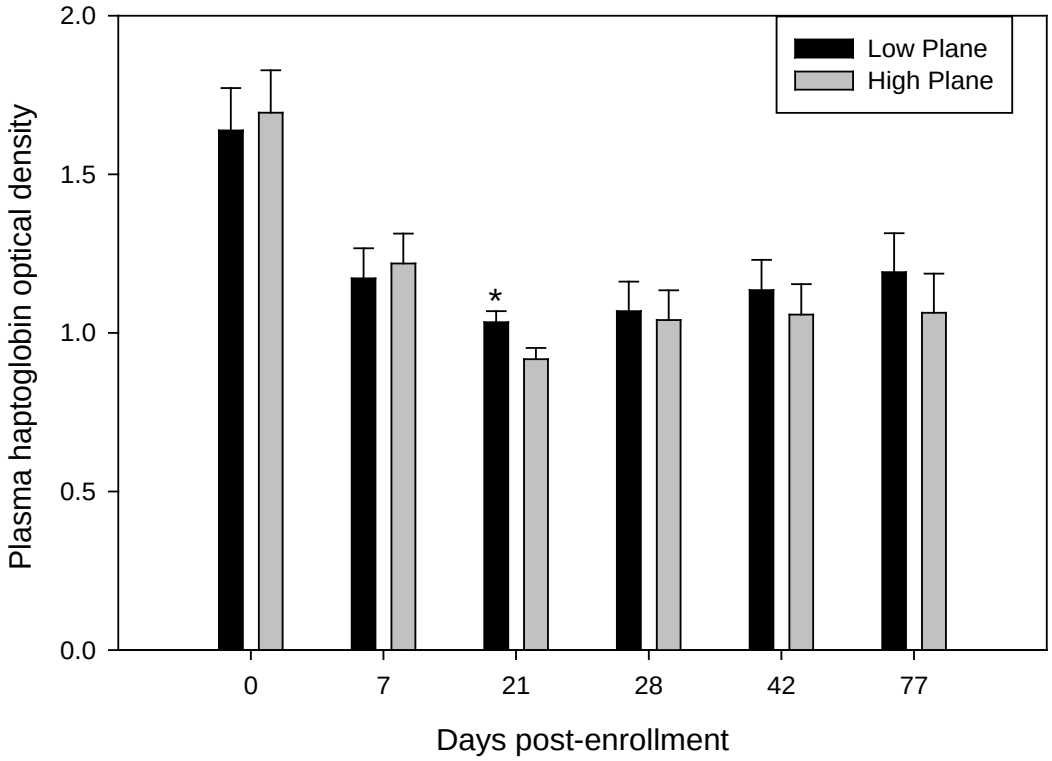

Figure 3. Plasma haptoglobin optical density in calves provided milk and a starter ration at either a low or high Plane of nutrition. There was a time effect (P<0.05) on plasma haptoglobin concentrations. Dietary treatments were Low Plane calves consuming 409 g/DM per day and High Plane calves consuming 610 and 735 g/DM per day for weeks 1 and 2-6, respectively. Log-transformed sliced time effects reported as * P<0.05. Data are reported as LSmean ± SEM.

Figure 4

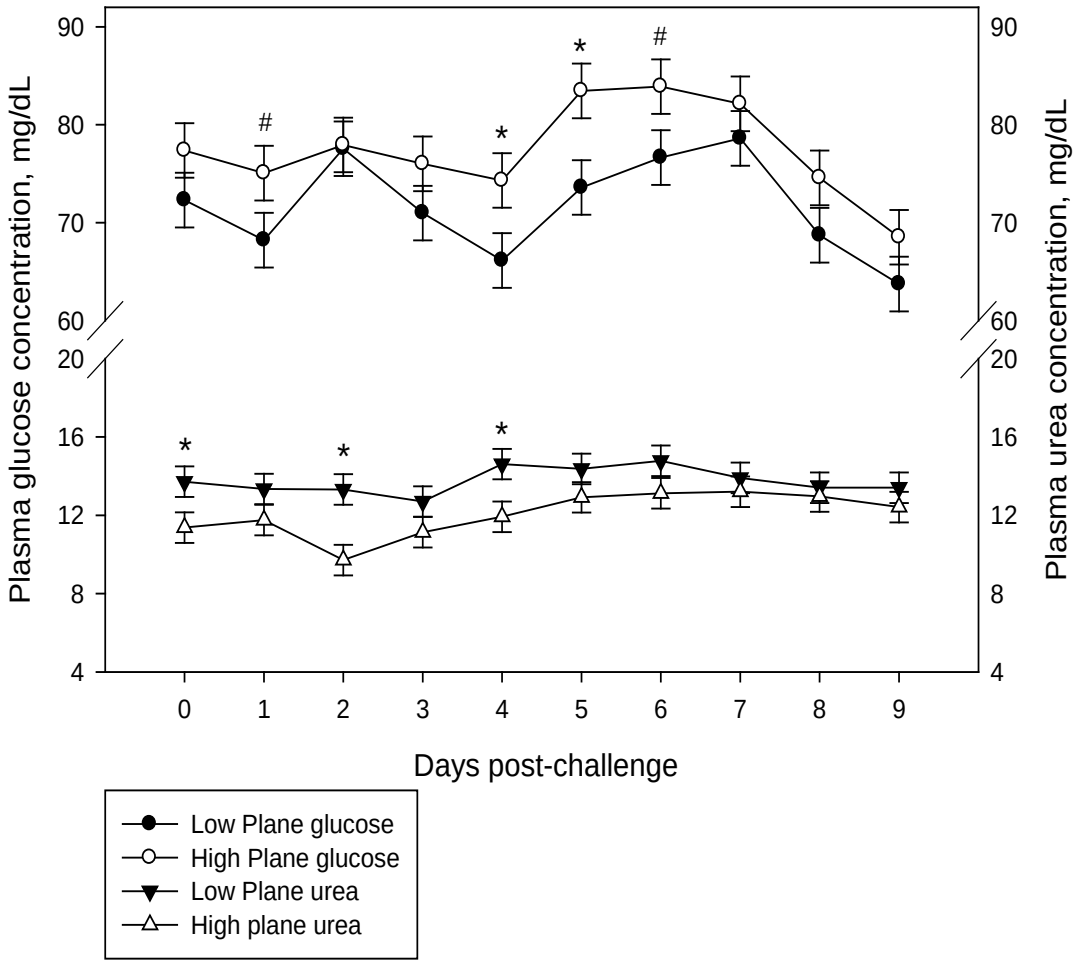

Figure 4. Plasma concentration of glucose and urea nitrogen in calves provided a starter ration of either a low or high Plane of nutrition. There was a treatment and time effect (P<0.05) on plasma glucose and a treatment effect on urea nitrogen levels. Low Plane calves were provided a starter ration consisting of 18/2.2/12.5% crude protein, fat, and fiber and High Plane calves were fed a starter ration consisting of 20/2.2/16% crude protein, fat, and fiber. Sliced time effects reported as * P<0.05; # P<0.10. Data are reported as LSmean ± SEM.

Figure 5a

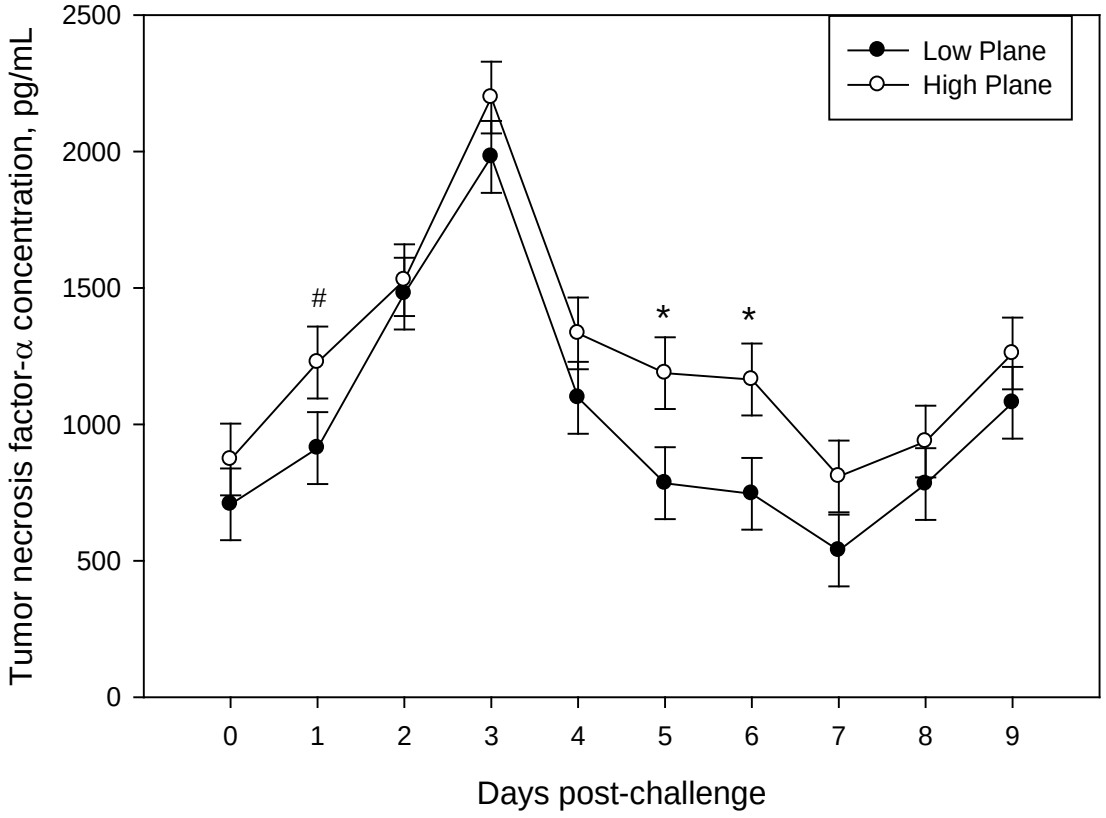

Figure 5a. Tumor necrosis factor-α concentrations in calves provided a starter ration of either a low or high Plane of nutrition There was a treatment and time effect (P<0.05) for tumor necrosis factor-α concentrations. Low Plane calves were provided a starter ration consisting of 18/2.2/12.5% crude protein, fat, and fiber and High Plane calves were fed a starter ration consisting of 20/2.2/16% crude protein, fat, and fiber. Sliced time effects reported as * P<0.05; # P<0.10. Data are reported as LSmean ± SEM.

Figure 5b

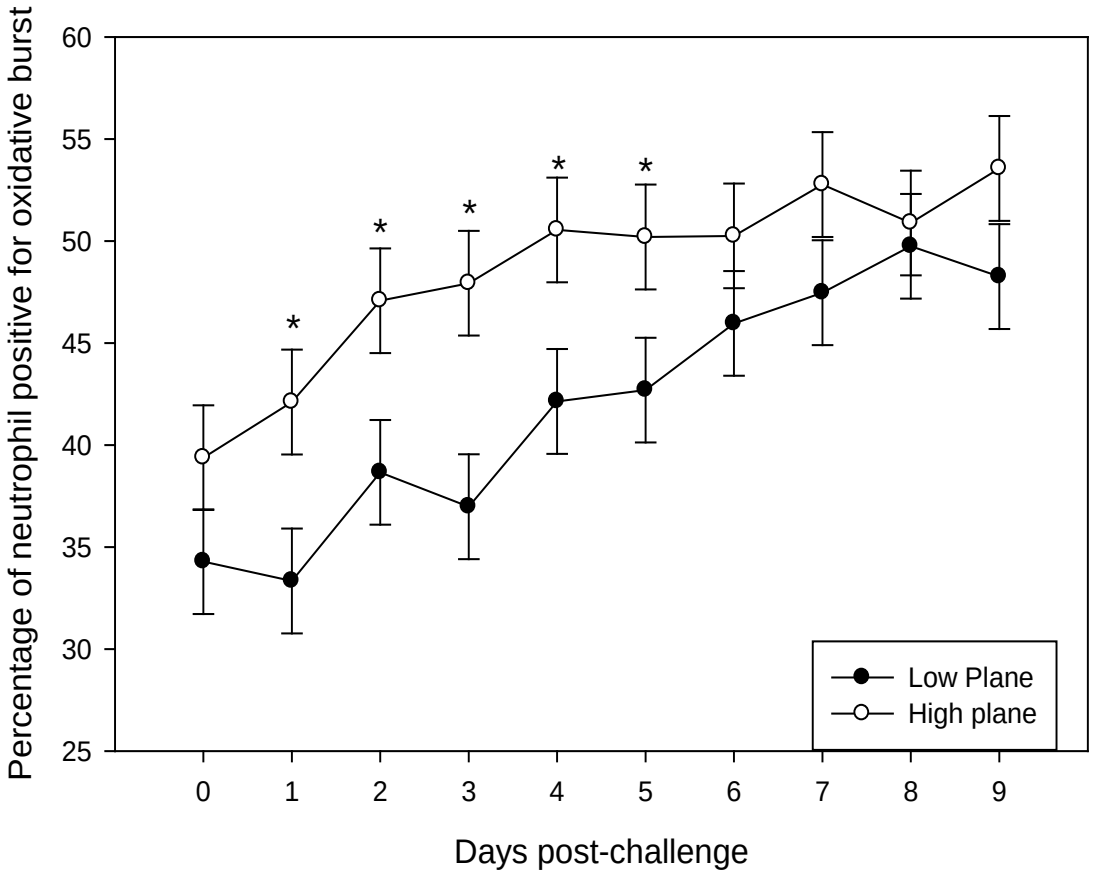

Figure 5b. Percentage of neutrophils positive for oxidative burst in calves provided a starter ration of either a low or high Plane of nutrition. There was a treatment and time effect ($P<0.05$) on percentage of neutrophils positive for oxidative burst. Low Plane calves were provided a starter ration consisting of 18/2.2/12.5% crude protein, fat, and fiber and High Plane calves were fed a starter ration consisting of 20/2.2/16% crude protein, fat, and fiber. Sliced time effects reported as * $P<0.05$. Data are reported as LSmean ± SEM.

Figure 5c

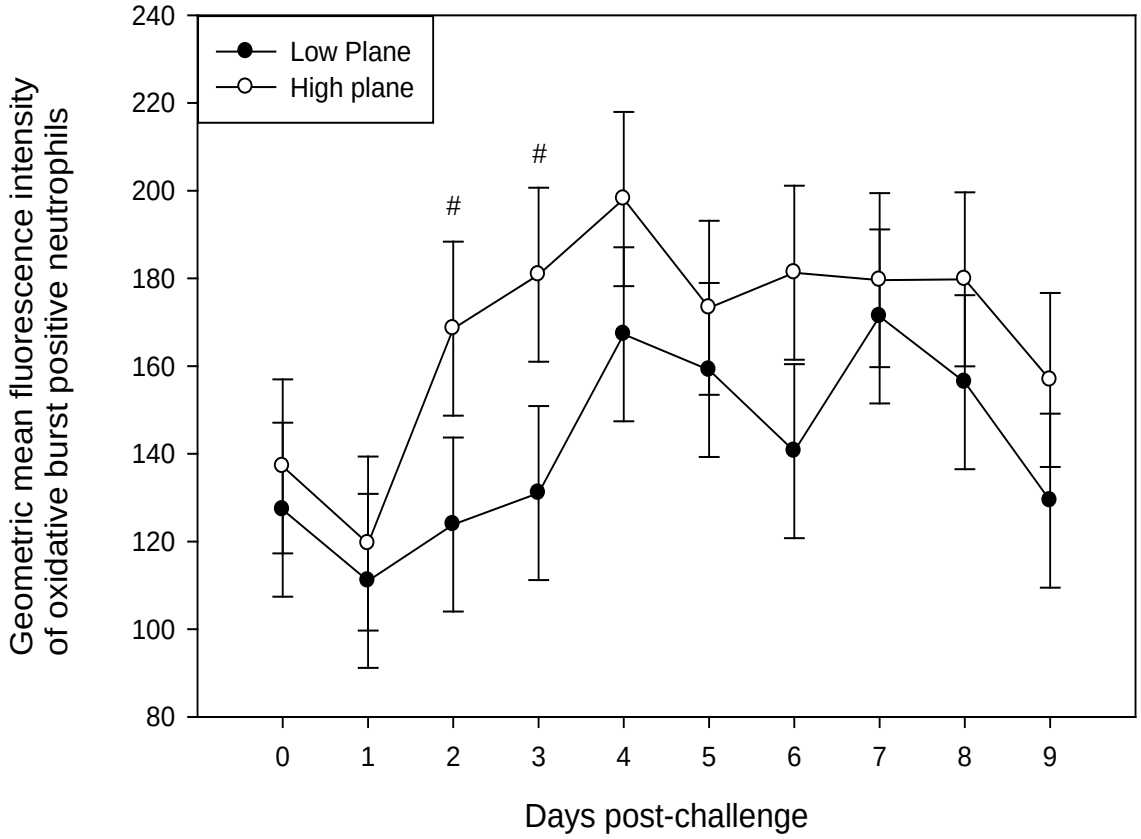

Figure 5c. Geometric mean fluorescence intensity of oxidative burst positive neutrophils in calves provided a starter ration of either a low or high Plane of nutrition. There was a time effect ($P<0.05$) on geometric mean fluorescence intensity of oxidative burst intensity. Low Plane calves were provided a starter ration consisting of 18/2.2/12.5% crude protein, fat, and fiber and High Plane calves were fed a starter ration consisting of 20/2.2/16% crude protein, fat, and fiber. Sliced time effects reported as # $P<0.10$. Data are reported as LSmean ± SEM.

Figure 6

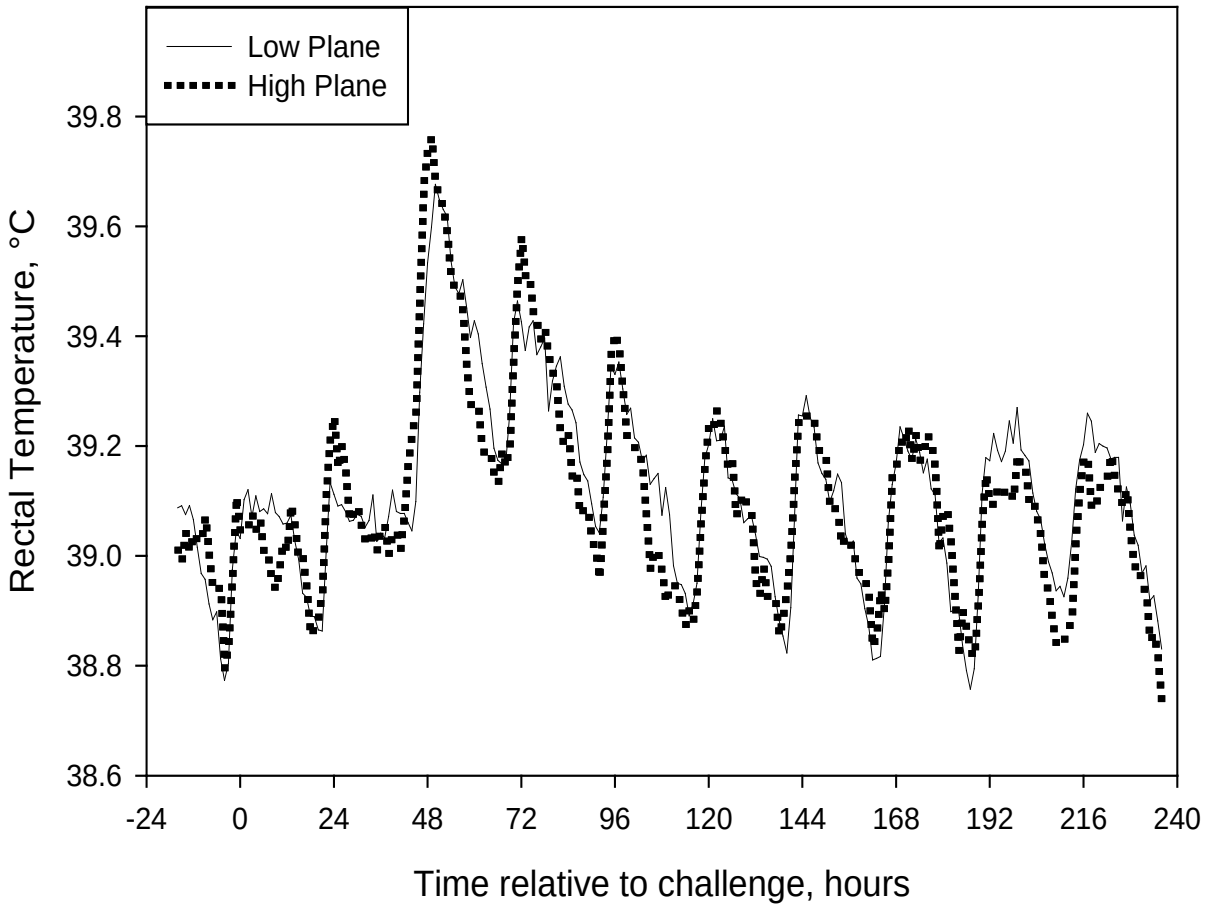

Figure 6. Rectal temperatures in calves provided a starter ration of either a low or high Plane of nutrition. There was a time effect ($P<0.05$) on rectal temperature data. Low Plane calves were provided a starter ration consisting of 18/2.2/12.5% crude protein, fat, and fiber and High Plane calves were fed a starter ration consisting of 20/2.2/16% crude protein, fat, and fiber.. Data are reported as LSmean ± SEM.

Figure 7

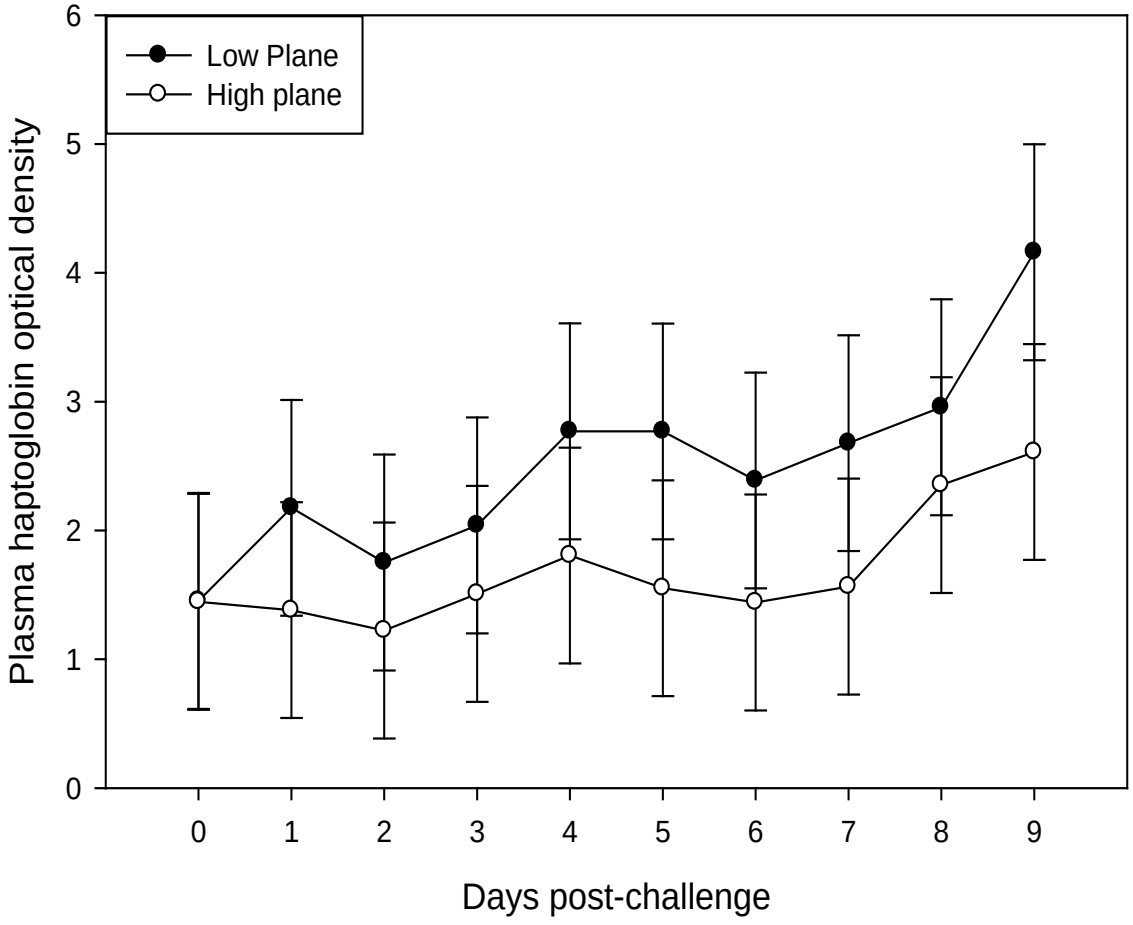

Figure 6. Plasma haptoglobin optical density in calves provided a starter ration of either a low or high Plane of nutrition. There was a time tended to effect (P<0.10) on plasma haptoglobin concentrations. Low Plan calves were provided a starter ration consisting of 18/2.2/12.5% crude protein, fat, and fiber and High Plane calves were fed a starter ration consisting of 20/2.2/16% crude protein, fat, and fiber. e Data are reported as LSmean ± SEM.