

COBALT SUPPLEMENTATION AFFECTS HUMORAL IMMUNE RESPONSE IN
BEEF CALVES

By

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DEDICATION

My Doctor of Philosophy degree and my graduate education is dedicated to my father, H.B. Sager, who guided me and taught me as a young student to appreciate beef cattle, the skills required for beef cattle production, and the rewards of success in using these skills. My appreciation of beef cattle developed as a young boy while growing up on the family ranch near Manhattan, Montana. Experience in ranching as a young student further developed my appreciation of beef cattle and beef cattle production. With the adaptation of skills required in beef cattle ranching, I acquired a passion for beef cattle ranching and improved beef cattle production which resulted in my life-long career as a beef cattleman. As a college student I developed a goal to become a veterinarian which resulted in a forty year career as a beef cattle practitioner and now as a beef cattle consultant and the Miratrog Senior Veterinarian with Miratrog Holdings, the largest vertical intergraded beef cattle operation in the world, near Bryansk, Russia. My appreciation and learning continues each day in achieving optimum beef cattle production.

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ABSTRACT

Economic losses from morbidity and mortality associated with bovine respiratory disease (BRD) in beef cattle are approaching \$2 billion annually in the United States. Incidence and severity of BRD is increasing despite advances in animal health programs in prevention and treatment compared to twenty years ago. Mineral supplementation during pre-weaning has potential to reduce sickness and improve health. Cobalt (Co) is used by rumen-inhabiting microbes for the production of vitamin B₁₂. Vitamin B₁₂ is a cofactor for vital metabolic pathways in tissue carbohydrate and lipid metabolism required for maintenance and growth. Vitamin B₁₂ is also vital for B-cell proliferation to form plasma cells that secrete antibodies. National Research Council (NRC) recommendations for Co are 0.1 ppm (0.1 mg/kg; DM dry matter basis). Beef production has changed tremendously since NRC recommendations were set in the 1950's. The hypothesis of these three studies is NRC Co concentrations need to be increased to meet today's beef cattle metabolic requirements and production needs. The objectives of these studies were to evaluate if an orally-supplied Co dosed at nursing, pre-weaning, or post weaning affects humoral immune response during the post-weaning feeding period and reduces the incidence of BRD. *Mannheimia haemolytica* is a major pathogen of BRD which causes increased pathophysiological pulmonary tissue severity, increased treatment time, and increased mortality in beef calves. Calves were vaccinated with *M. haemolytica* in all studies as an indicator of immune response. Different dosages and forms of Co were administered to evaluate humoral immune response. Results indicate increased NRC Co concentrations affect humoral immune response and potentially improve beef calf health. Study results suggest current NRC Co concentrations should be increased to improve post-weaning health in beef calves.

Key words: bovine respiratory disease, humoral immune response, *Mannheimia haemolytica* leukotoxin

CHAPTER ONE

INTRODUCTION

In the past 50 years, United States beef cattle production has increased nearly 50% due to improved genetics, advances in nutrition, bio-technology, advances in animal health, and value added management (Paterson, 2010). The National Research Council (NRC) requirements for Co is 0.1 mg/kg (DM intake) and were first published in the 1950's when production requirements for beef cattle production were two-thirds of present day production expectations (NRC, 2005). Recommended NRC Co levels were derived from experiments during the 1950's using cattle that were genetically different, raised with a different production focus, and fed different diets. Today beef cattle production involves animals that are 35 to 40 % larger anatomically, grow at increased rates, are developed with more focus on muscle growth with efficient gain than 50 years ago. Beef cattle are grown in larger feeding facilities with increased population densities and increased microbial environments that potentially magnify pathogen densities and increased risk for bovine respiratory disease (BRD). Modern beef cattle feedlots do increase efficiency of production but inadvertently cause increases in BRD. Since these factors have all changed, possible increased requirements in Co could be required in beef cattle production.

The hypothesis was increased NRC Co concentrations affects humoral immune response and improves beef calf health. Three studies were conducted to test this hypothesis.

CHAPTER TWO

LITERATURE REVIEW

Beef Cattle Production and Bovine Respiratory Disease

Feeder cattle occur many physiological and psychological stresses during weaning, transportation, and change of environment into the feedlot (Grandin, 1997). Transportation and handling is generally regarded as stressful to cattle and includes both physical and psychological stimuli that might be aversive (Swanson and Morrow-Tesch, 2014). The majority of beef cattle raised in the United States undergo transportation at least once in their lifetime. Trucks are the primary form of transport for beef cattle in the United States. Recent data indicate at least 934,000 loads of cattle are transported annually in the US (Tim O'Byrne of Calico Beef Consulting).

Any novel or rare experience for an animal can be a source of stress causing physiological and psychological changes in the animal. Physiological changes include activation of the hypothalamo-pituitary-adrenal axis resulting in increases in cortisol concentrations, immune suppression, and increases in heart and respiration rate. Key factors influencing level of transport stress are critical in initiating stress; including pre-transport management, noise, vibration, novelty, social regrouping, crowding, temperature, humidity, restraint, feed and water deprivation, and time of transit. Fear is also an important psychological stressor during handling and transport of cattle. Previous experience with handling and transport as well as genetic composition determines level of fear response of the animal (Grandin, 1997).

Preconditioning was not found to reduce weight loss following transport in several studies (Cole, 1985; Pritchard and Mendez, 1990). Stresses of deprivation in feed and water, inclement weather, antagonistic encounters, increased pathogen environment, and transportation from one production unit to another are often additive and critical to the immune system. Stress during transport and marketing can compromise the immune system and predisposes cattle to develop certain infectious diseases, including the bovine respiratory disease complex (Dubeski et al., 1996). Humoral immune response is decreased by stresses of transportation and remains suppressed for several weeks (Mckenzie et al., 1997). Abrupt weaning does increase plasma cortisol and noradrenaline concentrations that decreases interferon- γ production up to 7 days after weaning (Hickley et al., 2008). Abrupt weaning with transportation has additive effects on humoral immune response (Mckenzie et al., 1997; Pollard et al., 1997). Stressors associated with transportation affect the acute-phase protein response in newly weaned beef calves (Arthington et al., 2003). Changes in environment, complicated stress factors, and cattle handling management often lead to calves not eating. In general, the calf's immune system responds to transport stress by increasing the number of total white blood cells (WBC) and specific types of WBC (neutrophils, eosinophils, and mononuclear cells) in circulation (Kent and Ewbank, 1986a,b; Murata et al., 1987). Lymphocyte numbers are decreased (Kent and Ewbank, 1986a; Murata and Hirose, 1991), which, along with increasing numbers of neutrophils, increases a particular measure of stress, the neutrophil:lymphocyte ratio (N:L). Kent and Ewbank (1986b) found, in very young calves (1 to 3 week old), that changes in WBC numbers occurred after approximately a 6-

hour transport. In calves transported for 18 hours, WBC were back to baseline levels near the end of the trip, although observation of increases in neutrophils and decreases in lymphocytes were reported.

A widely accepted stressor of beef cattle is commingling.. When calves from various sources are commingled in feedlot pens, the social hierarchy is destroyed, and additional stress is imposed (Loerch and Fluharty, 2000). Calves with “trainer” cows in their pens had improved dry matter intake (DMI) during the first few days after arrival, and in some cases had improved gains and a decreased incidence of BRD (Loerch and Fluharty, 2000). In contrast, Gibb et al. (2000) reported that trainer cows did not improve calf performance, feeding behavior, or health, with calves actually avoiding cows at the bunk during the first few days in the pen. Perhaps surprisingly, Arthington et al. (2003a) reported a tendency for commingled calves to consume more daily DM over a 21-d period than noncommingled calves. Although the background of the commingled calves was unknown, previous exposure to feed bunks was offered as a possible explanation for the increased DMI.

It has been reported as much as 67% percent of received calves are not on full feed for 3e days after arrival (Smith, 2005, Sager, 2012). Nutritional deprivation drastically affects immune response predisposing to immunosuppression. All of these stresses promote and induce hormonal changes, fever, altered glucose metabolism and other nutritional metabolism, dehydration, anorexia, exhaustion, behavioral changes, and immunosuppression. Estimates of 10 to 13% increase in body metabolism for every degree increase in body temperature are associated with an immune response (Hermson,

2013). Addition energy expenditure associated with the increase in body temperature is an energy cost associated with antibody production and synthesis of liver proteins. Redirecting energy resources to support an immune response means that energy is not available for production. This is complicated on new arrival calves by nutrient deprivation of being off feed. The most critical factor is time in transit. Table 2.1 estimates shrink with respect to time.

Table 2.1. Relationship of % shrink to hours of transport in a truck (Fox et al., 1985)

Hours in truck	Percent shrink	Days to recover weight
1	2	0
2-8	2-4	4-8
8-16	5-6	8-16
16-24	8-10	16-24
24-32	10+	24-30

Behavioral observations may have value in diagnosing BRD (Sowell et al., 1999). Using a feeding behavior system with radio frequency technology (GrowSafe Systems Inc., Calgary, Alberta, Canada), Sowell et al. (1999) suggested that the daily number of feeding bouts was a better predictor for steers that subsequently were identified as morbid than was watering behavior. Results showed a 30% decrease in time at the feed bunk for morbid vs. healthy steers, and differences in feed intake seemed most pronounced during the first 4 days after arrival (Sowell et al., 1999; Buhman et al., 2000). Sick calves had a greater frequency and duration of drinking 4 to 5 days after arrival than animals that were

not sick and further suggested that drinking behavior 4 to 5 d after arrival may be an indicator of BRD (Buhman et al., 2000).

Micro-minerals affect immunity and health primarily through their function in important enzyme activity associated with energy metabolism, cellular protein-dependent synthesis, and DNA replication. During inflammation and infection there exists increased binding capacity in plasma indicating an increased cellular demand for vitamin B₁₂. This increased binding process has been proved in vitro by greatly increased cobalamin uptake by lymphocytes stimulated during inflammation (Quadros et al., 1976) and decreased neutrophil phagocytosis in Co deficient cattle (Mac Pherson et al., 1987). Stress or “moderate” injury may increase requirements of vitamin B₁₂ to double or quadruple (Dubeski et al., 1996). Plasma B₁₂ concentrations increase with food deprivation and during infection, probably due to release of vitamin B₁₂ from liver reserves. The liver contains high concentrations of vitamin B₁₂ and liver tissue is catabolized during food or water deprivation. A mild respiratory (bovine herpes virus – 1 [BHV-1]) infection in steer calves markedly decreases plasma concentrations of vitamin B₁₂ (Dubeski et al., 1996).

Restricting feed intake and depriving food increased concentrations of plasma B₁₂, because of depleted water-soluble vitamins when stress or immune challenge increase physiological requirements often during shipping and marketing processes (Dubeski et al., 1996). Depletion of B vitamins during shipping and marketing may contribute to the enhanced susceptibility of cattle to infectious disease during the first few weeks after arrival.

Stresses lead to increased BRD and reduced performance. These require special management adaptations in health, nutrition, and treatment which are costly and often late in adaptation. Recent interest has grown into pre-weaning nutritional supplement to reduce stress induced immunosuppression and increase health at entry into the feedlot (Smith, 2005; Sager, 2012). Inadequate nutrition can increase immunological suppression and promote infection to pathogens of BRD. Specific micro-minerals have been studied to reduce immunological suppression and increase health, thus decrease stress induced effects and enhance recovery from stresses (Cole, 1996).

Bovine respiratory disease (BRD) is the most common disease among feedlot cattle in the United States. It accounts for approximately 75% of feedlot morbidity and 50 to 70 % of all feedlot deaths (Edwards, 1996; Galvayan et al., 1999; Loneragan et al., 2001). The majority of deaths due to BRD occur shortly after arrival to the feedlot or within the first 45 days (Edwards, 1996; Loneragan et al., 2001). In fact, Buhman et al. (2000) reported that about 91% of calves diagnosed with BRD were diagnosed within the first 27 days after arrival. Bovine Respiratory Disease causes an estimated \$800 million to \$900 million in economic losses from death, reduced feed efficiency, and treatment costs annually (Chirase and Greene, 2001).

Veterinary medical costs attributable to the treatment of BRD are substantial, and the economic impacts of BRD on carcass merit and meat quality further increase the economic costs. Medicine costs accounted for 21% of the decrease, while 79% was attributable to lower carcass weight (8.4% less) and lower quality grade (24.7% more USDA Standard quality grade carcasses). Bovine respiratory disease alone is estimated to

cost feedlot management over \$500 million annually even with preconditioning of calves and new health programs in place (Taylor et al, 2010). Economic losses due to decreased gain and carcass values can be attributed to BRD (Duff and Galyean, 2007). A Texas A & M Ranch-to-Rail Study found BRD morbidity accounted for 8% higher production costs, not including losses related to decreased performance (Griffin et al., 1995). They found cattle with BRD had a 3% decrease in gain compared with healthy cattle and cost the program \$111.38 per sick animal. Snowden et al (2006) estimated economic losses in a 1,000-head feedlot from BRD infection to be approximately \$13.90 per animal due to lower gains and treatment costs.

The total health costs in beef cattle production in the United States from sickness and loss of production are projected to be greater than \$2.2 billion annually (Jacaub, 1984; Confer, 2005; Radostits, 2007). An estimated 25% of all calf deaths before weaning can be attributed to BRD (USDA NAHMS, 2007) and BRD is the largest single cause of etiological mortality reported in feedlots and accounts for more than 28% of all beef calf mortality problems (Van Eenennaam, 2011).

Nutritional management decisions toward improved health status decreases sickness and increases profitability (Maas, 2005; Smith, 2005). In the event that the immune system is activated, nutrient partitioning is altered, which subsequently decreases growth rate (Klasing and Korver, 1997). The concentrations of trace minerals need to be maintained within narrow limits if the functional integrity of cells and tissues vital to normal immune function of the animal are to remain unimpaired (McDowell, 1989, 1992; Underwood and Suttle, 1999; Engle, 2001). Trace mineral deficiencies in beef cattle have

showed altered immune system function (Blezinger, 2010). Deficiencies of trace minerals have been shown to alter specific components of both the innate and adaptive portions of the immune system and deficiencies require the animal to metabolically compensate for the nutrient deviation. In the process of compensation immune system function can be depressed and thus decreasing animal performance and health. Interactions between the immune system and micro-minerals in beef cattle are extremely complex as micro-minerals are necessary for normal immune response to infection and disease. The differences in micro-mineral metabolism of beef cattle often determine the status of response to infection and disease because of breed, physiological status (pregnancy), feed or water antagonists, environmental factors, and influence of stress (Engle, 2001). Gestation and lactation decrease Co concentration levels in the liver (Kincaid et al., 2003). Also vitamin B₁₂ levels decrease with age in cattle (Kincaid et al., 2003; Tiffany et al., 2003; and Weiss et al., 2005).

Nutritional management efforts to minimize health problems affect production throughout life (Smith, 2005). Demand for higher quality beef products and increased value-based marketing have heightened producer's awareness of health management practices with potential to increase profitability and beef product quality. Feedlot producers able to purchase calves more likely to remain healthy during the feeding period could potentially increase profits through reduced costs and higher revenues.

There is a distinct trend of increasing mortality, evident since 1994, from respiratory disease among cattle (Langeragan, et al., 2001; Smith, 2005, Radostits, 2007, Taylor, et al., 2010). The reason for bovine pneumonia is complex, but no other factor is

as important as the anatomy of the bovine lung. Anatomically the bovine lung has increased compartmentalization and is under constant stress as the area of lung volume per body size or per weight is small compared to other mammals. This in turn creates a physiological stress, because of smaller gaseous exchange capacity. This constant stress predisposes beef calves to pulmonary disease problems (Smith, 2005; Radostits, 2007). Because of the importance of the anatomy of lung tissue, and the complex immune system response to infection, much lung tissue is often destroyed permanently. This inflammatory damage causes permanent tissue loss, decreased alveolar gas exchange, a chronic disease state, and resulting decreased longevity. Advanced treatment therapy and preventative vaccine programs have improved calf health yet the problem is still vast and economically important to the industry. The stress of inclement or stormy weather during the normal calving period is a constant factor inducing calf pneumonia. Weather related stresses require larger energy resources that often cannot be supplied by the cow's milk and result in disease problems. Susceptibility to calf pneumonia is determined by many factors such as genetics, nutrition, and the calf's resistance (passive transfer of immunoglobulin G [IgG₁]) to infectious agents that cause or predispose the calf to the disease syndrome (Smith, 2005; Taylor, 2010). Impairing innate and adaptive resistance of immunity are large factors in the disease syndrome (Smith, 2005; Taylor, 2010). Also, multiple factors which contribute or predispose the calf to the disease process are: often an energy deficient calf, a causative agent (bacterial or viral), and respiratory tissue damage predisposing inflammation and infection to BRD. Therefore, BRD is a complex disease syndrome of cattle, involving viral, bacterial, and stress factors with bacterial,

especially *Mannheimia haemolytica*, being one of the most severe etiology factors. (Smith, 2001; 2005).

In the past few years a significant increase in resistance to antimicrobial treatment has occurred with *Mannheimia haemolytica*. This resistant pattern causes concern among operations in the beef production industry as many antimicrobial treatments that were effective a few years ago are now ineffective due to *Mannheimia haemolytica*'s ability to change and resist antimicrobial treatment. "In 2009, just over 5% of isolates were resistant to 5 or more antimicrobials (pan-resistant). Diagnostic results show more than 35% of the isolates of *Mannheimia haemolytica* were characterized as pan-resistant during 2011" (Lubbers, and Hanzlicek, 2013). *Mannheimia haemolytica* has become more important as a single antigen than in past years and other attempts to prevent *Mannheimia haemolytica* as a factor in BRD should be considered.

With increased BRD and resistance to antimicrobial therapy of *Mannheimia haemolytica* interest has grown to increase management efforts and nutritional supplementation to reduce BRD problems. Nutritional supplementation during pre-weaning has potential to reduce sickness and improve health in beef calf production (Fisher and Mac Pherson, 1990; Engle, 1999; Drake, 2000, Fernandes, 2003, Maas, 2005). Nutritional management efforts to minimize health problems affect production throughout life (Lofgreen, 1988; Engle, 1999; Smith, 2005). Sickness and decreased health have lasting effects on feedlot performance and carcass quality at slaughter (Stovall et al. 2000; Mc Beth et al. 2001, Smith, R.A., 2009).

Predisposing viral factors cause release of cytokines that stimulate CD11a / CD18 expression on leukocytes that enhance leukotoxin binding. This causes a release of leukocyte inflammatory mediators (mediating chemicals, enzymes, and reactive oxygen intermediators) that destroy leukocytes and damage lung tissue. This reaction causes additional inflammatory reaction by the host. Severe inflammatory tissue damage decreases active leukocyte migration to the pathogen and interrupts normal leukocyte phagocytosis which increases the duration of the infection. This additional inflammatory reaction decreases normal lung capacity and predisposes the host to chronic pneumonia. The ability of *Mannheimia haemolytica* to induce this severe virulence and pathophysiology promotes this microbe as the most important microbe in BRD. While viral microbes initiate inflammatory reaction, damage respiratory mucosa, induce febrile and off feed behavior, the bacteria, *Mannheimia haemolytica* is the most important microbe in the BRD complex. The ability of *Mannheimia haemolytica* to destroy leukocytes and initiate a cascade of inflammatory reactions is unique among bovine microbes.

Pre-weaning nutritional supplementation that would reduce BRD sickness and improve bovine health during the feeding period has large implications in improving bovine health and increase productivity. Public awareness against feed additive antimicrobials, increased resistance, and resistant microbial zoonosis all are important factors to consider nutritional management in reducing BRD problems in beef cattle.

Trace minerals are important for immune function in highly stressed, newly received calves where trace mineral levels may be deficient during BRD (Richeson and

Kegley, 2011). Providing essential minerals through the diet can be challenging because feed intake of newly received cattle is typically low (Galvayan et al., 1999). Calves administered a trace mineral injection had reduced BRD compared with control calves (Richeson and Kegley, 2011). Antibiotic treatment cost was greater for control than treated calves with trace mineral injections at arrival to a feedlot. Administration of a trace mineral injection during initial processing of highly stressed, newly received heifers improved average daily gain (ADG), feed efficiency, BRD, and antibiotic treatment cost (Richeson and Kegley, 2011). In another study completed at Oklahoma State University an injectable trace mineral solution (Multimin) increased feed intake after arrival and increased ADG during the feeding period. Reduction in antibiotic treatment cost exceeded the cost of administering either injectable trace mineral solution during processing, which was less than \$1.50 per animal ((Richeson and Kegley, 2011). Consideration should also be made to evaluate the differences in ADG, feed efficiency, and carcass quality with trace mineral treatment compared to non-treated arrivals at a feedlot.

Nutritional supplementation pre-weaning without the need to rely on normal intake after arrival needs to be considered. In recent years preconditioning programs have added value to beef calf producers to establish marketing advantages and increased return profit from management decisions to immunize before weaning and shipment.

Preconditioning programs are designed to reduce stress associated with weaning, enhance the immune systems of calves, and teach calves to eat from a feed bunk and drink from a fountain or trough while remaining at their birth location for a 30- to 45-days post-weaning period. Benefits of preconditioning to stocker and feedlot operators are well

established with less morbidity and mortality, improved post weaning performance, and higher carcass quality. Approximately 50% of calves sold at weaning do not have any preconditioning program in place. Nutritional supplementation at pre-weaning has shown advantages post-weaning (Smith, 2005; Taylor, 2010). Calves from a single source that are retained on the ranch for 45 days after weaning exhibit less morbidity and less health costs during the receiving period at the feedlot than when cattle are commingled or trucked to the feedlot immediately after weaning (Step et al., 2008). Factors affecting immune response to mineral supply from Spears, 2000, are listed below.

Many times inconsistent results are obtained from studies dealing with mineral trials. The reasons include: differences in the nutrient status of the animals; differences in the concentration of the nutrient in the basal diet because of variability of the the mineral in the soil and forages; differences in bioavailability of the mineral; and differences in the physiological and metabolic demands of the animals in the study. Stresses and high performance requirements have higher demand of specific minerals by the animal.

During 2001 and 2002 several vaccine-failure problems in nursing beef calves initiated diagnostic investigation to determine causes of BRD in four month old calves. Confirmation of primary Cu (copper) deficiency causing failure of normal immune response was determined with liver biopsy analysis. Cu deficiency was determined to be a result of antagonistic interactions with iron (Fe), Mo (molybdenum), and SO_4 (sulfates). Geological soil analysis showed Mo: Cu of 8: 1 with Fe in soil and water several times higher than recommended levels. Sulfate levels were 3 times higher than previous analysis due to 6 to 7 years of draught. BRD signs decreased within 1-week after CuSO_4

supplementation in water tanks at a dosage of 300 ppm for 10 days and 150 ppm for another 30 days. Bovine respiratory disease decreased and calves grew at a normal rate until weaning in October. Weaning weights were 97% of normal and BRD during the feeding period was normal compared to past years. Follow up liver biopsy analysis 90 days post treatment resulted in normal liver Cu levels but Co remained abnormally low. This led to determining why Co remained low. A literature search resulted in finding no research with Co affecting immune response in beef cattle.

Minerals in beef cattle production

All animal feeds contain inorganic or mineral elements utilized by animals for maintenance and production of normal metabolism. Mineral elements occur in the tissues and cells of animals in functional proportions, in chemical combinations of different concentrations, dependent on the element and the desired function. Concentrations need to be maintained within a narrow limit if the tissue functional and structural integrity for growth, health, and productivity of the animal is to be unimpaired. Deficiencies of mineral elements are dependent on many different properties causing altered physiological pathways, biochemical lesions, and structural abnormalities. Homeostatic body mechanisms can delay or minimize deficiency signs because of age, sex, and species of the animal (Underwood and Suttle, 2004). Desired prevention of mineral deficiency requires supplementation in the diet in a palatable and non-toxic form of the required mineral of proper amount, proportion, and bioavailable forms.

Recommendations of nutrients in beef cattle production in the United States have been developed by volunteer committees of the Board on Agriculture and Natural Resources of the National Research Council (Galvayan, 2014). Estimates of requirements for beef cattle are founded on understanding of digestive physiology and metabolism that are typically determined by evaluation and approaches based on reviews of the literature and analysis of derived and experimental data sets of nutritional research. “Systems for describing nutrient requirements of animals are intrinsically composed of 2 parts: (1) estimates of animal requirements for nutrients and (2) estimates of the ability of feedstuffs to meet those requirements” (Galvayan, 2014). These derived systems contribute to animal health and well-being and also need to provide a means to predict animal performance and adjust feeding and management practices to achieve economic goals.

Recognizing trace mineral requirements of grazing ruminants is essential when the forage is the primary food source. Areas of the United States have forages that meet energy and protein requirements, but animals fail to gain weight or grow normally because of a nutritional deficiency of trace minerals or toxic levels of a specific trace mineral. Trace mineral deficiencies required by grazing ruminants exceed those found of soil or plant deficiencies found in nature (Kuboto et al., 1985). Large numbers of livestock graze forages that are deficient of specific mineral elements in certain geographical areas of the world (McDowell et al., 1993). As a consequence certain deficiencies occur, which range from acute or severe to marginal. Clinical signs vary from toxicity diseases which show characteristic clinical signs, pathological changes, and

high mortality, to more subtle un-thriftiness or unsatisfactory growth, production, and fertility. These deficiencies attract much importance and interest in livestock production because of confusion of clinical signs similar to starvation, protein deficiency, or parasitic infestations (Underwood and Suttle, 2004). Deficiencies of minerals are increasingly more important now than in the past because of the magnified importance of the economical production focus in animal production. In certain parts of the world limited animal productivity is caused by shortages of energy and protein, parasitic or infectious diseases, or genetic traits. Deficiencies can be amplified by climate changes in rainfall and sunlight influencing forage uptake of soil elements and minerals. As these limitations are corrected, local mineral imbalances and deficiencies are more apparent and more critical toward production goals.

Daily nutritional status of cattle has important implications for productive outputs including growth, lactation, and reproduction. Nutritional status determined simply as adequate feed intake or specifically as mineral status affects multiple immunological functions. Physical barrier maintenance, antibody production, and cellular based immunity are controlled and influenced by nutrition (Hermson, 2013).

Adequate trace mineral, protein, energy, and vitamin status are nutritional variables that are quantitatively affected by cattle producer management. Pasture, forage storage, energy-protein supplementation, and vitamin- mineral supplementation programs are critical affecting bovine immune function. Association of minerals with fiber fractions in feedstuffs and/or binding of minerals to undigested fiber constituents in the gastrointestinal tract may alter bioavailability of some trace minerals in ruminants

(Spears, 2003). The pH in the rumen environment is only slightly acidic (6.0 to 6.8), and many trace minerals exist largely in an insoluble form in the rumen. Some metal complexes that are formed in the rumen remain insoluble even under the acidic conditions found in the abomasum (Waghorn et al., 1990).

Mineral deficiencies and imbalances for cattle are reported from almost all regions of the world (McDowell, 1996). The mineral elements most likely to be lacking under normal grazing conditions for ruminants are Ca, P, Na, Co, Cu, I, Se and Zn. In some specific regions, under specific conditions, Mg, K, Fe and Mn may be deficient and excesses of F, Mo and Se can be extremely detrimental. The principle means by which cattle producers attempt to meet mineral requirements of their grazing herds is through use of free-choice dietary minerals. As a low cost insurance to provide adequate mineral nutrition, a modified 'complete' mineral supplement is recommended and be available free-choice (McDowell, 1996). Calcium, Cu, or Se, when in excess, can be more detrimental to cattle production than any benefit derived by providing a mineral supplement. The major disadvantage to free-choice minerals is lack of uniform consumption by animals (McDowell, 1996). Factors influencing consumption of mineral mixtures include: (1) soil fertility and forage type, (2) season of year, (3) available energy and protein, (4) individual requirements, (5) salt content of water, (6) palatability of mineral mixture, (7) availability of fresh minerals and (8) physical form of minerals. Mineral supplements need to be evaluated for accuracy of formulation and suitability for cattle when optimizing production goals. Recent studies have shown positive responses

of mineral chelates and complexes when compared to inorganic sources for specific production goals.

Functionality of minerals can be defined in four basic types: structural, regulatory, physiological, and catalytic. Many mineral properties can be discharged by the same element in the same animal (Underwood and Suttle, 2004). Basic mineral functions are: 1) regulatory; specific minerals regulate cell replication and differentiation with transcription and transduction properties; 2) catalytic: minerals act as catalysts in enzyme and hormone functions with metalloenzymes that are important components of metabolic functions of cells; 3) physiological: electrolyte functions required by tissues contain specific minerals that function as membrane osmolality and permeability, acid-base balance, and tissue irritability; and 4) structural: formation of structural organ and tissue components in stabilization of molecules and membranes within the body.

As a metalloenzyme the mineral is attached firmly to a protein moiety with a specific number of metal atoms per mole of protein. At rare instances the mineral can be replaced by another mineral with little loss of function. Minerals are replaced in some instances related to deficiencies or toxicity of those specific minerals within the body. Occasionally serious clinical and pathological disorders arise (Underwood and Suttle, 2004).

Cattle derive a large proportion of their mineral content from feeds and forages during grazing. Mineral content is highly dependent on vegetative components and seeds of specific forages and grains consumed. Concentrations are dependent on: genus and species of the plant, type of soil on which the plant grows, climate conditions during

growth, and the stage of maturity of the plant when consumed. All of these affect concentration of mineral elements. Variations exist in natural sources as genetic variations result in species of forages with different mineral composition, differences between legumes and grasses, variations among seeds and grains, effects of soil fertilization, consumption of water interactions, and differences of diets all affect mineral absorption or bioavailability (Underwood and Suttle, 2004). Human interaction with treatment of the plant is a large factor in availability of the mineral: as with fertilization, soil amendments, irrigation, cultivation of different varieties of plants for increased production. The use of inorganic geological compounds is widely used in animal production as supplements to minerals supplied by natural forages and grains.

Ideally mineral supplements should only be used when requirements can't be made by normal consumption of feeds. Use of animal requirements, knowledge of mineral composition of feeds, and the availability of minerals toward desired production of specific animals are formulated to meet ideal needed requirements. Interactions between minerals are a major cause in availability and influence the nutrient value or change the potential toxic effects. Interactions forming un-absorbable complexes between dissimilar ions, the competition of similar ions for metabolic pathways, or non-specific metal binding proteins occur that interrupt availability and absorption of minerals. The nutrient value of a mineral is determined by the sum of all these interactions with dietary consumption (Underwood and Suttle, 2004). Many interactions have not been discovered and multiple interactions between different minerals make availability difficult to estimate requirements. As stated, the natural supply of minerals to animals is an outcome

of a complex set of chemical events with availability of the soil, plant, and animal bioavailability. Assessing supply is often confusing and difficult to assess. Seasonal differences, differences of farm or ranch availability, ecosystem influences, and different management systems have large influences in availability that change requirements of supplemented minerals for desired production.

The NRC lists 17 minerals required for beef cattle production. Minerals are classified into two broad groups: macrominerals and microminerals. Macrominerals are minerals that are required in levels of greater than 100 mg per day for normal growth and reproduction. Microminerals are those required in levels less than 100 mg per day. Macrominerals include the following: calcium, phosphorus, magnesium, potassium, sodium, chlorine, and sulfur. These minerals are required in the largest amounts and are the most commonly listed in deficiency syndromes. Microminerals necessary for normal production of beef cattle include: cobalt, chromium, iron, iodine, manganese, molybdenum, nickel, selenium, zinc, and copper. These minerals required in smaller amounts, are often found to be deficient because of antagonistic interactions between specific minerals.

Minerals not required but can be toxic in small amounts by cattle include the following; aluminum, arsenic, bromine, cadmium, fluorine, lead, mercury, and strontium. The balance of minerals within the physiological boundary of the animal is critical for normal or optimum performance. Dietary intake is critical as many elements may be antagonistic to one another and may cause deficiencies even in proper amounts for requirements in production (Suttle, 2004). Recent attempt to increase absorption has led to

production of chelated minerals. Chelated minerals are produced in an attempt to increase the utilization of dietary minerals. By formation of metal ions with an organic ligand, an effort is made to enhance mineral absorption across the intestinal mucosa. The effectiveness of synthetic mineral complexes for increasing mineral availability requires an understanding of the concepts of stability and constant ligand molecular weight (Underwood and Suttle, 2010). The stability constant is a measure of the affinity between a metal ion and a ligand. The stability constant must be high enough to allow intact absorption of the metal-ligand complex, yet low enough to allow metal ion removal at the metabolic point of use. In addition, the ligand molecular weight must be low enough to permit intact absorption of the metal complex (Hess et al., 1990). Reports of organic mineral bioavailability in livestock are inconsistent (Hess et al., 1990).

Of all the minerals required in beef cattle production the mineral that has been studied the least is cobalt (Co). Of the 17 minerals required for normal beef cattle production, knowledge of Co still remains the least understood. Recent research suggests that NRC recommendations for Co might underestimate requirements (Spears and Weiss, 2014).

The element cobalt can be accounted for by a specific single function within the body; that being the formation of vitamin B₁₂ involved in many metabolic processes. Vitamin B₁₂ is important as a coenzyme in lipid and carbohydrate energy metabolism in ruminants. Vitamin B₁₂ is utilized by both rumen microbes and by the host ruminant for critical metabolic functions necessary for growth and production.

Cobalt was first recognized as an essential nutrient for normal ruminant production as a result of studies conducted by Australian scientists identifying causes of naturally occurring diseases as “coast disease” of sheep (Lines, 1935; Marston, 1935) and “wasting disease” (Underwood and Flimer, 1935). Progress was slow until 1948 when vitamin B₁₂ was discovered and found Co as the nucleus of the molecule. Cobalt deficiency in ruminants is essentially vitamin B₁₂ deficiency. Only ruminants require Co as a dietary essential element in the synthesis of Co into the nucleus of cobalamin molecules and the vitamin B₁₂ molecule by ruminant bacteria species. Rumen bacteria synthesize many forms of cobalamin analog molecules of which vitamin B₁₂ is the most important for bacteria activity and the host ruminant (Underwood and Suttle, 2004). Most of the other cobalamin analogs are not absorbed or not active in ruminants (Bigger, 1976). Non-ruminants do not possess microbes to incorporate Co into vitamin B₁₂ in a physiological form for absorption and must therefore ingest preformed vitamin B₁₂ or ingest fecal material that has been activated on by large intestinal microbes previously.

Cobalt is also required for nitrogen fixation by legumes (Kubota, Welch, and Van Campen, 1985). Early work on Co in New Zealand and Australia where Co deficiency exists have led to the knowledge that Co deficient areas exist in the United States (Massachusetts, Maine, Michigan, Florida, New Hampshire to name a few specific areas) (Puls, 1992). The western U. S. has more of a problem with antagonistic Co areas than true deficient areas caused by iron or iodine antagonistic interactions (Puls, 1992).

Four trace minerals affect common production problems in the United States; Co, Cu, Se, Mo. These trace minerals are tied to availability in plants that grazing ruminants

consume or occur in toxic amounts (Kubota, Welch, and Van Campen, 1985). In the United States, the Humaquad class of soils has the lowest Co content of any type (Kubota, Welch, and Van Campen, 1985). These soil classes are found in glacier drifts and coastal plains of the United States and the world (Kubota, Welch, and Van Campen, 1985). An appreciable amount of Co has been leached below plant root uptake which causes deficiency in plant composition.

Historically cobalt has been studied to evaluate the element's influence in growth and performance of livestock. Cobalt has been studied for influences on growth and production using NRC recommendations that were developed over 60 years ago in the 1950's (National Research Council Beef Cattle Requirements, 2004). Recent studies have shown above NRC (National Research Council) requirements of Co fed to dairy cows resulted in increased milk production (Kincaid et al., 2003) and steers fed above NRC requirements of Co have increased HCW (hot carcass weights; Tiffany et al., 2003). Research previously done by Stangl et al. (2000) has demonstrated increased NRC Co levels are needed for minimizing metabolites of propionate metabolism that negatively affect intake and growth of beef cattle. Early studies by Fisher and MacPherson (1989) showed ewes fed above recommended NRC Co in late gestation had lambs with greater vitality and survival ability. MacPherson et al. (1989) showed Co deficiency in cattle had increased parasitism (*Ostertagia ostertagia*) and Co deficiency altered neutrophil function in innate immunity. Work by Tamura et al. (1999) reflected vitamin B₁₂ (cobalt dependent) has vital importance in B cell proliferation and differentiation for plasma cell formation necessary for antibody production important in adaptive immune function.

Previous recommended NRC Co requirements for normal growth and production were placed at 0.10 mg/kg/day or 0.10 ppm DM intake (NRC 2005). Recently, experiments have shown improved growth and performance at 0.20 mg/kg/day (Underwood, 1977; Smith, R.M., et al., 1997; Schwartz, 2003, Tiffany, et al., 2003, Suttle, 2010).

Cobalt is utilized by rumen microbial organisms for the synthesis of vitamin B₁₂ which is necessary as a cofactor for vital metabolic pathways in lipid and carbohydrate energy metabolism. Presently the only known requirement for the element cobalt, in ruminants, is as a constituent of vitamin B₁₂, (Maas, 1996; McDowell, 2000) which is approximately 4% Co by chemical weight (Miller, 1984; Berger, 1984). Cobalt is utilized by rumen microorganisms for synthesis of the large corrin ring molecule of vitamin B₁₂ (Schwartz, Kirchgessner, Stangl, 1999). Cobalt is also necessary as a function for the storage of copper in bovine liver tissue which copper is an essential element in the immune system of beef cattle (Kreplen, 2009). Ruminant Co requirements are higher than in non-ruminants because of the microbial need for cobalt and for non-metabolic vitamin B₁₂ factors such as involvement with cofactors in milk fat depression, ketosis, or both (Elliot, 1980).

Determination of Co tissue analysis in beef cattle is done by liver biopsy. Liver biopsy for Co mineral analysis is considered the gold standard for evaluation as blood concentrations fluctuate and can be misleading during deficiency (Underwood and Suttle, 2004). Cobalt liver biopsy concentrations have been found low or deficient in diseased animals with pneumonia when copper deficiencies have influenced disease and sickness

(Sager, 2002). Field investigation work done to determine causes of vaccine failure problems in beef calves showed marked Co deficiency (by liver Co biopsies) in bovine respiratory disease (BRD; Sager, 2002).

Cobalt deficiency reflects varying signs based on severity due to lack of soil and forage concentration of Co, antagonistic interference by other minerals, and the physiological requirements of the animal (Engle, 1999; Underwood and Suttle, 2004). Depending on Co tissue stores, deficiency can be slow and subtle in reflecting clinical signs. Clinical signs of cobalt deficiency may not show for weeks until liver storages are depleted and enzymes are depleted resulting in decreased appetite and failure of growth. Continual deficiency leads to un-thriftiness, loss of body weight, and acute emaciation. Necropsy signs reflect severe emaciation and total lack of visceral adipose tissue (Smith, 2005). Cobalt deficiency leads to metabolic changes occurring in the liver tissue, resulting in a fatty liver syndrome, with hemosiderization of the spleen. Hypoplasia of the bone marrow results in low red blood cell (rbc) production with lower hemoglobin concentration. Normocytic hypochromic red blood cells in lambs and microcytic and hypochromic rbc in calves result with chronic Co deficiency (Radostits, 2005; Smith, 2005). There is also a decrease of cellularity of the bone marrow in ovine Co deficiency. Animals also have hypoglycemic (<60 mg/dL glucose) and have low levels of alkaline phosphatase enzymes (<20 UI; Radostits et al., 2005). Liver lesions include accumulation of lipid droplets and lipofuscin of the hepatocytes predisposing to disassociation and necrosis of hepatocytes. Inflammation reflects infiltration of neutrophils, macrophages, and lymphocytes. Ultrastructural hepatocyte alterations include; swelling, condensation

and proliferation of mitochondria, hypertrophy of smooth endoplasmic reticulum, vesiculation, altered rough endoplasmic reticulum with lipid accumulation within the hepatocytes (Radostits, et al., 2005). Reduction of vitamin B₁₂ dependent enzymes predispose increases of methylmalonic acid (MMA) and formiminoglutamic acid (FMGA) because of deficiencies of methylmalonyl CoA mutase, methionine synthase, and lipid peroxidase enzymes. This Co deficiency leads to cellular and tissue pathophysiological lesions (Radostits et al., 2005).

Differential diagnosis of malnutrition, parasitism, copper or selenium deficiency needs to be considered with Co deficiency (Scott, Penny, and Macrae, 2011). Treatment with vitamin B₁₂ injections show quick response and almost complete reversal of the syndrome within days to weeks in both lambs and calves (Smith, 2005; Maas, 2005).

Ovine white liver disease (OWLD), a neonatal lamb disease of the United Kingdom, and very problematic in specific areas of Scotland, was later determined to be associated with Co deficiency (Maas, 2005). Concentrations of very low dense lipoproteins (VLDL) that build up in liver tissues are caused by Co deficiency as vitamin B₁₂ is required for VLDL metabolism and export from liver tissues (Suttle, 2010). With B₁₂ deficiency triglycerides build up in liver tissue giving the characteristic white tissue appearance as triglycerides are not metabolized into VLDL's to be exported from liver tissues.

Availability of Co is increased in acid soils of higher moisture content (Van Soest, 2005). Main sources of Co are forages (legumes higher than grasses), ingested soil, concentrates (poor sources), and cobalt salts in commercially mixed minerals supplied as

a trace mineral source to livestock (Suttle, 2010). Milk is an important source for pre-ruminant calves in the form of vitamin B₁₂ (derived by rumen microbial synthesis of Co) and can be increased by Co supplementation of the mother (Hart and Andrews, 1959, Quirk and Norton, 1988). Vitamin B₁₂ in cow's milk is more bioavailable than in other forms and thus is an excellent source in the neonatal diet (Matte, Guay, and Girard, 2012). Bioavailability of vitamin B₁₂ in milk is the highest of all sources with approximately 51% to 79% absorption (Tucker, 2000). There exists a large genetic variation in the mammary synthesis of vitamin B₁₂ in milk (Rutten, et al., 2013).

The absorption of vitamin B₁₂ in ruminants is complex. Vitamin B₁₂ is bound to proteins and is released from proteins by the action of a high concentration of hydrochloric acid present in the abomasum. This process results in the free form of the vitamin, which is immediately bound to a mixture of glycoproteins secreted by the stomach and salivary glands. These glycoproteins, called R-binders (or haptocorrins), protect vitamin B₁₂ from chemical denaturation in the stomach. The stomach's parietal cells, which secrete hydrochloric acid, also secrete a glycoprotein called intrinsic factor. Intrinsic factor binds vitamin B₁₂ and ultimately enables its active absorption. At an acidic pH the affinity of the intrinsic factor for vitamin B₁₂ is low whereas its affinity for the R-binders is high. When the contents of the stomach enter the duodenum, the R-binders become partly digested by the pancreatic proteases which cause them to release their vitamin B₁₂. Because the pH in the duodenum is more neutral than that in the stomach, the intrinsic factor has a high binding affinity to vitamin B₁₂, and it quickly binds the vitamin as it is released from the R-binders. The vitamin B₁₂-intrinsic factor

complex is absorbed during phagocytosis by specific ileal receptors in the small intestinal mucosa (Weir and Scott, 1999).

As Co is required for rumen microbe synthesis of vitamin B₁₂ as a cofactor in lipid and carbohydrate metabolism (for energy required by both rumen microbes and the host ruminant), a deficiency of Co intake affects rumen bacteria in total numbers, in types present, and in cultural qualities (Smith and Loosli, 1957). Among the multitude of bacteria-strains in the rumen, only a few of them are able to synthesize cobalamin compounds or molecules such as vitamin B₁₂ analogues and vitamin B₁₂ (Dryden et al. 1962). *Propionibacterium shermanii* is one of the more prolific and efficient cobalamin synthesizers in the rumen. *Propionibacterium shermanii* is also used in industry to produce vitamin B₁₂ for commercial use (Friedrich, 1987). The biosynthesis of cobalamin involves a complex series of reactions and must take place under anaerobic conditions (Costigan and Gerdes, 1991). Microbial biosynthesis is supplied to the mammalian host tissues as an organic vitamin B₁₂ /cobalamin complex.

Vitamin B₁₂ is an important factor for growing bacteria (especially for some strains of *Propionibacteria* species and *Selenomonas ruminantium*) which are the most prolific and efficient in vitamin B₁₂ synthesis. *Selenomonas ruminantium* is necessary for propionate synthesis vital for gluconeogenesis in bovines. *Selenomonas ruminantium* has a high affinity for Co and high ability for conversion into a complex form. *Selenomonas ruminantium* contains a very high affinity transport system for cobalt. One of the most important reductive reactions in the rumen is propionate formation, and a reciprocal relationship is generally observed between methanogenesis and propionate production

(Asanuma and Hino, 2001). Among propionate-producing bacteria, *Sel. ruminantium* is one of the most predominant bacteria in the rumen and accounts for $22 \pm 51\%$ of the total viable bacterial counts in the rumen (Caldwell and Bryant, 1966). Stemmea (Stemmea, et al., 2008) concluded that an elevated dietary cobalt supply (0.29 mg Co/kg DM) had no influence on ruminal parameters such as pH-value, short chain fatty acids and microbial protein synthesis in comparison to the native cobalt content in the ration (0.17 mg Co/kg DM). Although feeding 0.29 mg Co/kg DM resulted in higher amounts of vitamin B₁₂ in the duodenal digesta, with individual differences considerable.

Cobalt synthesis occurs in the anaerobic areas of the lower rumen. There may be two factors that contribute to ruminants' susceptibility to cobalt deficiency. The first is the inefficient conversion of cobalt to vitamin B₁₂ by rumen microorganisms (Suttle, 2010). Smith and Marston (1970) showed that under cobalt deficiency conditions about 15% of dietary Co was converted to cobalamin in the rumen whereas only 3% was converted when Co was at an adequate level in the diet. Also, Gawthorne (1970) showed that 44% of the vitamin B₁₂ activity in sheep fed a cobalt sufficient diet (0.34ppm) was the physiologically inactive form, 2 - methyladenyl cobamide. This proportion remained the same even when the dietary cobalt was dropped to 0.04ppm. Active 5, 6 - dimethyl benzimidazolyl cobamide produced in this deficient cobalt state, increased from 35 to 63% at the expense of other vitamin B₁₂ analogues. It is apparent by these studies that a greater proportion of the 'vitamin B₁₂' synthesized by rumen microorganisms growing under cobalt deficient conditions was in a form that could be utilized by the host animal. Overall the total quantity produced was far less than that produced when on a cobalt

sufficient diet (Costigan and Gerdes, 1991). The second factor contributing to the ruminant's susceptibility to cobalt deficiency is the degradation and poor absorption of vitamin B₁₂ produced in the rumen with as low as 3% of vitamin B₁₂ absorbed in the ileum of the host (Costigan and Gerdes, 1991).

A deficiency of cobalamin may decrease microbial protein synthesis in the rumen necessary for propionate synthesis vital to gluconeogenesis. There appears to be an optimum Co level for microbe growth and production of cobalamin compounds. Elevated dietary cobalt supply (0.29 mg Co/kg DM vs. 0.17 mg Co/ kg DM) had no influence on ruminal parameters such as pH-value, short chain fatty acids and microbial protein synthesis in comparison to the native cobalt content in the ration (0.17 mg Co/kg DM). Feeding 0.29 mg Co/kg DM did result in higher amounts of vitamin B₁₂ in the duodenal digesta contents (Stemmea et al., 2008).

The amount of synthesized cobalamin depends on the composition of the ration (relation of roughage to concentrate; fiber content) and the dry matter intake (Smith and Marston, 1970). The most important factor for the production of vitamin B₁₂ seems to be the concentration of cobalt in the diet. Little is known about the efficiency of cobalamin production in the rumen. Smith and Marston (1970) found that Co was converted to cobalamin more efficiently in Co-depleted sheep than sheep that access to normal Co intake. In cattle a positive correlation between the amount of dietary cobalt and the vitamin B₁₂ content in the ruminal fluid exists (Singh, et al., 1995).

Cobalt in the form of vitamin B₁₂ is interrelated with iron and copper in hematopoiesis and, thus, indirectly involved with molybdenum. Vitamin B₁₂ may

function in the formation of excretion products of selenium and thereby reduce the animal's susceptibility to selenium toxicity (Ammerman, 1970). There is a direct antagonist relationship with iron and iodine with iron as the most common antagonist. Iron can be fed to ruminants with Co toxicity as Fe competes for intestinal absorption sites in the intestine (Puls, 1999). Co interacts with other micro-minerals; manganese, zinc, and iodine as antagonists (Puls, 1992).

Cobalt is extremely safe as a supplement fed orally (Puls, 1992). Toxicity and deficiency signs are almost identical. Clinical toxicity signs often show reduced growth rate, rough and dull hair coat, muscular incoordination, and increased packed cell volume (PCV) and hemoglobin (Puls, 1992). The most pronounced toxic sign is reduced weight gains. As sheep have a higher Co requirement than cattle toxic signs are more prone to cattle than sheep. Levels of 1000 times recommended Co levels did not produce toxic signs in deficient sheep until after 8 weeks (Puls, 1992). Toxic symptoms in cattle are reached when cobalt is fed orally at rates of 300 times NRC (Puls, 1992). Iron is the most common antagonist and can be fed in Co toxicity as Fe competes for intestinal absorption sites in the intestine.

Concentrations of trace minerals need to be maintained within narrow limits if the functional integrity of cells and tissues vital to normal growth and performance of the animal is to remain unimpaired (McDowell, 1992; Engle, 2001; Underwood and Suttle, 2004). Deficiencies of trace minerals have been shown to alter specific components of the growth and performance of ruminants (Engle, 1999; Drake, 2000, Fernandes and Jolly, 2003, Maas, 2005). Growing and production bovines require large amounts of glucose

derived from gluconeogenesis. Vitamin B₁₂ is critical for normal gluconeogenesis in ruminant growth and production.

Ruminants utilize volatile fatty acids (VFA) produced by fermentation of fiber and cellulose by microbes in the rumen. Volatile fatty acids are transported to the liver for glucose metabolism. While all VFA's can be metabolized for gluconeogenesis the most important is propionate a three carbon molecule that can be metabolized into a six carbon molecule of glucose. Ruminant gluconeogenesis is a slow process with steady metabolism from fermentation of fibers and cellulose that require longer period of times for production of VFA's. The ruminant does not go through the change of gluconeogenesis by catabolism of amino acids or adipose tissues only in starvation. Substrate formation is in the form of VFA's with propionate as the primary substrate (75%) with lactate formation in anaerobic fermentation (13%) and alanine and glutamine amino acids as substrate precursors (12%). The liver is the primary organ (75%) with the kidney (25%) involved in gluconeogenesis. the nervous system of the bovine requires 15-20% of the glucose needs where the mammary gland during lactation requires more than 60% of all glucose requirements.

In ruminant gluconeogenesis from propionate, valerate, amino acids, lactate, and glycerol ruminant gluconeogenesis is of great importance at all times. Lactation requires increased rate of gluconeogenesis. Ruminant gluconeogenesis increases after feeding but gluconeogenesis rates are slow and continuous with the fermentation process of VFA from cellulose and hemi-cellulose. Ruminant gluconeogenesis can take hours to days for gluconeogenesis to be completed. Lactation is a huge requirement for glucose through

gluconeogenesis as it has been calculated that 7.4 kg of glucose is needed daily by a dairy cow producing 90 kg of milk per day and that 6.6 kg of that glucose was derived via gluconeogenesis, hence illustrating the quantitative importance of gluconeogenesis (Young, 1977). Ruminant liver releases glucose into blood during both fed and fasted states, whereas the liver of non-ruminants in the fed state has a net uptake of glucose and a net release of glucose during the fasting state. Gluconeogenesis occurs at greater rates when ruminants are in positive energy balance (Young, 1977).

Inefficient use of dietary Co utilized by the ruminant is due to microbial partitioning of Co into active and inactive Co-vitamin B₁₂ compounds referred to as corrinoids. These compounds cannot be absorbed or used in this form (Gawthorne, 1970). Microbial rumen production of vitamin B₁₂ with increased Co supplementation responds within hours to change, but the efficiency of absorption decreases with increased intake levels (Underwood and Suttle, 2001).

Dietary corrinoids bind with R proteins from saliva, but are digested by pancreatic enzymes in the small intestine while cobalamine compounds synthesized in the rumen bind to an intrinsic factor produced by parietal cells in the abomasum (McKay and McLeay, 1981). Bile salts increase binding of cobalamine: vitamin B₁₂ complexes to the receptor sites in the ileal mucosal brush border (Smith, 1997). Cobalt and vitamin B₁₂: corrinoid compounds are absorbed more slowly and less completely than in monogastric animals (Rothery et al., 1953). The corrinoid cobalamine- intrinsic factor-complex enter the enterocyte by endocytosis, with the cobalamine compound released from the intrinsic

factor protein. It is then bound to a carrier protein (transcobalamin), and exits into the circulating blood.

During inflammation and infection there exists increased binding capacity in plasma indicating an increased cellular demand for vitamin B₁₂. This increased binding process has been proved in vitro by greatly increased cobalamin uptake by lymphocytes stimulated during inflammation (Quadros et al., 1976) and decreased neutrophil cytolysis in Co deficient cattle (Mac Pherson et al., 1987). With increased NRC levels of Co of 0.20 ppm (Schwartz, 2000) rumen micro-organisms synthesized increased vitamin B₁₂ (Bishenhsari, 2010), but constant intake is needed as liver storage ability and mobilization are limited in the ruminant (Underwood and Filmer, 1935). Because ruminants make poor use of dietary cobalt as rumen microbes partition the Co particles between cobalamin active components and inactive forms of vitamin B₁₂ - like compounds (corrinoids) are un-absorbable to the ruminant (Gawthorne and Smith, 1968). Of all the total microbial production of vitamin B₁₂ produced by rumen microorganisms, only 1 to 3 % is absorbed in the ileum of the gastrointestinal tract (Miller, 1984).

Cobalt is essential for the formation and production of vitamin B₁₂ for coenzyme functions in metabolism. Cobalamin synthesis in the rumen results in the formation of methylcobalamin and adenosylcobalamin compounds each vital in different metabolic pathways in ruminant energy production.

Vitamin B₁₂, in the form of methylcobalamin, is necessary in methyltransferase metabolic enzyme functions and acts as a donor of methyl groups and is involved in one-carbon addition by building carbon chains in metabolism. Methylcobalamin is necessary

in methane, acetate, and methionine synthesis by rumen microbes (Poston and Stadman, 1975). Methylcobalamin enables the enzyme, methionine synthase, to supply methyl groups to a wide range of reactions including formate, noradrenaline, myelin, and phosphatidyl ethanolamine. As adenosylcobalamin, Co is a factor in energy metabolism assisting in glucose formation and assisting methylmalonyl-coenzyme A mutase to form succinate from propionate in the liver for gluconeogenesis.

The importance of VFA as sources for energy in ruminants has been understood for many years. As much as 60 to 80% of the metabolizable energy (ME) can be from VFA'S in rations consumed (Phillipson, 1969). Of the three common VFA'S (acetate, butyrate, and propionate) the most important for gluconeogenesis and energy utilization in ruminants is propionate. Vitamin B₁₂ is utilized in this metabolic pathway for gluconeogenesis described below. Very little glucose is absorbed from the intestinal tract so propionate metabolism for glucose is vital in ruminants. Propionate is converted to pyruvate first, then through the gluconeogenesis pathway, to produce glucose in liver (90%) and kidney tissues (Underwood, 1977). The first important enzyme, pyruvate carboxylase, is activated by both acetyl-COA and propionyl-COA. With Co deficiency rumen fluid changes result in propionate microbes decreasing and less propionate is produced leading to less availability of propionate for gluconeogenesis but the most compounding factor is the relationship of Co needed for vitamin B₁₂ synthesis required for metabolic pathways for methylmalonyl-COA to be changed to succinate-COA to enter the TCA cycle for energy (Figure 7 on next page). As a result methylmalonyl- COA

builds up in the blood and depresses appetite causing “wasting disease”. Propionate and acetate clearance rates increase as vitamin B₁₂ levels decrease (Underwood, 1977).

In the early stages of Co deficiency, biochemical changes occur in fluids and tissues of the ruminant, because of the lack of Co storage in body tissues. Cobalt levels below 0.5 ug / ml are considered deficient and levels of vitamin B₁₂ start to decline (Underwood, 1977). Increase in succinate in rumen fluids within 2 weeks of Co deficiency affect concentrations by decreasing specific rumen microbes such as *Selenomonas ruminantium* necessary for propionate synthesis.

Deficiency of Co also influences defects in lipid metabolism involving cobalamin dependent pathways and can be seen in fatty liver syndrome in sheep (OWLD) due to accumulation of methylmalonyl-COA which is an inhibitor of beta oxidation of fatty acids (Suttle, 2010). The loss of normal fatty acid metabolism to form triglycerides in the liver and the export of VLDL is due to the need for methylcobalamin formation of VLDL in liver tissue, therefore the accumulation of triglycerides and a fatty liver syndrome result. Homocysteine levels also increase and initiate an accumulation of oxidized products, depletion of vitamin E and damage to mitochondria (Kennedy et al. 1997). The main energy source in ruminants is volatile fatty acids not glucose. The breakdown of propionate in the metabolic pathway requiring adenosylcobalamin for methylmalonyl-COA to a converted succinyl-COA is a necessary reaction in energy production (page 37). The ability for propionate synthesis to occur in the rumen is normal in deficient Co animals but the ability to metabolize propionate in the blood is greatly inhibited with Co deficiency and methylmalonyl -COA accumulates causing decreased feed intake

(Gawthorne, 1968). Acetate clearance in the blood is impaired (Sommers, 1969) yet the propionate buildup has the greater inhibition of appetite in the ruminant (Farmingham and Whyte, 1993).

The essential defect in Co deficiency in ruminants is an inability to metabolize propionic acid produced by microbial carbohydrate fermentation in the rumen (Radostits et al., 2007). Adenosylcobalamin is a coenzyme needed for the reaction of methylmalonyl (MMA) coenzyme A to succinyl coenzyme A (Paterson and Mac Pherson, 1990). Methylmalonyl-CoA to succinyl CoA involves methylmalonyl-CoA isomerase or mutase (Gropper. et al. 2007) and is a B₁₂ requiring enzyme that catalases the conversion of methylmalonyl-CoA to succinyl CoA (Underwood and Suttle, 2004). In carbohydrate fermentation propionic acid is a by-product and the rate of clearance is dependent on the above coenzymes (Underwood and Suttle 2004; Fisher and Mac Pherson, 1990b). In absorption of propionic acid in the blood, methylmalonic acid accumulates and depresses appetite, therefore decreasing feed intake with Co deficient animals (Underwood and Suttle, 2004). This concept is thought to be the factor in Co deficient steers on corn diets, low in Co, have lower final BW and lower carcass weights than in Co supplemented feedlot steers with rations containing 0.15 to 0.20 ppm (Tiffany et al., 2003). Co supplemented steers showed increased propionate, higher blood glucose levels, and greater vitamin B12 plasma concentrations than control steers (Kincaid et al., 2003).

Ruminant gluconeogenesis, lipid metabolism, nucleic acid synthesis, and hemopoiesis are critically decreased in deficient Co cases (Radostits, 2007). Co-induced

B₁₂ deficiencies result in lowered red blood cell production causing anemia and later in the reduction of intake, ADG, and protein synthesis causing decreased growth (Wang et al., 2007). Co may alter growth rate and lipid metabolism in finishing steers (Sharman, et al. 2008).

Increased vitamin B₁₂ synthesis by ruminal microbes may reduce stress (Shelley et al., 2013). As Co is used to synthesize B₁₂ and Smith (1987) showed that the conversion of dietary Co to vitamin B₁₂ is generally very low especially in “off feed” calves it has been hypothesized that receiving calves may benefit from increased Co supplemented in diets therefore supplemental Co may be needed. In addition to vitamin B₁₂ synthesis supplemental Co may also impact the rumen environment and increase fiber digestion and energy production. Cobalt can be supplemented using inorganic or organic sources.

Co deficiency induced vitamin B₁₂ deficiency affects digestibility of other nutrients as there were significant increases ($P < 0.05$) in digestibility coefficients of dry matter, organic matter, crude protein, ether extract and energy respectively, in goats from a vitamin B₁₂-treated group (Kadim et al., 2003). Recent Irish research suggests that cobalt deficiency may directly affect the metabolism of rumen bacteria which in turn may affect the digestion process. Kennedy et al. (1991) reported large increases in succinate concentration in rumen fluid within two weeks after sheep were fed a cobalt- deficient barley-based diet. They suggested that propionate producing bacteria like *Selenomonas ruminantium* may be especially susceptible to a cobalt deficiency. Kennedy et al. reported that succinate concentrations increased within two days after being fed a diet containing

0.02 ppm cobalt, but not when 0.04 ppm cobalt was present (Kennedy et al., 1991).

Florida researchers also reported rapid changes in the rumen microbial population of animals grazing cobalt-deficient forages (Gall et al., 1949).

Lopez-Guisa and Satter (1992) reported that cobalt supplementation above that required for B₁₂ synthesis may improve the utilization of poor quality forages. The rate of fiber digestion in the rumen is a major factor affecting voluntary intake on high forage diets. Supplementation of cobalt above animal requirements may increase the ability of bacteria to digest fiber. Divalent cations such as cobalt may allow bacteria to connect to plant cell walls. The cellulose enzymes produced by bacteria are retained on the cell membrane and are not released into the environment. Consequently, the bacteria must physically attach to the fiber particle for the enzymes to digest the cellulose. It appears that when a negatively charged bacteria has a difficulty attaching to a negatively charged fiber particle, cobalt with two positive charges can serve as a means of linking the two surfaces (Lopez-Guisa and Satter, 1992). In one experiment, cobalt increased the rate of in situ corn fiber digestion from 3.4 to 6.2% per hour. In other experiments cobalt supplementation above that required by the animal increased volatile fatty acid concentrations in the rumen fluid suggesting that the rate of fiber digestion was improved. Total VFA concentrations and molar proportions of individual VFA were not affected by a treatment × day interaction. Cultures fed the control diet supplemented with 0.10 mg of Co/kg of DM had lower total VFA concentrations than those supplemented with 0.05 or 1.0 mg of Co/kg of DM.

Another change in the ruminal environment is a change in ruminal pH due to Co supplementation (Table 2.2). However, neither methane nor ammonia were affected with Co supplementiation (Tiffany et al., 2006).

Table 2.2. Effects of Co concentration on: methane, ammonia, and pH in continuous cultures of ruminal microbes from Tiffany, et al. 2006.¹

Item	Added Co, mg/kg of DM				SEM
	0	0.05	0.10	1.0	
Methane, mmol/d	10.3	10.7	12.4	13.4	1.9
Ammonia N, mg/dL	22.8	22.8	23.2	23.0	1.3
pH ^{2,3,4}	5.58	5.43	5.59	5.44	0.03

¹Data are means of 3 fermentors.

²Control vs. cobalt ($P < 0.10$).

³0.05 vs. 0.10 mg of Co/kg of DM ($P < 0.01$).

⁴0.10 vs. 1.0 mg of Co/kg of DM ($P < 0.01$).

Clinical signs of Co deficiency in cattle have serum vitamin B₁₂ values of < 0.2 ug/mL. Vitamin B₁₂ serum levels increase after feed deprivation for short periods of time. Serum vitamin B₁₂ values increase within 2 days after vitamin B₁₂ treatment (Radostits et al., 2005). Normal liver vitamin B₁₂ values have been established in past liver biopsy analysis and the mean vitamin B₁₂ value of the 350 liver samples was 1.18 mg/kg, with a range of 0.58 to 2.70 mg/kg. The mean value for livers from each of the 35 samples varied from 0.95 to 1.43 mg/kg (Rammell and. Poole, 1974). Lower levels of vitamin B₁₂ < 1.00 mg/k were generally found in liver samples from geographical areas previously defined as cobalt-deficient areas (Rammell and. Poole, 1974).

Cobalt has been reported to affect both plasma and liver cobalt concentrations (Figure 2.1). In addition, plasma methylmalonic acid (MMA) varies with Co ($\mu\text{g}/\text{kg DM}$; Stangl et al., 2000; Figure 2.2).

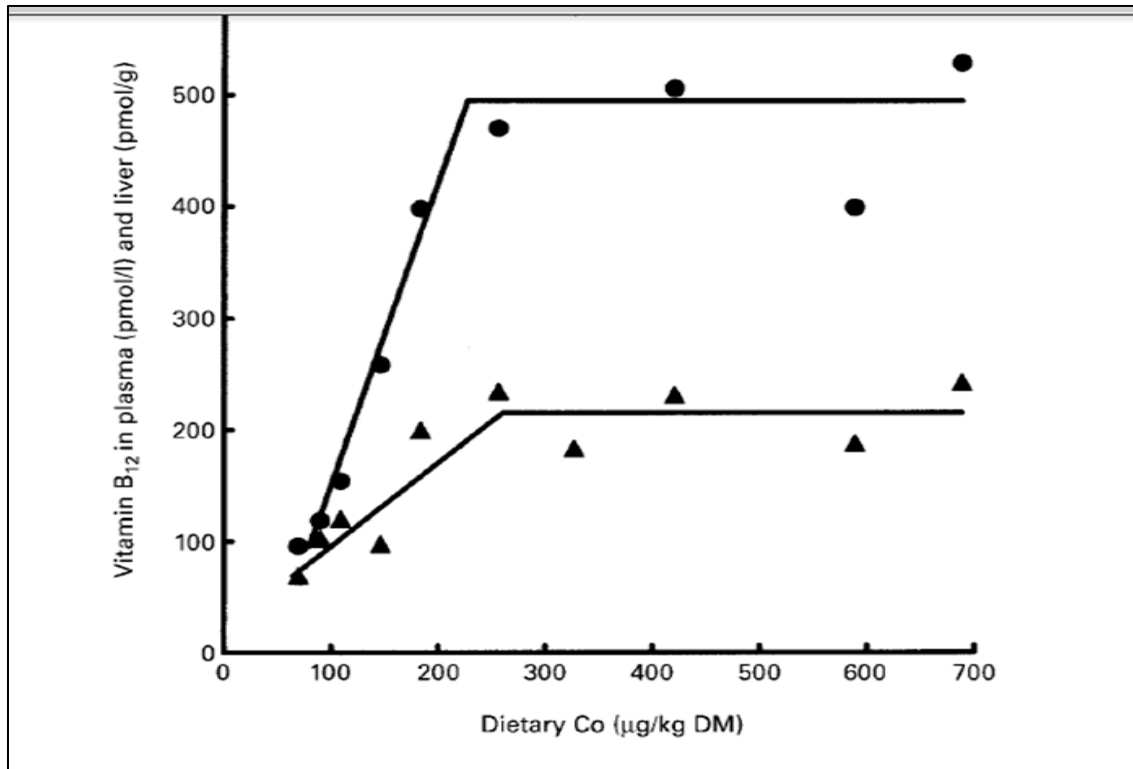


Figure 2.1. Relation of bovine vitamin B₁₂ levels in plasma and liver (oval marks represent liver concentration and triangle marks indicate serum levels (Courtesy of Stangl et al., 2000).

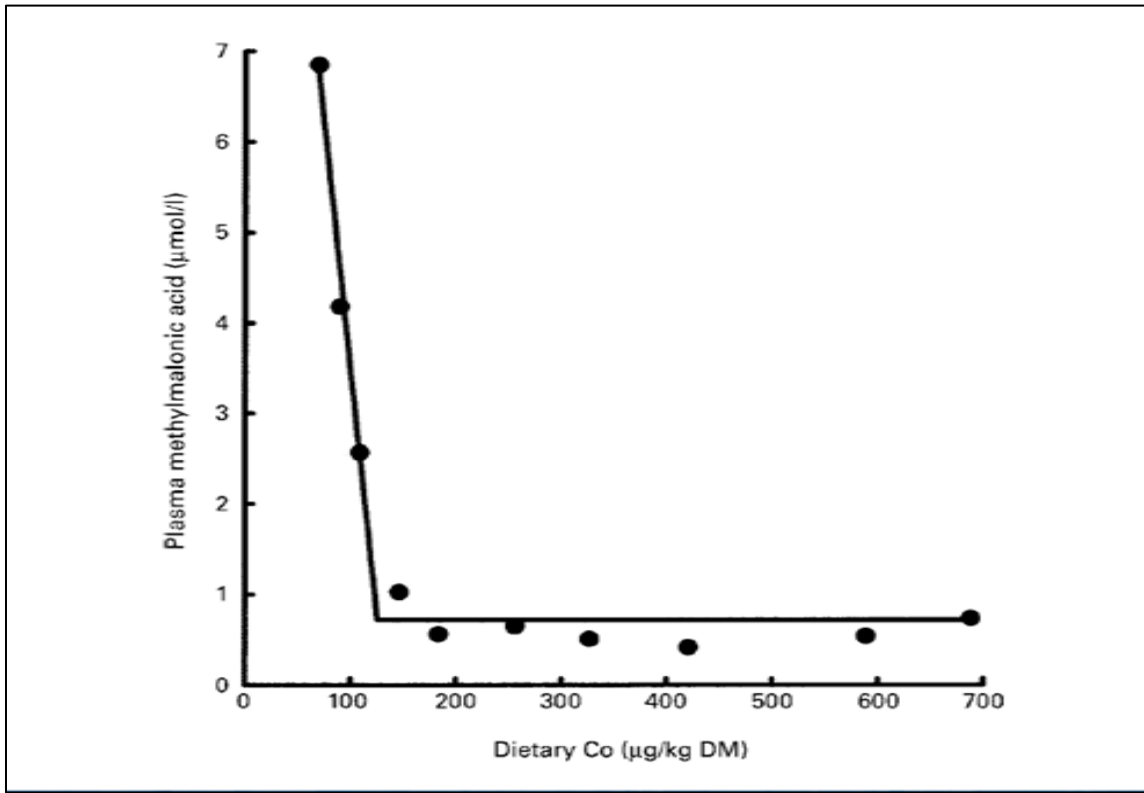


Figure 2.2. Relation of plasma methylmalonic acid (MMA) compared to Co (ug/kg DM) (Courtesy of Stangl et al., 2000).

Importance of Cobalt in Bovine Immune Response

Trace minerals are also important to immune function (Engle, 1999; Fernandes and Jolly, 2003). Normal immune function is critical for the ruminant to remain healthy and perform at an optimal level desired. A deficiency of any trace mineral will alter immunoglobulin production and the normal response to infection (Engle, 1999). Co, as a necessary trace mineral for rumen microbial synthesis of vitamin B₁₂, can be a limiting factor in immune function as vitamin B₁₂ has been shown to be involved in phagocytosis of microbes by neutrophils and macrophages (Drake, 2000). Vitamin B₁₂ is vital to B cell proliferation needed for DNA synthesis and immunoglobulin production (Tamura, 2001; Fernandes and Jolly, 2003). During inflammation and infection there exists increased

binding capacity in plasma indicating an increased cellular demand for vitamin B₁₂. This increased binding process has been proved in vitro by greatly increased cobalamin uptake by lymphocytes stimulated during inflammation (Quadros et al., 1976) and decreased neutrophil cytolysis in Co deficient cattle (Mac Pherson et al., 1987).

Nutritional supplementation during pre-weaning has potential to reduce sickness and improve health in beef calf production (Fisher and Mac Pherson, 1990; Engle, 1999; Drake, 2000, Fernandes, 2003, Maas, 2005). Nutritional management efforts to minimize health problems affect production throughout life (Lofgreen, 1988; Engle, 1999; Smith, 2005). Sickness and decreased health have lasting effects on feedlot performance and carcass quality at slaughter (Stovall et al. 2000; Mc Beth et al. 2001, Smith, R.A., 2009).

Importance of Cobalt During Gestation

Concentrations of trace minerals need to be maintained within narrow limits if the functional integrity of cells and tissues vital to normal immune function of the animal is to remain unimpaired (Mc Dowell, 1992; Engle, 2001; Underwood and Suttle, 2004).

First, trace minerals are vital for normal fetal development. Trace minerals have important requirements for health of the new-born calf through immunoglobulin production of the bovine mammary gland. Deficiencies of trace minerals have been shown to alter specific components of the immune system (Engle, 1999; Drake, 2000, Fernandes, 2003, Maas, 2005).

Trace minerals are important to immune function (Engle, 1999). A critical component in adaptive immunity is the production of antibodies. Antibody production, by B cell proliferation, forms immunoglobulins (IgG). Immunoglobulins are secreted

through mammary tissues in late gestation for colostrum production in milk at parturition (Radostits, 2005). A deficiency of any trace mineral will alter immunoglobulin production (Engle, 1999; Fernandes, 2003; Smith, 2005, Taylor, 2010). Trace mineral intake during late gestation is critical for normal colostrum production (Engle, 1999). Co as a necessary trace mineral for rumen microbial synthesis of vitamin B₁₂ can be a limiting factor in immune function as vitamin B₁₂ has been shown to be involved in normal immune function (Fernandes, 2003). B₁₂ is critical for B cell proliferation, DNA synthesis, and antibody production necessary for vital colostrum production. Co deficiency can lead to poor colostrum quality and lowered quantity vital to neonatal immune function (Engle, 1999). Neonatal ruminants derived the entire supply of immunoglobulins through colostrum as the ruminant placenta will not allow transfer of immunoglobulins to fetal tissues during gestation (Radostits, 2005). Co supplied during gestation is critical for colostrum production quality and is critical for neonate health during the first four months before the neonate immune system develops (Smith, 2005).

Nutritional supplementation during pre-partum has potential to reduce sickness, improving health, and increasing profits in beef calf production (Fisher and Mac Pherson, 1990; Engle, 1999, Drake, 2000, Fernandes, 2003, Maas, 2005). Nutritional management efforts to minimize health problems affect production throughout life (Lofgreen, 1988; Engle, 1999; Smith, 2005). Colostrum IgG affects growth and performance throughout life. Sickness and decreased health have lasting effects on feedlot performance and carcass quality at slaughter (Stovall et al. 2000; Mc Beth et al., 2001). Supplemental dietary Co did not affect secretion of Co in milk, tissue retention, or subcellular

distribution of Co within the liver (Kincaid, et al., 2003). Primiparous and multiparous cows differed in their milk yield response to dietary Co supplementation with primiparous cows secreting more Co in milk but decreasing faster than in multiparous cows (Kincaid, et al., 2003). Cobalt supplementation or the use of vitamin B₁₂ injections during lactation did not influence plasma or liver measures of energy metabolism. Injections of vitamin B₁₂ increased plasma, liver, and milk vitamin B₁₂ content (Kincaid, et al., 2003). Dietary Co addition did not affect plasma vitamin B₁₂ concentrations. Increase milk vitamin B₁₂ concentrations throughout lactation and liver vitamin B₁₂ at calving resulted with no effect of source or level of Co. Overall, Co supplementation (inorganic and organic) or vitamin B₁₂ injections improved measures of vitamin B₁₂ status, but not lactation performance compared with controls (Akins, et al., 2013).

Interaction of nutrition and the immune system reflects a complex and intimate relationship that involves an understanding of cellular and molecular metabolism. Nutritional science and the immune system sciences are closely linked together (Drake, 2010). Nutrients provide both energy and metabolic components for maintenance, normal regulation, growth, and repair of tissues involved in the immune system. Amino acid use, especially lysine, increases six fold during robust immune response (Klasing, 2007). Methylcobalimin is needed for methylation to form lysine and many other amino acids with methylcobalmin required methionine synthase synthesis for methionine important in all immune responses. Requirements for minerals during immune response are vital to immune response activity to prevent sickness and disease. The beneficial responses to certain organic trace minerals are most importantly decided by the form of the mineral

absorbed not the quantity of mineral absorbed (Spears 1996). Studies in poultry show the immune system accounts for approximately 9% of nutrient use (Klasing, 2007). Immune system tissues are very sensitive to feed deprivation, decreased energy metabolism, and specific nutrient intake (Klasing, 2007). Thus the thymus responsible for CD4+ T cells, results in lower IgG (antibody) production with nutrient deprivation especially vital mineral deprivation.

The immune system relies on anti-oxidants necessary for normal function that are micro-mineral dependent. During stress antioxidants are used up quickly as body supplies in fat, muscle, and liver tissues are minimal for immune system use (Kaplring, 2007; Hermson, 2013). Antioxidants are immune-modulatory because of the effects on free radical formation in leukocytes. Stimulation of the immune system results in a series of metabolic changes that are antagonistic toward growth (Klasing and Johnstone, 1991). Cytokines, including interleukin-1, tumor necrosis factor, and interleukin-6, are released from cells of the monocyte-macrophage lineage after recognition of antigen stimulating immunogens (Klasing and Johnstone, 1991). Immune response alters the partitioning of dietary nutrients away from growth and skeletal muscle accretion in favor of metabolic processes which support the immune response and disease resistance. Alterations include: 1) decreased skeletal muscle accretion due to increased rates of protein degradation and decreased protein synthesis; 2) increased basal metabolic rate resulting in increased energy utilization; 3) use of dietary amino acids for gluconeogenesis and as an energy source instead of for muscle protein accretion; 4) the synthesis of acute phase proteins by the liver; 5) redistribution of iron, zinc, copper and cobalt in tissues due to the

hepatic synthesis of metallothionein, ferritin, and ceruloplasmin; 6) Impaired accretion of cartilage and bone; and 7) release of hormones such as insulin, glucagon, and corticosteroid-corticosterone

Cytokine response also influences the differentiation of cells. Tumor necrosis factor suppresses the differentiation of myoblasts and adipocytes (Klasing and Johnstone, 1991). A Co study in goats suggest that low dietary levels of cobalt lead to a reduction in both phagocytic activity as well as in T cell responses and are sensitive indicators of developing vitamin B₁₂ deficiency (Johnson, et al., 2009).

There exists an indirect connection with Co deficiency and the impairment of the immune system as Co deficiency directly affects feed intake resulting in deficiencies of vital nutrient groups such as fatty acids and amino acid metabolism through lipid and protein intake and absorption. A deficiency of any nutrient essential for immune function will result in impairment of most adaptive and innate immune function. This deficiency impairs the ability of the immune system in normal phagocytic function, in innate immunity, and cytokine production in adaptive immunity with irregular function of humeral (antibody production) and cell mediated immunity (Drake, 2010). Dendritic cell uptake, necessary for antigen presentation to macrophages and to lymph nodes, is required for B cell production of antibodies (Liebler et al., 1995).

Bovines live in an environment of pathogenic micro-organisms of bacteria, viruses, fungi, and parasites commonly referred to as antigenic pathogens. The bovine host plays a large part in the reproduction and life of these micro-organisms and constantly defends itself from these micro-organisms through both innate and adaptive

immune responses. In the adaptive immune response white blood cells of hematopoietic stem cell origin are responsible for phagocytosis (neutrophils, dendritic cells, and macrophages) and by a more specific response mediated by lymphocytes (B and T lymphocytes) which the B cell is activated by T cells to stimulate proliferation to form plasma cells which secrete antibodies. Activated B cells migrate to a lymph node or other lymphatic tissue and undergo rapid proliferation within a germinal center of the lymphoid tissue into a plasma cell. The plasma cell is capable of producing over 1500 specific antibody molecules per second (Nossal and Makela, 1962). Antibody production is one of the hallmarks of the adaptive immune system that is complex to a specific antigenic pathogen. Antibody response is selective and attachment to the surface of the antigen directs phagocytosis or destruction by other immune cells. This action provides for a “memory response” for faster action with reinfection of the same antigen.

B cell production varies between species and with cattle B cell proliferation occurring in the Peyer’s patch, an organ of intense B cell follicles, located in the ileal submucosa (Tizard, 2009). The ileal immunoglobulin gene rearrangement of B cells occurs with unknown knowledge of DNA rearrangement and post-recombinant modification such as somatic hypermutation or gene conversion in this area. These result in promoting generation of specific antibody modifications of the immunoglobulin genes in these B cell follicles. B cell lymphopoiesis occurs in fetal bone marrow but not in adult bovine tissues resulting in no new immunoglobulin rearrangement during bovine adult life. Therefore the bovine responds immunologically with the fetal and neonatal peripheral B cell production.

Vitamin B₁₂ is utilized for B cell proliferation to form plasma cells which secrete antibodies necessary for normal adaptive immune response (Tamura, et al., 1999).

Plasma cells are a result of antigen stimulated B cells formed in the lymph node cortex and the marginal center of the spleen (Tizard, 2009). Plasma cells undergo migration to different tissues of the body but are found in greatest numbers in the spleen, bone marrow, and medulla area of lymph nodes which respond to antigen stimulus producing 10^4 immunoglobulin molecules per second (Tizard, 2009). These cells are removed from the body by apoptosis except for a small number that survive as precursor memory cells (under the expression of bcl-2 which protects against apoptosis) which lie in the cortex and form germinal centers for further development of plasma cells. This second antigen stimulation results in a faster and more robust response.

The ability of high-affinity antibodies to neutralize toxins, viruses, or bacteria pathogens and their products from the body are very complex specific reactions of the foreign protein, antigen, and the immune system. Many pathogens cannot be neutralized by antibody and must be destroyed by other immunological means. Many pathogen-specific antibodies do not bind to neutralizing targets on pathogen surfaces and need to be linked to other effector mechanisms. Another important defense mechanism is the activation of a variety of accessory effector cells bearing receptors called Fc receptors because they are specific for the Fc portion of antibodies of a particular isotype. Through these receptors, accessory cells (macrophages and neutrophils, often referred to as antigen presenting cells) dispose of neutralized microorganisms and attack resistant extracellular pathogens. This immune mechanism presents maximum effectiveness of all

antibodies regardless of where they bind. Accessory cells the phagocytic cells (macrophages and neutrophils), which ingest antibody coated bacteria and phagocytize them. Other immune cells; natural killer (NK) cells, eosinophils, basophils, and mast cells which are triggered to secrete stored mediators when their Fc receptors are engaged to react to antigen protein (Janeway, 2001). These accessory cells (AC) are activated when their Fc receptors are aggregated by binding to the multiple Fc regions of antibody molecules coating a pathogen. They can also be activated by soluble mediators, which include products of the complement cascade, which can itself be activated by antibody.

Antibody-coated pathogens are recognized by accessory effector cells (macrophages and neutrophils) through Fc receptors that bind to the multiple constant regions of the bound antibodies. Binding activates the accessory cell and triggers destruction of the pathogen. Fc receptors comprise a family of proteins, each of which recognizes immunoglobulins of particular isotypes (Janeway, 2001). Fc receptors on macrophages and neutrophils recognize the constant regions of IgG or IgA antibodies bound to a pathogen and trigger the engulfment and destruction of IgG- or IgA-coated bacteria. Binding to the Fc receptor also induces the production of microbicidal agents in the intracellular vesicles of the phagocyte. NK cells, tissue mast cells, and blood basophils also release their granule contents when their Fc receptors are engaged (Janeway, 2001).

Cobalt trial results from Vellema et al. (1994) showed B₁₂ deficient Trexel lambs had a marked decrease of cell mediated immunity, specifically T cell response. Immune responses against gastrointestinal nematodes are mainly cell-mediated (Haight al., 1989;

Gill, 1994). This explains the increased parasite loads in B₁₂ deficient animals studied by many scientists (Vellema, et al., 1994; MacPherson, Gray. and Mitchell.1987). Research completed related to immune system function reflects one study in evaluating the increased viability of lambs born to ewes supplemented with Co during gestation (Fisher and Mac Pherson, 1990a). A study on the effects of parasitism with *Ostertagia* infestation on decreased neutrophil function in Co deficient cattle was completed by Mac Pherson and Gray in 1987. The results of Johnson study demonstrated that low levels of dietary cobalt leads to an early impairment of phagocytic function (Johnson et al., 2010). Low levels of dietary Co had an early and significant effect ($P < 0.05$) on the oxidative respiratory burst of PMN (neutrophil activity), which was recorded one month after initial Co deficiency (Johnson et al., 2010). Johnson noticed that goats fed low levels of Co reflected a significant decline in their serum vitamin B₁₂ levels, red blood cell counts, hemoglobin, and red blood cell indices after four months on a ration with low Co. Low levels of serum vitamin B₁₂ have been shown to demonstrate lower rates of phagocytosis of bacteria and hexose monophosphate shunt activity of their neutrophils (Kaplan and Basford, 1976). Trials using a candidacidal assay reported that within a few weeks after feeding a Co-deficient diet that the neutrophils of calves experienced a significant decrease in their ability to kill *Candida albicans* and that this effect was noticed before there was a measurable decline in serum levels of vitamin B₁₂ (Patterson and Mac Pherson, 1990 and Mac Pherson et al., 1987). It has been proposed that because vitamin B₁₂ is known to play a role in DNA and protein synthesis that the production of one or more enzymes involved in the hexose monophosphate shunt might be reduced in cells

(Kaplan and Basford, 1976). This would dramatically affect the antibody response in Co deficient calves.

A cobalt study by Grotelueschen et al. (2001) showed higher *Mannheimia haemolytica* titers in cows supplemented with cobalt. Completed work by Sager resulted in increased antibody response to a single antigen (*Brucella abortus*) with increased NRC Co supplementation (Sager, 2011). More recent work with Co supplementation (above NRC requirements) in Co treated pre-weaned beef calves resulted in increased *Mannheimia haemolytica* leukotoxin titers compared to control calves (Sager, 2014 unpublished data). Feedlot health was improved in the treated calves versus the controls during the feeding period and analysis of final carcass traits will be analyzed during the summer of 2014.

Nutrients such as methionine, (dependent on Co for formation by methionine synthase a methyl cobalamine dependent enzyme), is required for production and mobilization of immune cells to combat invading pathogens (Fernandes et al., 2008). The immune system is dependent upon sensitive nutrients, such as methionine and fatty acids, for proper immune function such as antibody production from plasma cell secretion (Jutila, 2011).

Methionine as an amino acid absorbed by enterocyte cells, metabolized in the liver, and utilized by immune cell production reflects the complexity of methionine metabolism in the body. Methionine is important in the oxidative burst phagocytosis of neutrophils in innate immunity and is vital for pathogen infection to be controlled before systemic infection results (Fernandes et al, 2008).

Interaction of cobalamin-amino acid nutrition with the immune system, especially the amino acids of glutamine, methionine, and cysteine, are involved in the production of glutathione and vital immune cells (Kudsk, 2000). Methylcobalamin is necessary for methionine a precursor of cysteine and taurine, a methyl group donor for biosynthesis, is involved in nucleotide synthesis of immune cells (Lugton, 1999). Methionine is an important component in glutathione synthesis as glutathione, an antioxidant of extreme importance in phagocytosis, and oxidative burst of neutrophil activity. Methionine studies show evidence that additional levels of dietary methionine and cysteine can reduce adverse effects on immune system stimulation. The results lead to increase protein metabolism in skeletal muscle growth (Miles, 2010).

Methionine deficiency is a compromising factor in cell mediated immunity, by altering antibody response to antigens affecting humeral immunity. Phagocyte function is negatively impaired, decreased natural killer cell action, and decreased or altered cytokine signaling (Fernandes et al., 2008). There is well documented evidence that methionine modulates T-cell activation (Yaqoob, 2003). Amino acids, especially methionine, have shown to be involved in immune system response (Fernandes et al., 2008).

Previous research on Co has been done world-wide as described above but only related to growth, production, or nutritional performance. Until this date no studies have been completed on Co influencing beef cattle production or health by affecting immune response.

Summary

Given improvements in beef production since dietary Co concentrations were developed, research is needed to define Co concentrations using modern production practices. Cobalt is a cofactor in several key metabolic processes in the body, particularly processes involved in the immune function. Thus, evaluating Co concentrations deserves attention.

CHAPTER THREE

A PRELIMINARY STUDY ON THE EFFECTS OF COBALT
SUPPLEMENTATION ON RB51 BRUCELLA ABORTUS ANTIBODY RESPONSE
IN WEANED BEEF CALVES

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A PRELIMINARY STUDY ON THE EFFECTS OF COBALT
SUPPLEMENTATION ON RB51 BRUCELLA ABORTUS ANTIBODY RESPONSE
IN WEANED BEEF CALVES

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Abstract

Cobalt (Co) is utilized by rumen microbial organisms for the synthesis of vitamin B₁₂ which is necessary as a cofactor for vital metabolic pathways in lipid and carbohydrate energy metabolism. Co supplementation has recently attracted attention because of increased carcass weights in feedlot cattle and increased milk production in dairy cattle. Past Co supplementation studies have focused on performance and growth. The objective of this study was to evaluate the influence of different levels of Co supplementation on humoral immune response to *Brucella abortus* in weaned beef calves. Twenty seven castrated male calves (Angus x Hereford x Red Angus x Lowline cross) of initial BW 450 lb \pm 50 lb were randomly selected from eighty-five head of steer calves for the study. Calves were randomly assigned to receive supplemented Co treatment rates of 0.139 ppm, 0.489 ppm, or 0.889 ppm. These treatment levels correspond to approximately NRC, 4 X NRC, and 10 X NRC recommended levels. Overall Co supplementation resulted in increased antibody response ($P < 0.004$) to RB 51 *Brucella abortus*. Improved humoral immune function has potential of reducing sickness, improving health, and increasing profits in beef calf post-weaning production. Nutritional management efforts to minimize health problems affect production throughout life. Results from this study may suggest that increasing NRC Co supplementation increases beef calf health through improved immune response.

Key words: humoral immune function, *Brucella abortus*, supplemented cobalt, weaned beef calves.

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Objective of Study

The objective of this study was to evaluate the influence of different levels of cobalt supplementation on the humoral immune system in weaned beef calves. Co treatment rates were (0.139 ppm, 0.489 ppm, and 0.898 ppm. These treatment levels were approximately NRC, 4 times NRC, and 10 times NRC (recommended by Paterson, 2010). The study hypothesis was increased NRC Co will positively influence humoral immune function in weaned beef calves. Individual calves will be vaccinated and evaluated for antibody titer response to *Brucella abortus* RB51 vaccine and treated as experimental units. RB51 Brucellosis vaccine was selected as the vaccine (antigen) for immune response because of ease and cost in determination of serum antibody response and is a vaccine commonly used in beef cattle production. The titer levels will be evaluated (Department of Immunology and Infectious Disease, Montana State University), and different titer levels will be used to detect immune response. Differences in titer levels will be considered significant with $P < 0.05$.

Introduction

All animals require cobalt (Co) as an integral component in vitamin B₁₂. Co is utilized by rumen microbes for vitamin B₁₂ production. Vitamin B₁₂, which is absorbed in the ileum, is a vital cofactor in specific metabolic enzymes in lipid and carbohydrate energy metabolism. Cattle obtain cobalt from forages according to availability of Co from the soil (Van Soest, 1982). Cobalt deficiency is strongly geographically and geologically dependent on the element's concentration and availability in the soil (Suttle, 2010.). Early studies of Co were directed to diagnosing wasting disease syndromes;

clinically observed as ill-thrift, weight loss, anorexia, and poor production in ruminants in certain geographical areas of the world (Underwood and Suttle, 2004, Suttle, 2010).

White liver disease (OWLD) in sheep (common in Co deficient areas) is a result of Co deficiency limiting synthesis of vitamin B₁₂ resulting in abnormal lipid metabolism and accumulation of free fatty acid (FFA) deposits due to reduced β oxidation of FFA displacing normal liver tissue (Underwood and Suttle, 2004, Suttle, 2010). Normal mobilization of FFA into triglycerides for liver export is decreased as very low density lipoproteins (VLDL) require two vitamin B₁₂ derived cofactors in lipid metabolism. Vitamin B₁₂ deficiency (due to Co deficiency) causes accumulation of triglycerides to liver tissue.

Ruminant production was restricted in certain areas in the world because of unknown causes until the early 1900's when New Zealand scientists worked with "bush sickness" and "wasting disease" cases, first considered as iron deficiency. Later Underwood and Filmer (1935) discovered the syndrome was due to cobalt deficiency. Cobalt treatments with as little as 1 mg per day resulted in reversal of the disease syndrome (Maas, 2005). This discovery allowed the grazing of previously unusable large coastal areas of Australia and other Co deficient areas of the world (Suttle, 2010).

Differences in micro-mineral intake, absorption, and metabolism of beef cattle often determine response to infection and disease and are influenced because of age, breed, physiological status (pregnancy and lactation), and stress (feed or water antagonists, climatic, physiological, and environmental factors) (Engle, 2001). Stress stimulates cortisol production which negatively affects immune function. Deficiencies of

Co can result from breed differences as continental breeds require as much as twice the mineral requirements as British breeds (Puls, 1992). Cobalt availability is influenced by geographical areas with antagonistic interactions of soil and water minerals. A Co deficiency problem with additional stress can lead to sickness and disease. Beef cattle production has changed dramatically since NRC mineral recommendations were established in the 1950's. The objective of this study was to prove the hypothesis that increased NRC Co levels are needed for increased humoral immune response in weaned beef calves in beef cattle production and increased NRC Co will improve health post weaning.

Materials and Methods

Twenty seven spring born steer calves (Angus x Hereford x Red Angus x Lowline) of initial body weight (BW) 450 ± 50 lb were randomly selected from a group of eighty-five animals raised on a ranch in southwestern Montana. The calves were raised without antimicrobials, ionophores, or growth promoting implants. Calves were vaccinated October 20, 2010 with Vision 7+Somnus,^a Express5 PMH,^b and treated for parasites topically with Dectomax.^c Twenty days later (on November 09, 2010) calves were weaned, acclimated for 20 days, prior to the study, and randomly assigned to one of three dietary cobalt treatments.

Care, handling, and sampling of the animals were done with the approval of the Montana State University Animal Care Committee. The 90 day experimental phase was conducted at Muddy Creek Ranch, Wilsall, MT. The steers were housed in three pens of nine head each without shelter for the duration of the study. At day 1 of the trial, calves

were given IDS (individual electronic identification ear tags), weighed individually, bled via tail vein, liver biopsied, and randomly assigned to one of the three dietary treatments of 0.139, 0.489, and 0.898 ppm cobalt proteinate,^d added to a commercial cattle mineral supplement (Table 1). Supplements of Co provided NRC recommended level in treatment 1 and four and ten times NRC levels in treatment 2 and 3 respectively. (Recommended by my advisor, John Paterson PhD) The mineral supplements were fed ad libitum for the duration of the trial and were routinely monitored for consumption and wastage twice weekly. Mixed grass-alfalfa hay was fed ad libitum to meet requirements to afford weight gain between 1.25-2.0 lb. / day. Hay rations were fed once daily and refusals recorded every 14 days. On day 60, steers were weighed, and vaccinated with a *Brucella abortus* RB51^e by approval of APHIS and the office of the Montana State Veterinarian, Martin Zaluski. On day 90 cattle were weighed, bled via tail vein and cobalt liver biopsied.

Treatments consisted of 0.139 ppm of cobalt, 0.489 ppm cobalt, and 0.898 ppm cobalt (Cobalt proteinate, Balchem Corp, Salt Lake City, UT). Cobalt supplementation was completed with added cobalt in a commercial mineral mixture in granule form fed free choice (please see table 1 next page). During the 120 d growing period, steers were fed a mixed grass-alfalfa hay (free choice) under natural beef feeding guidelines to grow approximately 0.5-1.0 kg / d. Rations were fed once daily and refusals recorded every 14 d. On d 60, steers were weighed, ear tagged, and vaccinated with a *Brucella abortus* RB51 (2ml subcutaneously) by approval of APHIS and Montana State Veterinarian, Marty Zulaski (per. Comm., 2010).

Cobalt liver biopsies were obtained at d 1 and at d 90, by the Pearson and Craig technique. Before incision, biopsy sites were clipped of hair, scrubbed with betadine (Purdue Fredrick, Norwalk, CT) and flushed with Nolvasan (Pfizer Animal Health, Exton, PA) then blocked for anesthesia with 2% lidocaine (Vedco Pharmaceuticals, Nampa ID). A core liver sample was obtained using a modified Jan Shide bone marrow biopsy punch (0.4 cm diameter x 10 cm in length), via a true-cut technique (Pearson and Craig 1980). Liver biopsy samples were rinsed using a 0.10 M physiological buffered saline solution (pH 7.4). Samples were then placed in aluminum foil, folded in, and frozen in liquid nitrogen in a semen tank until Co analysis was completed by ICP analysis at the United States Fish Technology Center, Bozeman, MT.

Before liver biopsy incision, biopsy sites were clipped of hair, scrubbed with Betadine^h and flushed with Nolvasan^c then anesthetized with 2% Lidocaine HCL.ⁱ A core liver sample was obtained using the Shackleford-Courtney Liver Biopsy Instrument (0.2 cm diameter x 25 mm in length), via a true-cut technique. Liver biopsy samples were rinsed using a 0.10 M physiological buffered saline solution (pH 7.4). Samples were then placed in aluminum foil, folded in, and frozen in liquid nitrogen until analysis by Inductively-Coupled Plasma Mass Spectroscopy.

Sample analyses of the grass-alfalfa hay were prepared by Midwest laboratory^f (Table 3) and water analysis was completed by the Montana Diagnostic Lab^g (Table 4). Cobalt proteinate analysis was conducted by Balchem Corporation.^a Blood antibody titers for RB51 Brucella abortus was measured by ELISA and completed by the Department of Immunology and Infectious Diseases, Montana State University,

Bozeman, MT¹ (Figure 1). Liver mineral analysis was completed by Inductively-Coupled Plasma Mass Spectroscopy at the US Fish Technology Center, Bozeman, MT.

Statistical analysis of the antibody titers was completed using a one way ANOVA means (Fisher's Least Significant Difference Test). Difference was considered significant at $P < 0.05$. Prior to statistical analysis, assumptions were made that normality of the calves does exist and the titer measurements are independently distributed with the mean and the standard deviation (N, mean, and SD). Constant variance was assumed and independence is made as the pen of 9 calves was used as an experimental unit for both the immune study on *Brucella abortus* titers and for the Co study. Paired biopsy Co levels (first and last day liver Co biopsy data) were compared.¹ *Brucella abortus* ELISA titer data was evaluated by the same ANOVA method. Because of data obtained from animal repeatability in this study, the evaluation of Brucellosis data was considered the highest priority statistically.

There is no evidence that cobalt supplementation fed to steer calves at weaning time in the level of 0.139 ppm, 0.468 ppm, or 0.898 ppm did result in difference in mean liver biopsy cobalt levels (two-sided p value of $P < 0.05$, $df = 8$) from a paired t-test. However, supplemental cobalt fed to weaned steer calves did show a strong increase in titer response levels to RB51 Brucellosis vaccine (two-sided p-value of $P < 0.01$, $df = 26$). The 95% confidence interval for treatment 2 (0.468ppm) for the titer difference of RB51 *Brucella abortus* vaccine is +2.21 to +2.51 pg / ml or 1.02 to 1.225 times as much antibody response as in the control treatment group. Confounding variables to this study would be differences in Co mineral consumption (per head per day), social behavior

differences of calves in confinement, and the control pen being protected to weather or environmental stresses because of the wind break fence acting as a protection to wind and a shed for protection to wind and moisture.

Statistical analysis on immune system function was completed using a one way ANOVA means (Fisher's Least Significant Difference Test) variance as calves were experimental units and a difference was considered significant at $P < 0.05$. Calves were randomly assigned to one of three treatments, there are assumptions that normality does exist and they are independently distributed with the mean and the standard deviation (N, mean, and sd). Assumption of constant variance is made and independence is made as each calf is an experimental unit in the immune study on *Brucella abortus* titers and the pen of 9 calves was used as an experimental unit for the Co study. The data was grouped into two separate experimental collections; one involving immune system responses of RB51 *Brucella abortus* titers (40 days apart) and the second experimental collection of paired liver biopsy data > 90 days apart. First day liver biopsy Co levels and last day liver biopsy Co levels (paired liver Co biopsy data) were compared and the differences reported so a t-test (paired sample test) using the differences of data was used (SAS, Cary, NC). Differences in paired Brucellosis titer data were evaluated. Expected data did not show any outliers so a ranked sum test was not considered. The evaluation of Brucellosis data was considered the highest priority due to each animal was an experimental unit and it is repeatable in this study data collection.

Analytical Procedures

Feed samples for analysis of the grass-alfalfa hay were prepared by Midwest laboratory (Omaha, NE) (Figure 19, page 134) and water analysis was prepared by Montana Diagnostic Lab (Montana Diagnostic Lab, Bozeman, MT) (Table 4). Cobalt proteinate analysis was prepared by Balchem Corporation (Balchem Corporation, Salt Lake City, UT) and donated for the experiment. Blood titer analysis for RB 51 *Brucella abortus* were completed by the Department of Immunology and Infectious Diseases, College of Agriculture, Bozeman, MT and liver biopsy analysis for mineral profiles were completed by the United States Fish and Wildlife Service, Bozeman Fish Technology Center, Bozeman, MT.

Results and Discussion

Data were collected on initial and final BW for the experiment even though steers were fed a ration for minimal growth. There were no statistical differences in any of the three treatments ($P < 0.05$) groups in BW gain during the trial (Table 3.2).

Antibody response to *Brucella abortus* RB51 vaccination was higher in the 4 times and 10 times NRC treatment groups ($P < 0.004$). Results of antibody response between the 4 and 10 times NRC treatment groups did not differ statistically. Results from this study suggest that NRC recommended Co levels should be increased to provide increased antibody titers and immune system response in weaned beef calves. Starting Co liver levels did not significantly differ between the treatment groups but did vary between animals. Liver Co concentrations decreased during the experiment but did not differ between treatment groups ($P < 0.05$; Figure 3.2). This compares with similar trials

completed by other researchers (Kincaid et al., 2003; Tiffany, et al., 2003, Weiss, 2005).

Decreased concentrations were normal for growth and winter feeding stress.

Weaning is considered the most stressful time in the life of a calf and often results in sickness. During times of stress plasma cortisol suppresses the normal immune function in beef cattle (Smith, 2005). The total health costs in beef cattle production in the United States from sickness and lost production are over \$2.2 billion annually (Radostits, 2007). Economic losses from morbidity and mortality associated with bovine respiratory disease (BRD) in weaned beef cattle continue to plague the beef cattle industry (Galvayan, et al., 1997). Past research has shown micro-mineral deficiencies influenced humoral immune response in weaned beef calves (Swecker, et al., 1989). Nutritional management decreases sickness and improves profitability (Smith, 2005). Nutritional management efforts to minimize health problems affect production throughout life (Smith, 2005). Co supplementation during this period could decrease sickness, improve immune response, and increase performance in weaned beef calves.

In the past 50 years, United States beef cattle production has increased nearly 50% due to improved genetics, advances in nutrition, bio-technology, advances in animal health, and value added management (Paterson, 2010). The National Research Council states requirements for Co at 0.1 mg/kg (DM intake) since first published in the 1950's when production requirements for beef cattle production were two-thirds of present day production expectations (NRC, 2005). Recommended NRC Co levels were derived from experiments during the 1950's from cattle that were genetically different, were raised with a different production focus, and fed different rations. At present beef cattle are

grown in larger feeding facilities with increased population densities and increased pathogen challenge. Today beef cattle production involves animals that are 35-40 % anatomically larger, grow at increased rates, developed with an economical focus in muscle growth with efficient gain that were not considered fifty years ago. Since these factors have all changed, the dietary requirement for Co could be higher in beef cattle production of today. Previous research has demonstrated increased NRC Co levels are needed for minimizing metabolites of propionate metabolism that negatively affect intake and growth (Stangl, 2000).

Co is also necessary for the storage of copper (Cu) in bovine liver tissue (Kreplen, 2009). As Cu has been shown to affect function of the immune system of beef cattle, Co may play an indirect role in immune function by altering Cu metabolism (Kreplen, 2009; Sager, 2011; Figure 3.3). The study showed a linear correlation between liver Co and Cu levels supporting experiments that Co is needed for Cu storage in liver tissues (Kreplen, 2009). This study has shown increased NRC recommended supplement Co levels have positively affected ($P < 0.004$) humoral immune response to a single antigen in weaned beef calves.

Improved antibody production in weaned beef calves fed increased dietary Co is most probable a direct result of increased vitamin B₁₂ production by rumen microbial synthesis. Recent studies have shown vitamin B₁₂ does play an important part in immune system regulation with increases in NK (natural killer) cell activity and CD8⁺ cells effecting cytotoxic cells therefore acting as a immunomodulator in immunity (Tamara, et al., 1999). Vitamin B₁₂ is critical for folate metabolism in DNA synthesis required in B

cell proliferation and plasma cell production for antibody response (Tamara, et al. 1999). Vitamin B₁₂ is a compromising factor in cell mediated immunity by altering antibody response to antigens (Fernandes, 2003).

Ruminal microbial vitamin B₁₂ derived cobalamins (Cbl) are micronutrients essential for the synthesis of methylcobalamin (MeCbl) and adenosylcobalamin (AdoCbl), the respective cofactors in methionine synthase (MS) and mitochondrial L-methylmalonyl-CoA mutase vital to cystol metabolism. Methionine is required for production and mobilization of immune cells to combat invading pathogens (Fernandes, 2003; Underwood and Suttle, 2004).

This experiment has tested the hypothesis that increased NRC Co levels increased antibody response in weaned beef calves. Results determined by this study may be a fit for novel approaches to improve efficiency in beef production medicine by increased immune system response in weaned beef calves, and promote increased profitability in beef cattle production. Although there were no differences in health condition between the treatment groups during the study, the reduction of stress from weaning with no transportation to a feedlot (feedlot was on the ranch) was a factor. Also this ranch management has had excellent success post weaning in past years because of excellent health programs directed at reducing post weaning health problems. Calves were vaccinated at branding and again three weeks before weaning with both viral and *Pasteurella* spp. immunization components. This experiment was completed as a pilot study to test the hypothesis that Co supplementation in excess of NRC requirements positively affects humoral immune response in beef cattle. Individual Co intake levels

were not measured in this study as the pen of nine calves was the experimental unit (df = 0). Plans have been made for another study with individual feeding of calves, post weaning health in the feedlot, and evaluation of final carcass characteristics to evaluate Co fed pre-weaning affecting post weaning bovine respiratory disease (BRD).

Implications

Improved immune function as a result of Co supplementation could lead to improved welfare and health at weaning in beef calves. Increased supplementation of Co post weaning could improve immune function by increasing antibody production, reducing sickness at weaning, and improve beef calf production with added profit to production units.

Results from this study suggest that NRC recommended levels of dietary Co should be increased to provide improved antibody response in beef calves at weaning. Treatment levels of 4 X NRC Co levels showed increased RB51 Brucella abortus antibody levels ($P < 0.004$) in weaned beef calves in this study.

Cattle mineral was fed free choice and acted as the base mineral for the Co supplement to be added for treatments. The mineral was fed during the entire length of the trial and was checked on every two days for consumption and wastage.

Ingredient	Minimum	maximum
Salt (granular form)	17.5%	21.0%
Co	38 ppm	
Cu	2200 ppm	
Se	35ppm	
Zn	7500 ppm	

Scope of Inference

With the knowledge of this study supplementation of Co would not increase liver Co biopsy levels but would increase titer levels of RB51 vaccine. By supplementing Co into weaned feed rations or supplementing Co in mineral mixes, for weaned beef calves at weaning, Co could improve beef calf health at weaning and decrease sickness therefore increasing performance and profit. Since this study was a random assignment, therefore a strong causality can be made, and the difference in Co liver levels were due to increased supplementation of cobalt. *Brucella abortus* titer level differences can be associated with increased NRC recommended Co supplementation levels and differences can be inferred that Co supplementation above NRC recommended levels increases immune system function (antibody response) in weaned beef calves due to measured differences in RB51 *Brucella abortus* vaccination.

Results from this trial suggest that recommended NRC Co levels should be increased to provide increased antibody response to immunization of *Mannheimia haemolytica* in weaned beef calves. Results showed a statistical difference between NRC Co levels and the treatment groups of 2 X NRC Co and 4 X NRC Co levels fed free choice during this study. Differences between 2 X NRC Co and 4 X NRC Co were not statistically different. The treatment group of 2 X NRC Co showed a difference from the control group (NRC Co) at P= 0.004. The group provide some windbreak protection (7 foot solid fence built on the north side of the pen) from wind showed an improvement of 0.227 kg/ hd/ day which reflects the importance of minimal protection from wind chill at this latitude and altitude in beef cattle production.

Results from data obtained in this trial suggest NRC Co levels should be increased to provide increased immune response to vaccination in weaned beef calves. Treatment levels of 4X NRC recommended levels of Co provided increased RB51 antibody levels in weaned beef calves in this experiment.

Other trials need to be designed with individual experimental units to evaluate individual Co intake compared to results of Co concentration and AB production. A study with over 2000 weaned beef calves supplemented with Co 45 d before weaning and shipment to the feedlot is being considered for this fall and epidemiology data completed to evaluate pulls and morbidity of Co supplemented calves compared to controls in the overall feedlot population. This future study should be considered as to repeat results of this study and also evaluate results in an applicable and larger scale.

Liver biopsy samples were collected using humane treatment as sites were surgically prepped and areas were injected with Lidocaine 2% (Vedco pharmaceuticals, St. Joseph, MO) before biopsy liver samples were collected. Animals were treated with 10 ml of Benzathine penicillin (Agilabs, St. Joseph, MO) after biopsy collection.

Data was collected on initial and final BW for the experiment even though steers were fed a ration for minimal growth. There were no statistical differences in any of the three treatment groups in BW gain during the trial.

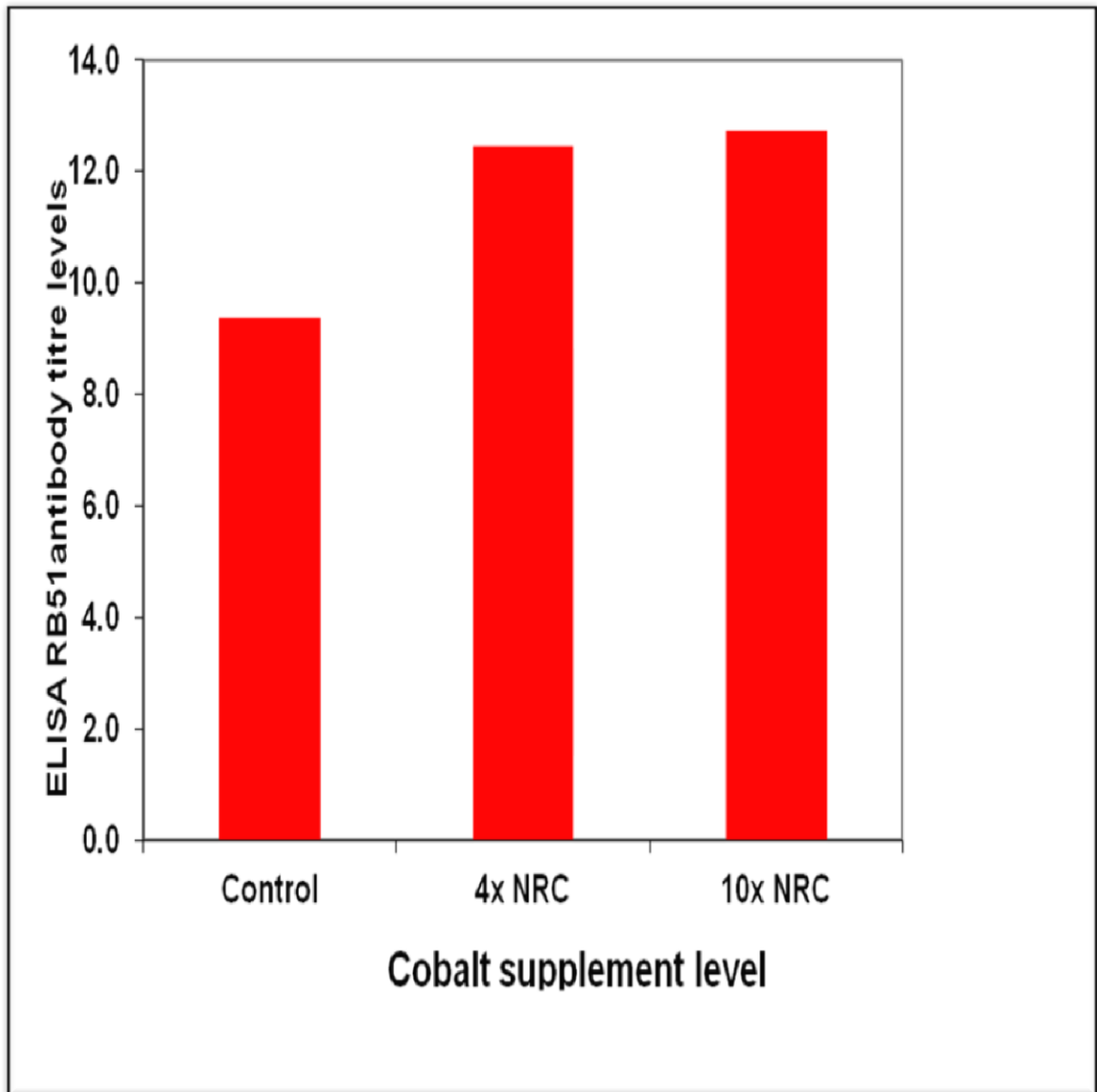


Figure 3.1. Comparison of control (NRC), 4X NRC, and 10X NRC levels of supplemented Co to RB51 antibody titre levels.

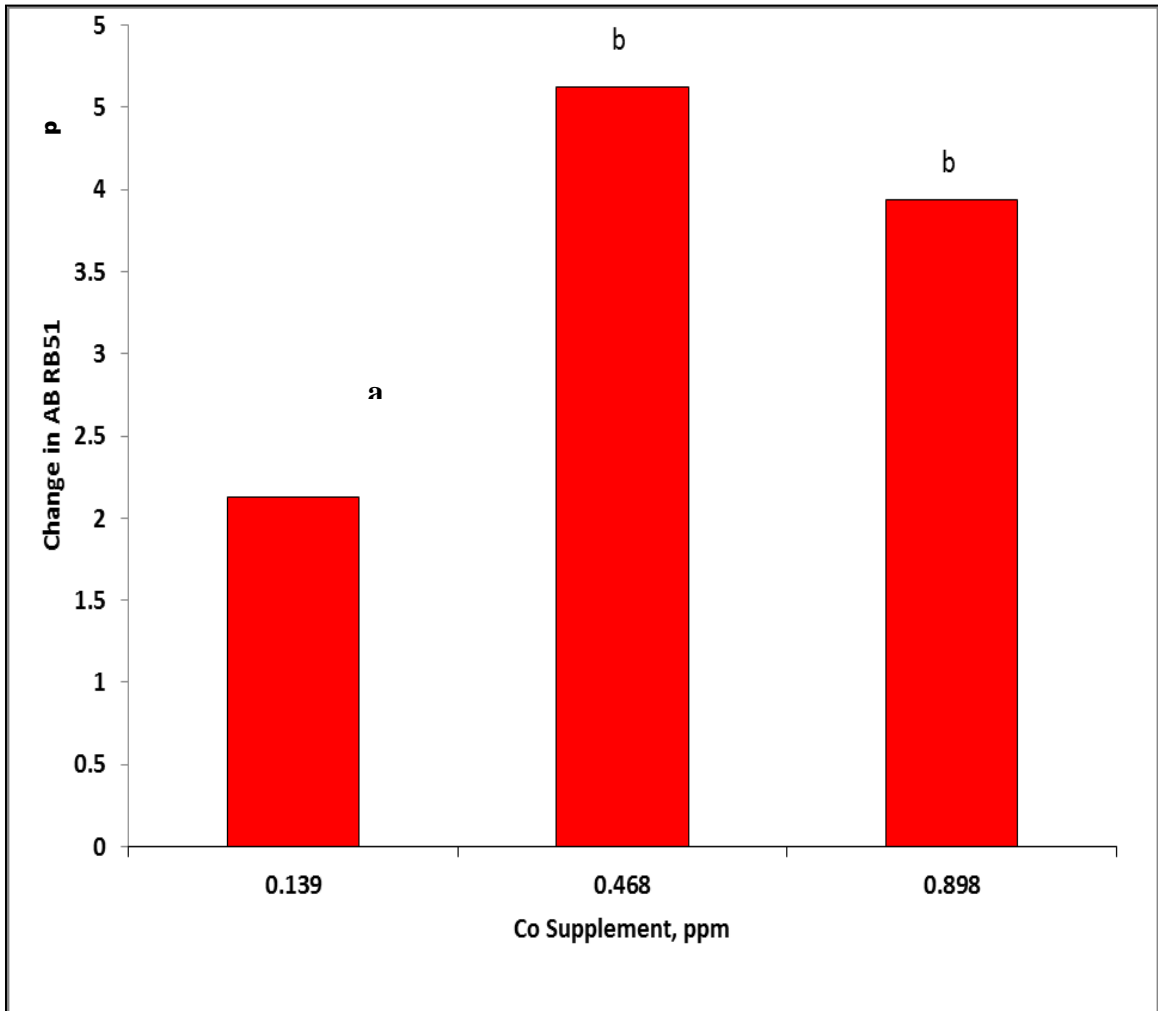


Figure 3.2 Changes in RB51 antibody titer

Table 3.2. Body weight gain over the 90-d cobalt supplementation period of weaned beef calves			
	Co supplementation, ppm		
Variable	0.139a	0.468	0.898
BW, kgb	44.5	42.4	51.6
Differences in concentration of liver Co compared to treatment group NRC group (0.139 ppm) 85.3% decrease = 1.94 $\mu\text{g}/\text{g}$ tissue, 4 X NRC (0.468 ppm) 81.4% decrease = 4.55 $\mu\text{g}/\text{g}$ tissue, and 10 X NRC (0.898 ppm) 74.1% decrease = 3.65 $\mu\text{g}/\text{g}$ tissue during the trial period			

Table 3.3. Liver cobalt concentration change over the 90-d cobalt supplementation period of weaned beef calves			
	Co supplementation, ppm		
Variable	0.139a	0.468	0.898
Co, ug/gb	-0.81	-0.57	-0.43

a Had access to windbreak fence. b SEM = 0.83.

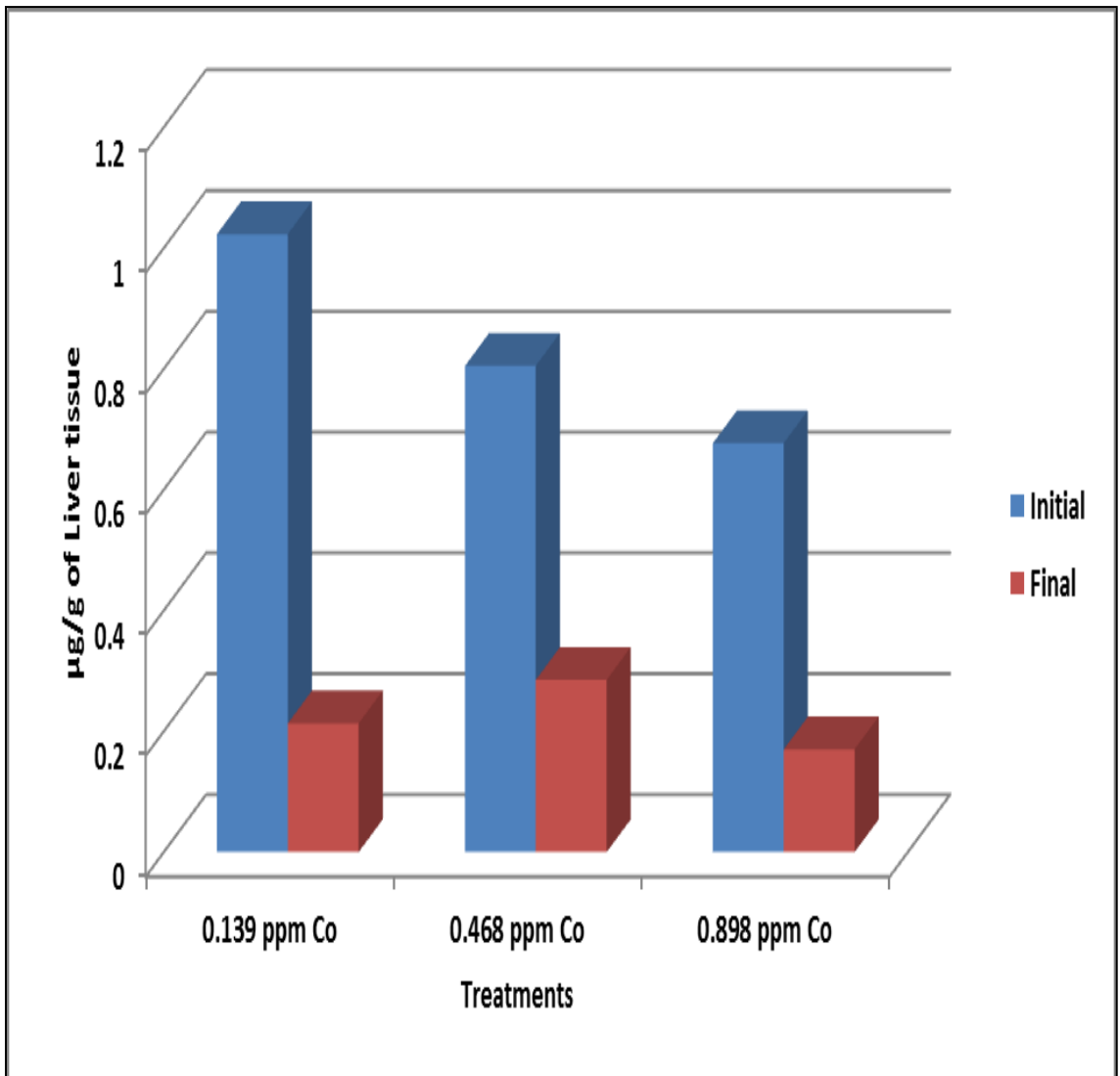

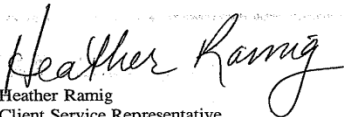


Figure 3.3. Histogram showing decrease in Co concentrations during trial.

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MONTANA STATE UNIVERSITY DR JOHN PATERSON 103 ANIMAL BIOSCIENCE BLD BOZEMAN MT 59717-			SAGER MUDDY CREEK	
FEED NUTRIENT ANALYSIS				COMMENTS
Date Sampled	Received	Reported	Lab #	
03/21/11	03/23/11	03/25/11	9711271	
Sample ID: 1 ALFALFA/GRASS				
Feedstuff: MIXED FORAGE				
ANALYSIS RESULTS				
Component	As Sent	Dry Wt.		
Moisture (%)	11.68	///////		
Dry Matter (%)	88.32	///////		
Crude Protein (%)	12.4	14.0		
Acid Detergent Fiber (%)	36.3	41.1		
Total digestible nutrients (%)	49.2	55.7		
Net energy-lactation (Mcal/lb)	0.50	0.57		
Net energy-maint. (Mcal/lb)	0.48	0.54		
Net energy-gain (Mcal/lb)	0.27	0.31		
Sulfur (%)	0.14	0.16		
Phosphorus (%)	0.25	0.28		
Potassium (%)	2.40	2.72		
Magnesium (%)	0.08	0.09		
Calcium (%)	0.17	0.19		
Sodium (%)	< 0.01	< 0.01		
Iron (ppm)	37	42		
Manganese (ppm)	27	31		
Copper (ppm)	5	5		
Zinc (ppm)	12	13		

1. Mineral analysis performed by ICAP using a wet digest procedure.
2. Midwest Labs uses wet chemistry methods for all forage and feed analyses. Forage and Silage testing methodology follows the National Forage Testing Association (NFTA) recommended methods.
3. Midwest Labs is certified by the National Forage Testing Association (NFTA) for wet chemistry methods and mineral analysis.
4. Analysis for:
(5678) MONTANA STATE UNIVERSITY
Phone: (406) 994-5562


 Heather Ramig
 Client Service Representative
 heather@midwestlabs.com (402)829-9891

The result(s) issued on this report only reflect the analysis of the sample(s) submitted. For applicable test parameters, Midwest Laboratories is in compliance with NELAC requirements.

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Figure 3.4. Analysis of grass/alfalfa hay mixture (Midwest Laboratories, Omaha, Nebraska)

Table 3.4 Nutrient analysis of common feedstuffs

Nutrient Analysis	Corn Stover	Alfalfa Hay	Grass Hay	Corn Silage	Grass Hay	Rx Mineral	Water (Well)
DM, %	85	89.4	89.5	48	81.3	94	-
Moisture, %	15	10.6	10.5	40.6	18.7	6	-
Protein, %	5.9	16.7	10	8.7	9.47	4.79	-
Ca %	0.6	1.5	0.71	0.86	0.68	0.38	65 ppm
P %	0.12	0.21	0.17	0.34	0.25	16.49	-
K %	1.8	2.37	1.2	1.38	2.46	-	-
Mg %	0.25	0.21	0.12	0.25	0.23	4.12	12 ppm
S %	0.17	0.22	0.12	0.13	0.2	0.28	-
Na %	0.08	0.09	0.01	0.03	0.04	12.67	85 ppm
Cl %	0.09	0.41	0.3	0.25	0.54	4.12	15 ppm
Zn ppm	18	21	28	84	22	17,073	-
Mn ppm	90	37	68	52	39	4,107	-
Cu ppm	5	9	5	15	6	3,566	-
Fe ppm	210	215	143	242	119	45	0.90 ppm
Al ppm	109	89	89	231	22	-	-
Si ppm	90	65	325	388	680	-	-
Co ppm	0.1	2.52	1.92	0.05	0.1	48	-
Se ppm	0.05	0.05	0.06	0.05	0.05	41	-
Mo ppm	1.8	3.62	2.64	2	0.98	-	-
I ppm	0.1	0.02	0.05	0.03	0.05	68	-
TDN %	55	66	55	69	52	-	-
NEg	190	325	189	512	185	Nitrate >	3 ppm
NEm	440	610	522	793	520	Sulfate >	350 ppm
NEI	504	609	541	833	539	-	-

Table 3.5 Analysis of CHS 12-6 Mineral used as base mineral for Co supplement addition for trial.			
Calcium	not less than	12.0	%
Calcium	not more than	14.0	%
Phosphorus	not less than	6.0	%
Salt	not less than	17.5	%
Salt	not more than	21.0	%
Magnesium	not less than	2.75	%
Cobalt	not less than	38	ppm
Copper	not less than	2,200	ppm
Iodine	not less than	200	ppm
Manganese	not less than	3,300	ppm
Selenium	not less than	35.0	ppm
Zinc	not less than	7,500	ppm
Vitamin A	not less than	250,000	IU/lb
Vitamin D	not less than	25,000	IU/lb
Vitamin E	not less than	250	IU/lb

Acknowledgements

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ELISA Brucella abortus antibody results were completed by Beata Clapp PhD, Department of Immunology and Infectious Diseases, Montana State University, Bozeman, Montana. Handling, processing cattle, and statistical help was provide by fellow graduate students; Billy Whitehurst, Blake Hauptman, and Ricardo Manzano PhD,. Editing was completed by C.W. Newman PhD and Ricardo Manzano PhD and is recognized as an important part of this paper.

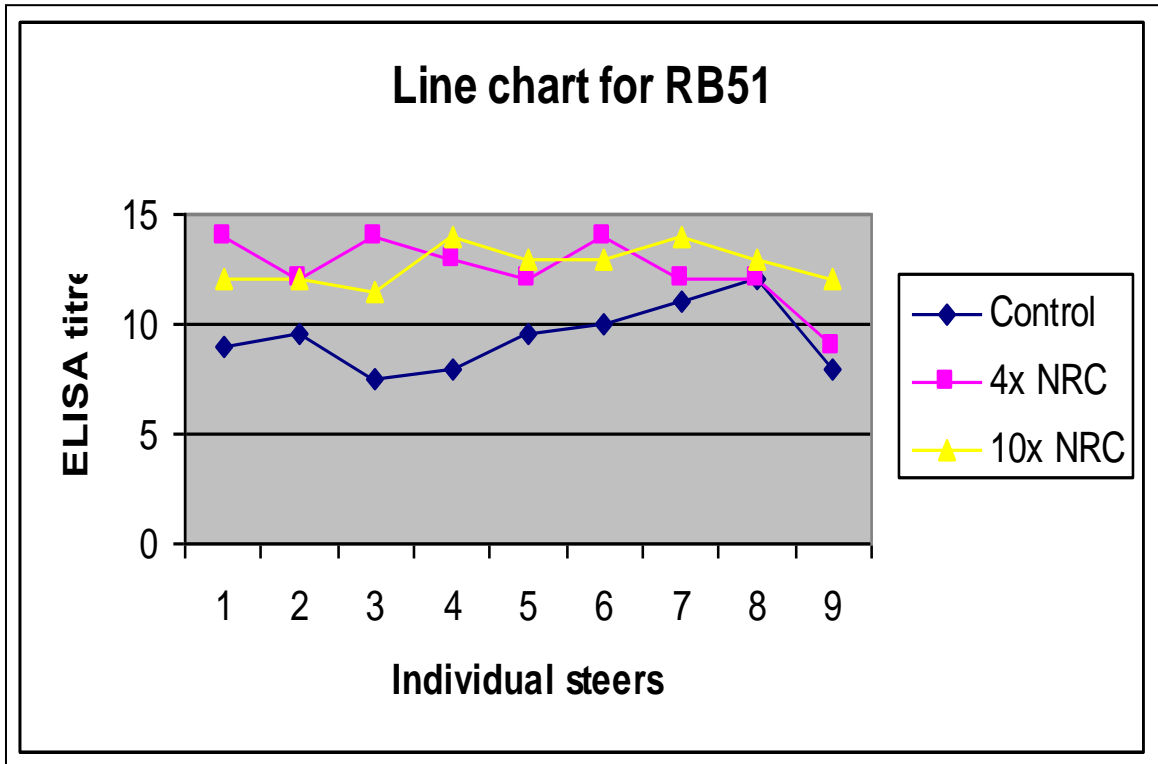


Figure 3.5. Line chart showing treatment results on Co supplementation influencing RB51 antibody response compared to treatment.

Endnotes

a Vision 7+ Somnus,® Merck Animal Health, Summit, NJ

b Express 5 +PMH,® Boehringer Ingelheim Animal Health, St. Joseph, MO

c Dectomax Pour On,® Pfizer Animal Health, Exton, PA

d Balchem Corp, Salt Lake City, UT

e Professional Biological Company, Denver, CO

f Midwest Laboratories, Omaha, NE,

g Montana Diagnostic Lab, Bozeman, MT

h Betadine,® Purdue Fredrick Laboratories, Norwalk,CT

i Lidocaine HCL 2% Vedco Pharmaceuticals, Nampa ID.

j College of Agriculture, Montana State University, Bozeman, MT

k United States Fish Technology Center, Bozeman, MT

l ICP-MS, Perkin Elmer, Optima 5300 DV, Optical Emission Spectrometer,
Waltham, MA.

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CHAPTER FOUR

COBALT SUPPLEMENTATION IN BEEF CALVES AFFECTING
HUMORAL IMMUNE RESPONSE

Contributions of Author

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ABSTRACT

Cobalt (Co) is utilized by rumen microbial organisms for the synthesis of vitamin B₁₂ which is necessary as a cofactor for vital metabolic pathways in lipid and carbohydrate energy metabolism. Historically, Co supplementation studies have focused on performance and growth. The objective of this study was to test the hypothesis that Co supplementation in milk replacer will not positively affect humoral immune response in nursing calves because of non- development of rumen microbes necessary for vitamin B₁₂ synthesis. The study evaluated the influence of different levels of Co supplementation on humoral immune response to *Mannheimia haemolytica*. Fifteen castrated male calves (Holstein) of initial BW 40kg were randomly selected from 50 calves for the study. Calves were randomly assigned to receive supplemented Co treatment rates of 0.10 ppm, 0.20 ppm, and 0.40 ppm in milk replacer fed twice daily. Calves were fed approximately 100 ml/ kg of 28% protein milk replacer with access to *ad libitum* 25% milk base pellets and free choice water. Calves were bled and vaccinated with *Mannheimia haemolytica* on d 40 to analyze existing *Mannheimia haemolytica* antibody titers and were bled on d 68 evaluate *Mannheimia haemolytica* titer response. Differences for *Mannheimia haemolytica* titers were analyzed by a one-way ANOVA means variance, and differences were considered significant at P < 0.05. Increased immune response to *Mannheimia haemolytica* in the treatment groups resulted, but was not statistically significant. Results from this study suggest that increasing NRC Co supplementation in milk replacer has no effect on humoral immune response.

Key words: humoral immune function, *Mannheimia haemolytica*, supplemented cobalt, nursing calves.

INTRODUCTION

Cobalt (Co), a trace mineral, is required by ruminants for rumen microbes to synthesize vitamin B₁₂. Co is utilized by microorganisms for synthesis of cobalamine compounds of which one formation has Co as the nucleus of the large corrin ring molecule of vitamin B₁₂ (Underwood and Suttle, 2004). Vitamin B₁₂ is a vital cofactor in carbohydrate and lipid energy metabolism for growth and maintenance. The only known requirement for the element, cobalt (Co), in ruminants is as a constituent of vitamin B₁₂, (Maas, 1996; McDowell, 2000) which is approximately 4% cobalt by chemical weight (Miller, 1984; Berger, 1984). This means cobalt deficiencies are actually vitamin B₁₂ deficiencies (Underwood and Suttle, 2004). Cobalt (Co) is also necessary for the storage of copper in liver tissue in ruminants as copper is an essential element in the immune system of beef cattle (Kreplen, 2009). Ruminant Co requirements are higher than in non-ruminants because of the microbial need for Co and for non-metabolic vitamin B₁₂ factors such as involvement with cofactors in milk fat depression, ketosis, or both (Elliot, 1980). Co analysis by liver biopsy is considered the gold standard for evaluation as blood concentrations fluctuate and can be misleading during deficiency (Underwood and Suttle, 2004). Co liver biopsy concentrations are low or deficient in diseased animals with pneumonia when copper deficiencies have influenced disease and sickness (Sager, 2002). Previous field

investigation work done on vaccine failure problems in beef calves showed marked Co deficiency in bovine respiratory disease (BRD) (liver biopsy analysis; Sager, 2013).

Cobalt is found in most plants, dependent on soil types and antagonistic properties, and is normally adequate in forages for required intake except for specific coastal areas of the world. Coastal areas have been known to have problems in livestock production with poor growth in neonates, progressive weight loss in adults with resulting poor reproduction, and “wasting” causing death (Filmer and Underwood, 1935; Suttle, 2004). Ruminant production was restricted in these geographical areas because of unknown causes until the early 1900’s when New Zealand scientists worked with “bush sickness” and “wasting disease” cases, first considered iron (Fe) deficiency and later found by Filmer and Underwood (Australian Scientists) that the syndrome was due to Co deficiency. Availability is increased in acid soils of higher moisture content (Van Soest, 2005). Cobalt treatments with as little as 1 mg per day resulted in positive reversal of the disease syndrome (Maas, 2005). This discovery led to the utilization of large coastal areas of Australia and other areas in the world to be used for ruminant grazing and livestock production with Co supplementation. Supplementation can be achieved by field dressing Co on pastures or Co supplementation in mineral supplied to beef cattle.

Cobalt deficiency reflects varying signs based on severity, soil and forage concentration of Co, antagonistic interference by other minerals, and the physiological requirements of the animal (Engle, 1999; Underwood and Suttle, 2004). Depending on Co tissue stores signs may not show for weeks until liver storages are depleted and

enzymes are depleted resulting in decreased appetite and failure of growth. Continual deficiency leads to un-thriftiness, loss of body weight, and acute emaciation. Necropsy signs reflect severe emaciation and total lack of visceral adipose tissue (Smith, 2005). Co deficiency leads to metabolic changes occurring in the liver tissue, resulting in a fatty liver syndrome, with hemosiderization of the spleen. Hypoplasia of the bone marrow results in low red blood cell (rbc) production with lower hemoglobin concentration. Normocytic hypochromic rbc's in lambs and microcytic and hypochromic rbcs in calves result in chronic Co deficiency (Radostits, 2005; Smith, 2005). Treatment with vitamin B₁₂ injections show quick response and almost complete reversal of the syndrome within days to weeks in both lambs and calves (Smith, 2005; Maas, 2005).

Ovine white liver disease (OWLD), a neonatal lamb disease of the United Kingdom and very problematic in specific areas of Scotland was later determined to be associated with Co deficiency (Maas, 2005). Concentrations of VLDL (very low dense lipoproteins) that built up in liver tissues are caused by Co deficiency as vitamin B₁₂ is required for VLDL metabolism and export from liver tissues (Suttle, 2010).

The main source of vitamin B₁₂, formed by ruminant microorganisms, is absorbed from iliem mucosa (in the last section of the small bowel) and transported as cobalamine (transcobalomine) with a carrier protein in the blood. Other sources are feeds or dietary vitamin B₁₂, as corrinoids that bind with R proteins from saliva, and are digested by pancreatic enzymes in the small intestine while cobalamine compounds synthesised in the rumen bind to an intrinsic factor produced by parietal cells in the

abomasum (McKay and Mc Leay, 1981). Bile salts increase binding of cobalamine: vitamin B₁₂ complexes to the receptor sites in the ileal mucosal brush border (Smith, 1997). Cobalt and vitamin B₁₂: corrinoid compounds are absorbed more slowly and less completely than in monogastric animals (Rothery et al., 1953). The corrinoid cobalamine- intrinsic factor complex enter the enterocyte by endocytosis, with the cobalamine compound released from the intrinsic factor protein, is then bound to a carrier protein (transcobalamin), and exits into the circulating blood (Gropper, 2007).

Inefficient use of dietary Co utilized by the ruminant is due to microbial partitioning of Co into active and inactive Co-vitamin B₁₂ compounds referred to as corrinoids. These compounds cannot be absorbed or used in this form (Gawthorne, 1970). Microbial rumen production of vitamin B₁₂ with increased Co supplementation responds within hours to change but the efficiency of absorption decreases with increased intake levels (Underwood and Suttle, 2001).

Because ruminants make poor use of dietary cobalt as rumen microbes partition the Co particles between cobalamine active components and inactive forms of vitamin B₁₂ - like compounds (corrinoids) are un-absorbable to the ruminant (Gawthorne and Smith, 1968). Of all the total microbial production of vitamin B₁₂ produced by rumen microorganisms, only 1-3 % is absorbed in the ileum of the ruminant gastrointestinal tract (Miller, 1984).

With increased NRC levels of Co of 0.20 ppm (Schwartz, 2000) rumen microorganisms synthesized increased vitamin B₁₂ (Bishenhsari, 2010) but constant intake is needed as liver storage ability and mobilization are limited in the ruminant (Underwood

and Filmer, 1935; Maas, 2005). More recent findings indicate the necessity to increase the amount of dietary Co for ruminants up to a level of 300 ± 500 mg/kg DM for optimum rumen microbial activity (Stangl, 2000).

Cobalt is essential for the formation and production of vitamin B₁₂ for coenzyme functions in metabolism. Cobalamin synthesis in the rumen results in the formation of methylcobalamin and adenosylcobalamin compounds each vital in different metabolic pathways in ruminant energy production and immune function.

Vitamin B₁₂ in the form of methylcobalamin is necessary in methyltransferase metabolic enzyme functions, acts as a donor of methyl groups and is involved in one-carbon addition by building carbon chains in metabolism. Methylcobalamin is necessary in methane, acetate, and methionine synthesis by rumen microbes (Poston and Stadman, 1975). Methylcobalamin enables the enzyme, methionine synthase, to supply methyl groups to a wide range of reactions including formate, noradrenaline, myelin, and phosphatidyl ethanolamine (Suttle, 2010).

Vitamin B₁₂, as adenosylcobalamin, is a coenzyme in energy metabolism assisting in converting succinate from propionate in the liver for gluconeogenesis (Suttle, 2010). Adenosylcobalamin is a coenzyme needed for the reaction of methylmalonyl (MMA) coenzyme A to succinyl coenzyme A (Paterson and Mac Pherson, 1990). Methylmalonyl-CoA to succinyl CoA involves methylmalonyl-CoA isomerase or mutase (Gropper. et al. 2007) and is a B₁₂ requiring enzyme that catalases the conversion of methylmalonyl-CoA to succinyl CoA (Underwood and Suttle, 2004).

Vitamin B₁₂ is vital as a cofactor in lipid metabolism and the formation of VLDL's that are mobilized and exported from liver tissues. The loss of normal fatty acid metabolism to form triglycerides in the liver and the export of very low density lipoproteins (VLDL) is due to the need for methylcobalamin formation of VLDL in liver tissue, therefore, the accumulation of triglycerides and a fatty liver syndrome result (Underwood and Suttle, 2004). A deficiency of vitamin B₁₂ causes a buildup of VLDL's in liver tissue giving a characteristic white color that is referred to as OWLD (ovine white liver disease) (Underwood and Suttle, 2004; Maas, 2005, Smith, 2005). This disease is important in the UK and Scotland where Co deficiency exists in the soil and adds to low growth performance and death in lambs.

The importance of volatile fatty acids (VFA) as sources for energy in ruminants has been well understood for many years. As much as 60-80% of the metabolizable energy (ME) can be from VFA'S in rations consumed (Phillipson, 1969). Vitamin B₁₂ is critical in ruminant gluconeogenesis from VFA (volatile fatty acids from microbial synthesis) formation in the rumen. With Co deficiency rumen fluid changes result in propionate microbes decreasing and less propionate is produced leading to less availability of propionate for gluconeogenesis but the most compounding factor is the relationship of Co needed for vitamin B₁₂ synthesis required for metabolic pathways for methylmalonyl-CoA to be changed to succinate-CoA to enter the TCA cycle for energy (Smith, 2005; Suttle, 2010). As a result methylmalonyl- CoA builds up in the blood and depresses appetite causing "wasting disease". Propionate and acetate clearance rates increase as serum vitamin B₁₂ levels decrease (Underwood, 1977; Underwood and

Suttle, 2004). The ability for propionate synthesis to occur in the rumen is normal in deficient Co animals but the ability to metabolize propionate in the blood is greatly inhibited with Co deficiency and methylmalonyl-COA accumulates causing decreased feed intake (Gawthorne, 1968). Acetate clearance in the blood is impaired (Sommers, 1969) yet, the propionate buildup has the greater inhibition of appetite in the ruminant (Farmingham and Whyte, 1993). The essential defect in Co deficiency in ruminants is an inability to metabolize propionic acid produced by microbial carbohydrate fermentation in the rumen (Radostits et al., 2007). In carbohydrate fermentation propionic acid is a by-product and the rate of clearance is dependent on the above coenzymes (Fisher and Mac Pherson, 1990; Underwood and Suttle 2004). In absorption of propionic acid in the blood, methylmalonic acid accumulates and depresses appetite, therefore decreasing feed intake with Co deficient animals (Underwood and Suttle, 2004). This was visually evident in the appearance of “wasting disease in ruminants” seen years ago in Co deficient areas.

This same concept is thought to be the factor in Co deficient steers on corn diets low in Co that have lower final BW and lower carcass weights than in Co supplemented feedlot steers with rations containing 0.15-0.20 ppm (Tiffany et al., 2003). Co supplemented steers showed increased propionate, higher blood glucose levels, and higher vitamin B₁₂ plasma levels than control steers (Kincaid et al., 2003).

In beginning Co deficiency, biochemical changes occur in ruminant fluids and tissues because of the lack of Co storage in body tissues. Cobalt levels below 0.5 ug / ml are considered deficient and levels of vitamin B₁₂ start to decline (Underwood,

1977). Increase in succinate in rumen fluids within 2 weeks of Co deficiency affect concentrations by decreasing specific rumen microbes such as *Selenomonas ruminantium* necessary for propionate synthesis (Smith, 2005).

Deficiency of Co also influences defects in lipid metabolism involving cobalamin dependent pathways and can be seen in fatty liver syndrome in sheep due to accumulation of methylmalonyl-CoA, which is an inhibitor of beta oxidation of fatty acids. Homocysteine levels also increase and initiate an accumulation of oxidized products, depletion of vitamin E and damage to mitochondria (Kennedy et al. 1997).

Ruminant gluconeogenesis, lipid metabolism, nucleic acid synthesis, and hemopoiesis are critically decreased in deficient Co cases (Radostits, 2007). Co-induced B₁₂ deficiencies result in lowered red blood cell production causing anemia and later in the reduction of intake, ADG, and protein synthesis causing decreased growth (Wang et al., 2007).

Previous recommended NRC Co requirements were placed at 10 mg/kg/d from experiments conducted during the 1950's. More recent studies show improved growth and performance at 20 mg/kg/d (Underwood, 1977; Smith, R.M., et al., 1997; Schwartz, 2003. Tiffany, et al., 2003, Suttle, 2010). Recent studies evaluating Co requirements from blood metabolites in beef cattle indicate 15 -20mg/kg/d as needed (Stangl, 2000). For maximum vitamin B₁₂ concentrations a level of 25 mg/kg/ d is required (Stangl, 2000). Furthermore, more recent findings also indicate the necessity to increase the amount of dietary Co for ruminants up to a level of 300±500 mg/kg DM for optimum rumen microbial activity (Stangl, 2000). Past studies with increased NRC

Co supplementation on immune function in beef cattle have not been completed. Today beef cattle production involves animals that are 35-40 % larger (anatomically), grow at increased rates, developed with an economical focus in muscle growth with efficient gain that were not considered fifty years ago. Since these factors have all changed, possible increased requirements in Co could be required in beef cattle production of today. A more accurate recommended Co requirement is needed for desired immune response in beef calf production (Maas, 2005). This concept is the basis of this study proposal of increased NRC Co supplementation in beef cattle.

Interaction of nutrition and the immune system reflects a complex and intimate relationship that involves an understanding of cellular and molecular metabolism. Nutritional science and the immune system sciences are closely linked together (Drake, 2010). Nutrients provide both energy and metabolic components for maintenance, normal regulation, growth, and repair of tissues involved in the immune system. There exists an indirect connection with Co deficiency and the impairment of the immune system as Co deficiency directly affects feed intake resulting in deficiencies of vital nutrient groups such as fatty acids and amino acid metabolism through lipid and protein intake and absorption. A deficiency of any nutrient essential for immune function will result in impairment of most adaptive and innate immune function. During inflammation and infection there exists increased binding capacity in plasma indicating an increased cellular demand for vitamin B₁₂ (Quadros et al., 1976). This increased binding process has been proved in vitro by greatly increased cobalamin uptake by lymphocytes stimulated during inflammation (Quadros et al., 1976) and decreased

neutrophil cytolysis in Co deficient cattle (Mac Pherson et al., 1987). Beef cattle with Co deficient feed intake have increased parasite infestations (Mc Pherson, et al., 1987).

Co deficiency impairs the ability of the immune system in normal phagocytic function, in innate immunity, and cytokine production in adaptive immunity with irregular function of humeral (antibody production) and cell mediated immunity (Drake, 2010). Dendritic cell uptake, necessary for antigen presentation to macrophages and to lymph nodes, is required for B cell production of antibodies (Liebler et al., 1995) and is altered in Co deficient cases (Drake, 2010).

Methionine (derived by methionine synthase from methylcobalamine) is also important in the oxidative burst phagocytosis of neutrophils in innate immunity and is vital for pathogen infection to be controlled before systemic infection results (Faernandes et al, 2008). Methionine is involved in the production of vital immune cells (Kudsk, 2000). Methionine, as a methyl group donor for biosynthesis, is also involved in nucleotide synthesis of immune cells (Lugton, 1999). Methionine is an important component in glutathione synthesis as glutathione, an anti-oxidant, is of extreme importance in phagocytosis and oxidative burst of neutrophil activity critical in innate immune response. Methionine studies show evidence that additional levels of dietary methionine can reduce adverse effects on immune system stimulation.

The use of *Mannheimia haemolytica* is indicated for study in this experiment because *Mannheimia haemolytica*, as a bovine pathogen, is associated with the most severe cases of BRD, is associated with permanent tissue damage, and is responsible for the greatest mortality in young beef calves (Smith, 2005; Taylor, et al., 2010).

During the log growth phase of *Mannheimia haemolytica* a leukotoxin (LKT) is released which causes necrosis, apoptosis, activation of ruminant leukocytes, as well as antibodies against bacterial cell surface antigens (Confer, 2011). As *Mannheimia haemolytica* is a normal microbial inhabitant of the nasal cavity of calves any stress induced precursor (as viral, bacterial, or environmental stress) responsible for decreased or impaired microcillary defense mechanisms of respiratory epithelial tissues will allow *Mannheimia haemolytica* to colonize lower respiratory tissues. Resulting pharyngeal, tracheal, and bronchoalveolar tissue inflammation occurs (Smith, 2005; Confer, 2011). Colonization of *Mannheimia haemolytica* predisposes bronchopneumonia and the BRD syndrome (Smith, 2005; Radostits, 2007, Taylor, 2010).

MATERIALS AND METHODS

Fifteen castrated male Holstein calves of initial BW 40kg \pm 4kg were randomly selected from 50 calves for the study. Calves were randomly assigned to receive supplemented Co (cobalt proteinate, Balchem Corporation, Salt Lake City, UT) treatment rates of 0.10ppm, 0.20ppm, and 0.40ppm in milk replacer fed twice daily. These treatment levels correspond to approximately NRC (National Research Council), 2 X NRC, and 4X NRC recommended levels. Calves were housed individually (6' x 6' open air partitions) inside a metal barn environment. Calves were fed approximately 100 ml/ kg of 28% protein milk replacer (ADM, Decatur, Il) with access to *ad libitum* 25% milk base pellets (CHS Nutrition, Sioux Falls, SD) after day 20 and free choice

water. Calves were bled and vaccinated with *Mannheimia haemolytica* (One Shot, Zoetis Animal Health, Exton PA) on d 40 to analyze existing *Mannheimia haemolytica* antibody titers. Calves were bled to evaluate *Mannheimia haemolytica* titers on d 68. Antibody titer differences for *Mannheimia haemolytica* were compared and analyzed by GLM Procedure Least Means Squared analysis, and differences are considered significant at $P < 0.05$.

RESULTS

Mannheimia haemolytica titers on d 68 showed increased antibody response to *Mannheimia haemolytica* leukotoxin compare to d 40 (Table 6 and 7, page 133-135; Figure 33, page 136). GLM Procedure Least Means Squared analysis showed no statistical difference. These statistical results may possibly be a result of low numbers in the treatment groups for this study. *Mannheimia haemolytica* leukotoxin antibody results did not show any statistical results but morbidity was lower in both treatments (2X and 4 X NRC CO) than in the controls at d68. B cell decrease in the treatment groups was slightly less (3.59% compared to 4.39%) than the control group (NRC) B cell percentage of total leukocytes decreases as calves age (Jutila, personal communication). This decrease appeared to be normal in this age group of calves. Appetite increased in the 2 X NRC and 4 X NRC Co treatment groups during the trial. This cannot be explained as vitamin B₁₂ should not have been synthesized by rumen microbes. Hair coat and visual health was visible in the calves receiving above recommended NRC Co levels at the end of the trial.

Table 4.1 <i>Mannheimia haemolytica</i> leukotoxin titers milk replacement study involving three different levels of Co Supplement			
Calf #	initial titer	final titer	difference
1	0.057	0.072	0.015
4	0.047	0.057	0.01
5	0.053	0.134	0.081
6	0.086	0.09	0.004
7	0.083	0.079	-0.004
8	0.019	0.038	0.019
9	0.065	0.079	0.014
10	0.082	0.81	-0.001
11	0.044	0.042	-0.002
12	0.06	0.301	0.241
14	0.047	0.05	0.003
15	0.082	0.103	0.021
16	0.086	0.084	-0.002
mean	0.062385	0.149154	0.030692

Table 4.2. Co supplement study on <i>Mannheimia haemolytica</i> leukotoxin titers comparison between treatment groups				
Control Co (NRC)				
Calf #	initial	final	difference	final-initial/initial (%)
4	0.047	0.057	0.01	0.212
6	0.086	0.09	0.004	0.0465
7	0.083	0.079	-0.004	-0.048
10	0.031	0.081	0.05	1.613
15	0.053	0.103	0.05	1.667
mean	0.06	0.082	0.022	0.366
2 x NRC Co				
8	0.019	0.038	0.019	1
16	0.086	0.084	-0.002	0.033
12	0.06	0.301	0.241	4.02
11	0.044	0.042	-0.002	0.045
14	0.047	0.05	0.003	0.0638
mean	0.0512	0.103	0.0518	1.012
4 x NRC Co				
1	0.057	0.072	0.015	0.263
9	0.065	0.079	0.014	0.215
2	0.058	0.095	0.037	0.638
3	0.053	0.091	0.038	0.717
5	0.053	0.134	0.81	15.28
mean	0.0572	0.0942	0.037	0.646

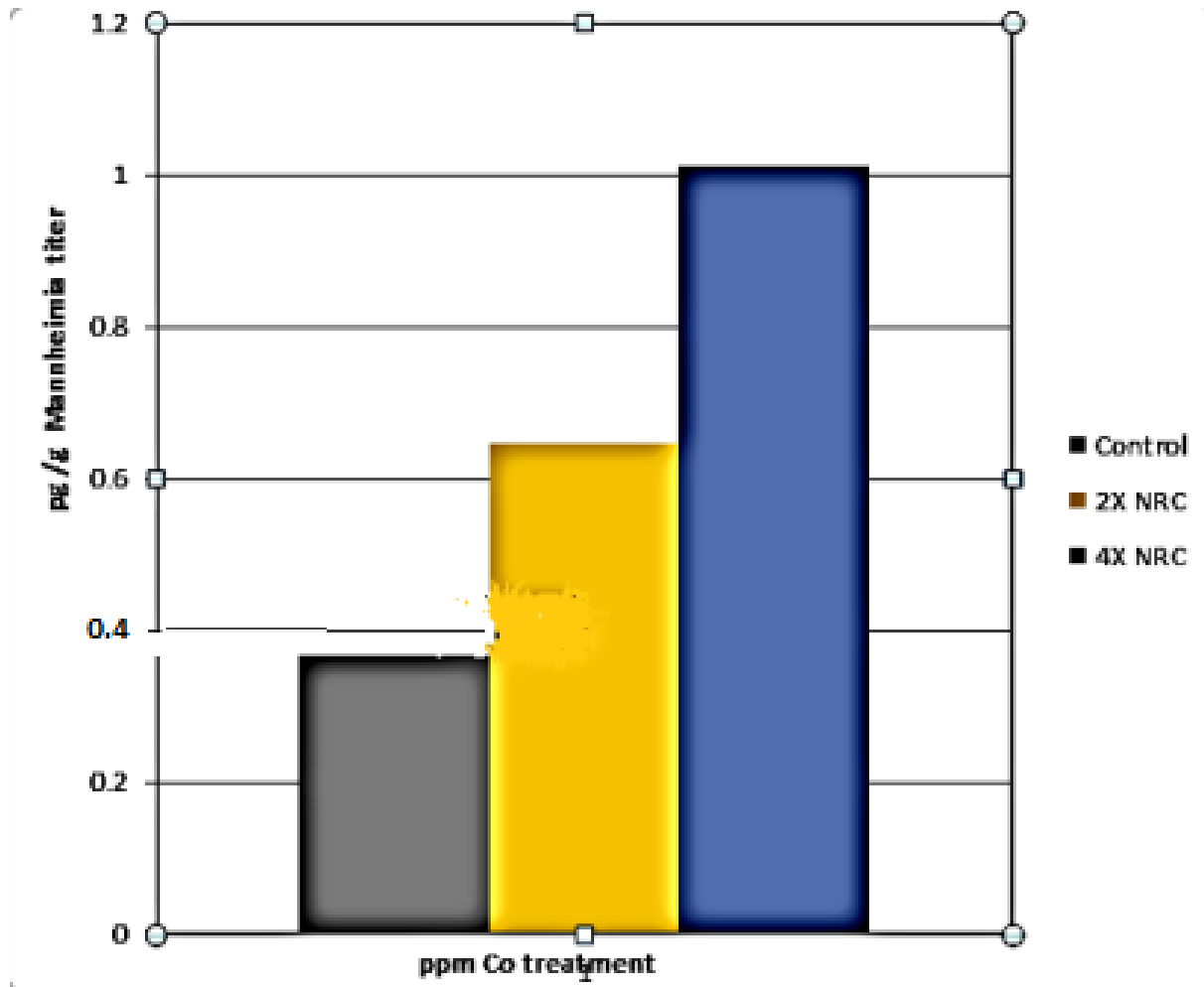


Figure 4.1. Histogram showing mean Mannheimia haemolytica leukotoxin antibody titers (pictogram / ml) in each treatment group; control, 2X NRC, and 4 X NRC Co in milk replacer.

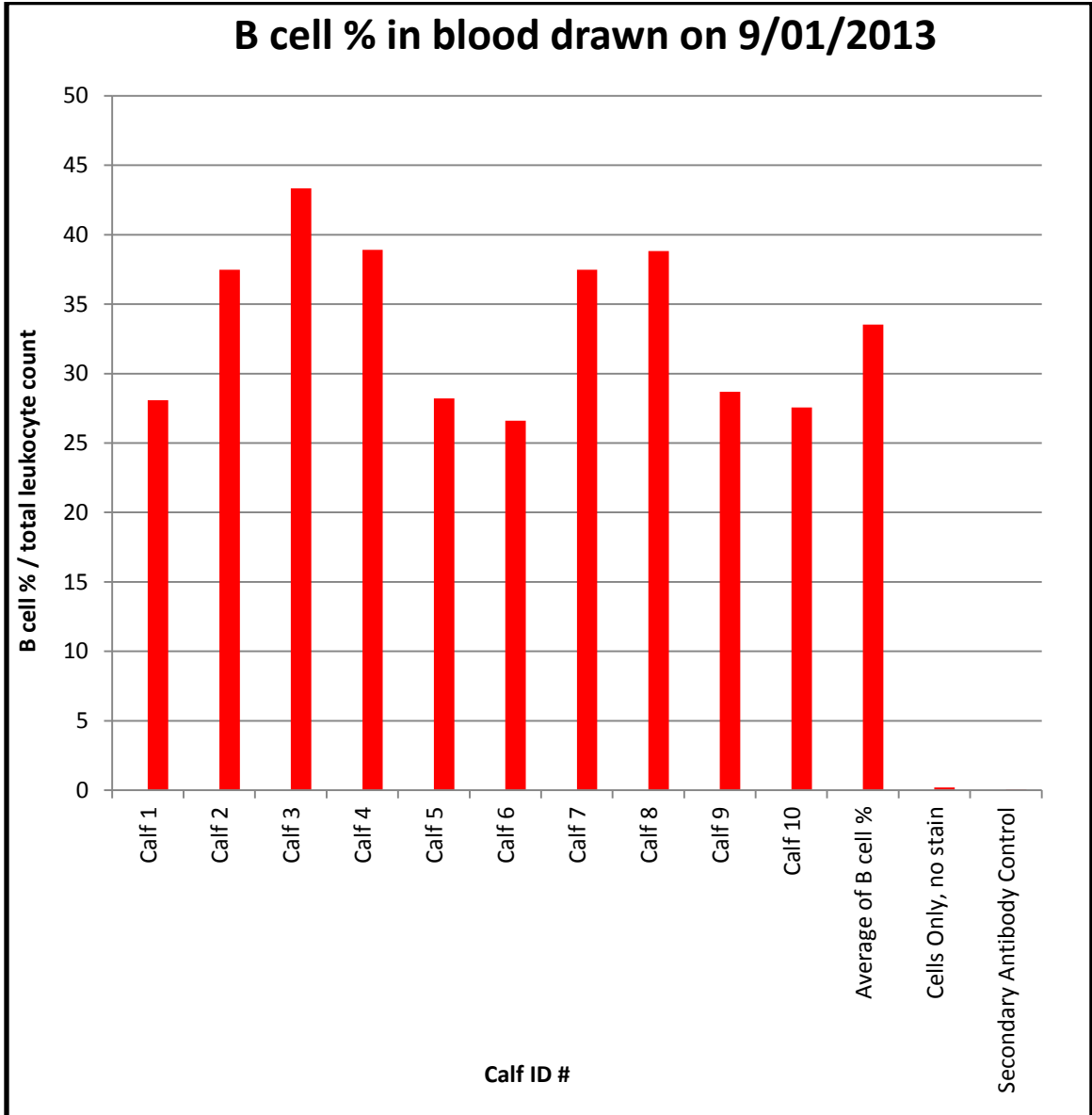


Figure 1 Individual calf % B cell compared to total leukocyte count /ml blood at first bleeding.

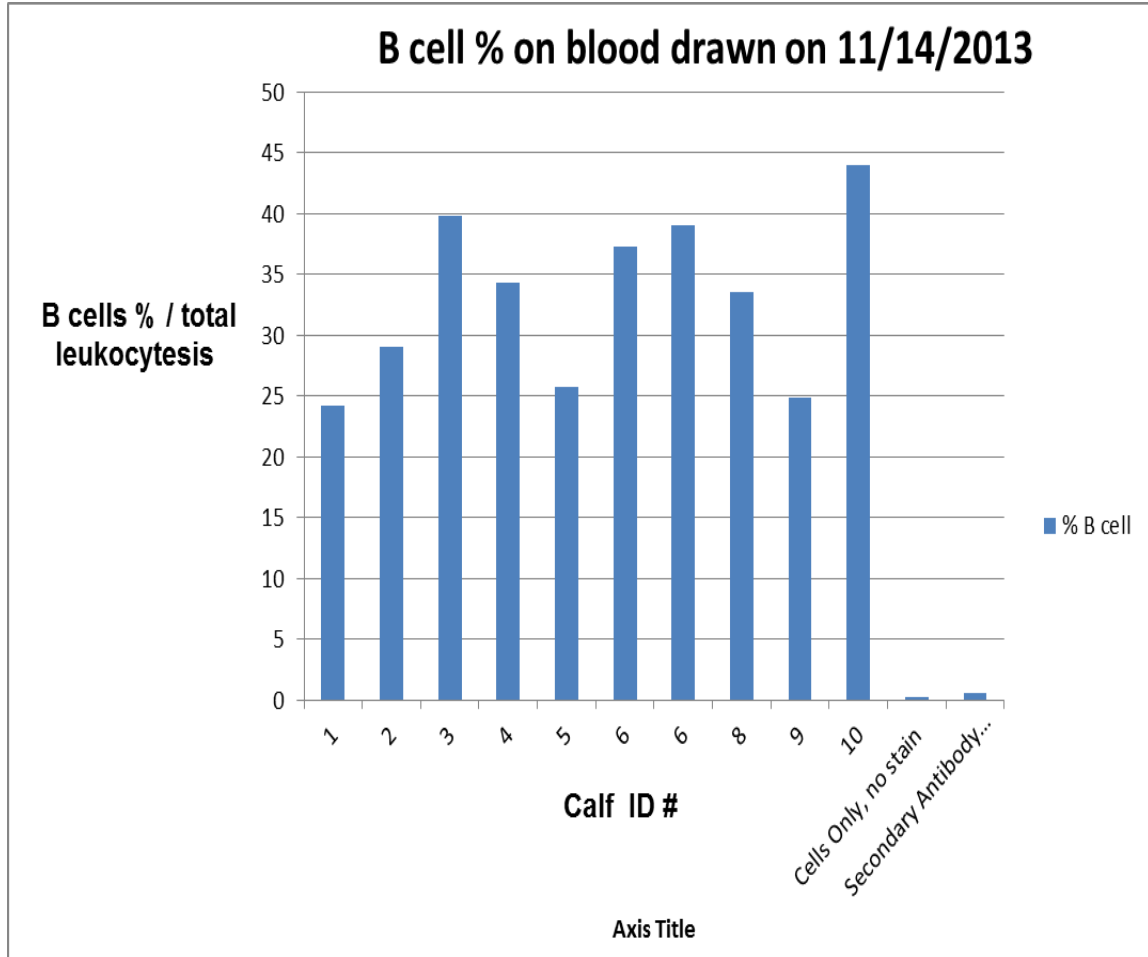


Figure 4.3. B cell % at last bleeding of Co study calves

DISCUSSION

Results from this study showed no statistical differences among treatment groups compared to controls. This result, as discussed before, may possibly be a factor of low numbers of experimental units in each treatment group. The original hypothesis that increased NRC Co supplemented in milk replacer would not improve humoral immune response in nursing calves was shown. Calves bled at d40 and d68 were young

enough that normal rumen development did not occur which was needed for rumen microbial synthesis of vitamin B₁₂. Vitamin B₁₂ synthesis normally occurs from *Propionibacterium* and other anaerobic bacteria normally not colonized in bovine rumens at this age. Among the B₁₂-producing species are the following genera: *Aerobacter*, *Agrobacterium*, *Alcaligenes*, *Azotobacter*, *Bacillus*, *Clostridium*, *Corynebacterium*, *Flavobacterium*, *Micromonospora*, *Mycobacterium*, *Nocardia*, *Propionibacterium*, *Protaminobacter*, *Proteus*, *Pseudomonas*, *Rhizobium*, *Salmonella*, *Serratia*, *Streptomyces*, *Streptococcus* and *Xanthomonas* (Perlman 1959).

Selenomonas ruminantium, a Gram-positive obligate anaerobe, isolated from cattle is the main contributor of vitamin B₁₂ to ruminant animals (Anderson, et al., 2001). Vitamin B₁₂ synthesis from rumen microbes apparently did not occur because of age and no or minimal rumen development. Hind gut microbial synthesis may have produced some vitamin B₁₂ but can't be absorbed from hind gut epithelium. Vitamin B₁₂ is absorbed in the ileum of the small bowel Miller, 1984, Underwood and Suttle, 2004).

IMPLICATIONS

Supplemental Co in milk replacer has not been studied previously. Increased humoral immune response to nutritional supplement would be cost effective and important to the calf raising industry because of health concerns due to BRD and other neonatal health concerns. Rumen development and specific rumen microbial populations need to be in place for Co synthesis for vitamin B₁₂ production. This does

not happen until rumen anatomy and microbe populations occur around 16-24 months of age after milk replacer is commonly used in beef and dairy calf production.

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CHAPTER FIVE

COBALT SUPPLEMENTATION IN PRE-WEANED CALVES AFFECTS
HUMORAL IMMUNE RESPONSE AND FEEDLOT HEALTH

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Contributions: Encouragement through the process with experimental design

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Contributions: Data interpretation

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COBALT SUPPLEMENTATION IN PRE-WEANED CALVES AFFECTS HUMORAL IMMUNE RESPONSE AND FEEDLOT HEALTH

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Abstract

Cobalt (Co) is utilized by rumen microbial organisms for vitamin B₁₂ production. Vitamin B₁₂ is a cofactor for vital metabolic pathways in tissue carbohydrate and lipid metabolism required for maintenance and growth. Vitamin B₁₂ is vital in B cell proliferation to form plasma cells that secrete antibodies. Recent studies have shown increased National Research Council Co positively affected antibody response in weaned beef calves. Economic losses from morbidity and mortality associated with bovine respiratory disease (BRD) in beef cattle are approaching \$2 billion annually. Mineral supplementation during pre-weaning has potential to reduce sickness and improve health. The objective of this study was to evaluate if a supplemented Co (30 g cobalt oxide) bolus dosed pre-weaning affects the humoral immune system during the post weaning feeding period and improve health. Five different ranches with similar genetics, forage, mineral, water aquifer bases, and the same preconditioning health program were utilized. Two hundred, six to eight month old beef calves were randomly selected from 2,000 head (BW 220 kg ± 24 kg). All calves were vaccinated for *Mannheimia haemolytica* three weeks before weaning. At vaccination one hundred calves were randomly selected to receive a Co (30 g cobalt oxide) sustained release bolus and one hundred calves randomly selected as controls. Both treatment and control calves were bled at vaccination to analyze initial *Mannheimia haemolytica* leukotoxin antibody and at d 70 in the feedlot. All calves were weaned and transported the same day. Calves were fed the same rations during the feeding period. Antibody response to *Mannheimia haemolytica* leukotoxin was analyzed by a Welch Two Sample t test with differences considered significant at $P < 0.05$. Differences between treatment and control calves showed treatment calves had a 42% increase to *Mannheimia haemolytica* leukotoxin antibody ($P = 0.0586$) compared to control calves. Feedlot health (morbidity and mortality) was evaluated pre-weaning and during the feeding period. Differences between treatment and control groups showed decreased BRD in the Co treatment group ($P = 0.0217$) compared to the control group. Final carcass characteristics were analyzed by chi square resulting in Yield and Grade characteristics not affected by treatment but HCW was increased in Co treatment by 17.86 kg ($P = 0.3714$).

Key words: humoral immune response, BRD, *Mannheimia haemolytica*, leukotoxin.

INTRODUCTION

Cobalt (Co) is utilized by rumen microbial organisms for vitamin B₁₂ production. Vitamin B₁₂ is necessary as a cofactor for vital metabolic pathways in tissue metabolism required for maintenance and growth. Among the B₁₂-producing species are the following genera: *Aerobacter*, *Agrobacterium*, *Alcaligenes*, *Azotobacter*, *Bacillus*, *Clostridium*, *Corynebacterium*, *Flavobacterium*, *Micromonospora*, *Mycobacterium*, *Nocardia*, *Propionibacterium*, *Protaminobacter*, *Proteus*, *Pseudomonas*, *Rhizobium*, *Salmonella*, *Serratia*, *Streptomyces*, *Streptococcus* and *Xanthomonas* (Perlman 1959).

Selenomonas ruminantium, a Gram-positive obligate anaerobe, isolated from cattle is the main contributor of vitamin B₁₂ to ruminant animals (Anderson, et al., 2001). There are both aerobic and anaerobic pathways for B₁₂ synthesis - *Selenomonas ruminantium*, uses an anaerobic pathway to secrete an enzyme for vitamin B₁₂ synthesis.

Recent studies have shown above recommended National Research Council (NRC) Co positively affected antibody response in weaned beef calves (Sager, 2011). The objective of this research proposal is to test the hypothesis that increased NRC recommendations of Co supplemented during pre-weaning will positively affect humoral immune response in beef calves by increasing antibody levels to *Mannheimia haemolytica* vaccination. Response to Co supplementation will be evaluated by measuring *Mannheimia haemolytica* antibody titers. Antibody response to *Mannheimia*

haemolytica will be analyzed by a Wilcoxon Rank Sum test and differences will be considered significant at $P < 0.05$. Humoral immune response would result in increasing serum immunoglobulin G (IgG1 antibody). With increased antibody levels, beef calves would be more resistant to bovine respiratory disease (BRD).

One of the most challenging factors affecting beef cattle production today is the problem BRD in beef calves. BRD causes 25% of all calf deaths before weaning (USDA NAHMS, 2007). Economic losses from morbidity and mortality associated with bovine respiratory disease (BRD) in beef cattle continue to plague the beef cattle industry (Galvayan et al., 1999) with economical loss and treatment costs approaching \$2 billion annually (Radostits, 2007). Calves less than eight months of age: BRD is stress induced by weather or by other environmental conditions, causing inflammation, thus predisposing BRD.

In the upper Great Plains of the United States one of the most common concerns for beef calf survivability is BRD with resulting calf pneumonia. Although scientific data cannot explain recent BRD morbidity and mortality, BRD cases are similar to those of 50 years ago, even with advances in pharmaceutical technology and in animal health (White, 2011). There is a distinct trend of increasing mortality, evident since 1994, from respiratory disease among cattle (Smith, 2005; Radostits, 2007, Taylor, et al., 2010). The reason for bovine pneumonia is complex, but no other factor is as important as the anatomy of the bovine lung. Anatomically the bovine lung has increased compartmentalization and is under constant stress as the area of lung volume per body size or per weight is small compared to other mammals. This, in turn, creates

a physiological stress, because of smaller gaseous exchange capacity. This constant stress predisposes beef calves to pulmonary disease problems (Smith, 2005; Radostits, 2007). Because of the importance of the anatomy of lung tissue, and the complex immune system response to infection, much lung tissue is often destroyed permanently. This inflammatory damage causes permanent tissue loss, decreased alveolar gas exchange, a chronic disease state, and resulting decreased longevity. Advanced treatment therapy and preventative vaccine programs have improved beef calf health, yet, the problem is still vast and economically important to the industry. The stress of stormy weather during the normal calving period is a constant factor inducing calf pneumonia. Weather related stresses require larger energy resources that often cannot be supplied by the cow's milk and result in disease problems. Susceptibility to calf pneumonia is determined by many factors such as genetics, nutrition, and the calf's resistance (passive transfer of IgG₁) to infectious agents that cause or predispose the calf to the disease syndrome (Smith, 2005; Taylor, 2010). Impairing innate and adaptive resistance of immunity are large factors in the disease syndrome (Smith, 2005; Taylor, 2010). Also, multiple factors which contribute or predispose the calf to the disease process are: often an energy deficient calf, a causative agent (bacterial or viral), and respiratory tissue damage predisposing inflammation and infection to BRD. Therefore, BRD is a complex disease syndrome of cattle, involving viral, bacterial, and stress factors with bacterial, especially *Mannheimia haemolytica*, being one of the most severe etiology factors.

The use of *Mannheimia haemolytica*, as an antigen for immune response, is

indicated for study in this experiment because *Mannheimia haemolytica*, as a bovine pathogen, is associated with the most severe cases of BRD. *Mannheimia haemolytica* is associated with permanent tissue damage, and is responsible for the greatest mortality in young beef calves (Smith, 2005; Taylor, et al., 2010). *Mannheimia haemolytica* is a normal microbial inhabitant of the nasal cavity of calves. Therefore, any stress induced precursor such as viral, bacterial, or environmental that is responsible for decreased or impaired microcillary defense mechanisms of respiratory epithelial tissues, will allow *Mannhemia haemolytica* to colonize lower respiratory tissues. Colonization results in pharyngeal, tracheal, and broncho-alveolar tissue inflammation. Colonization of *Mannheimia haemolytica* predisposes calves to bronchopneumonia and the BRD syndrome. In addition, colonization allows *Mannheimia haemolytica* to change serotypes in the lower respiratory tract and increases the virulence of the bacteria, thus producing more severe inflammatory responses. The change of serotype A₂ to A₁ allows for a potent leukotoxin, produced by the capsule, to be released, causing permanent pulmonary tissue damage (Taylor, 2010). *Mannheimia haemolytica* leukotoxin destroys neutrophils and macrophages responsible for normal phagocytosis, important in the immune response. This destruction along with the release of the leukotoxin, during the log phase growth of *Mannheimia haemolytica*, is the primary cause of permanent pulmonary tissue destruction (Smith, 2005, Taylor, 2010). Because of this permanent lung tissue damage calves do not grow normally with feedlot ADG (average daily gain) depressed. BRD caused changes by *Mannheimia haemolytica* are considered life long, the most severe, and the most economically important to the beef

cattle industry (Smith, 2005).

Complex inter-relationships exist between certain micronutrients, immune function and disease resistance in cattle (Spears, 2000). Several micronutrients have been shown to influence immune responses. The relationship between deficiencies of some micronutrients and disease resistance is less clear. Infectious, no matter how severe, have adverse effects on bovine nutritional status (Scrimshaw and SanGiovanni, 1997). Severity often is determined by the nutritional status before infection and diet during the recovery period. Almost any nutrient deficiency will impair resistance to infection and impair antibody production. Micro-minerals are particularly vital to immune response through antibody formation, decreased cutaneous hypersensitivity, reduced immunoglobulin concentrations, decreased thymic and splenic lymphocytes, reduced complement fixation, reduced T cell subsets (NK cells, helper T cells), decreased secretory IgA, and interferon (Scrimshaw and SanGiovanni, 1997). Cell mediated responses vital to antigen phagocytosis, APC (antigen presenting cells; dendritic cells, neutrophils, and macrophages) are more sensitive than humoral responses (Scrimshaw and SanGiovanni, 1997).

As stated above, Co is utilized by rumen microbial organisms for vitamin B₁₂ production. Recently microbial vitamin B₁₂ has been shown to be vital for DNA replication and repair for immune response (Tamara, et al. 1999). B₁₂-dependent ribonucleotide reductase catalyzes the conversion of ribonucleotides to deoxyribonucleotides, which is fundamentally important for DNA replication and repair (Banerjee and Ragsdale, 2003). This enzyme is important in antibody production

by the bovine host. A deficiency of Co will negatively alter vitamin B₁₂ synthesis decreasing humoral immune response.

Cobalt supplement to reduced calf stress and reducing BRD has been proposed in the past (Shelley et al., 2013). Economic losses can be substantial from newly weaned cattle entering the feedlot if health is compromised. Beef calves subjected to transportation and/or possible comingling are more susceptible to sickness (Duff and Galyean, 2007). Blecha et al. (1984) found that transportation stress causes suppressed immune function. Health management goals to reduce stress on newly weaned beef calves subjected to transportation could alleviate stress and may reduce morbidity and mortality (Shelley et al., 2013).

Providing supplemental cobalt stressed calves has mediated stress responses in recent studies (Shelley et al., 2013) Cobalt source does not appear to be a factor and because of other Co influences on ruminal microbial populations may increase intake on new arrival calves. Co supplementation has a potential to increase vitamin B₁₂ production, fiber digestibility and VFA production. Increased intake and energy supply may help newly arrived calves to manage transportation, fasting, and receiving stress more efficiently. Shelley et al. (2013) found Co treated heifers before transportation had stress related blood metabolites reduced compared to controls after transportation. Cortisol and haptoglobin tended ($P = 0.06$) to be lower for Co treated heifers than control heifers (Shelley et al, 2103). Reducing blood metabolites could improve feed intake and reduce sickness in newly arrived calves as 67% of newly arrived beef calves

do not show normal feed intake for the first three days after arrival (Texas A&M University Ranch to Rail, unpublished data, 1998).

BRD (bovine respiratory disease) is a complex disease-syndrome of cattle, involving viral, bacterial, and stress factors and is one of the most challenging factors affecting beef cattle production today. *Mannheimia haemolytica*, is an opportunistic pathogen; is a normal inhabitant of the nasopharynx of cattle, and is one of the most severe etiology factors of BRD, increasing mortality of beef calves. *Mannheimia haemolytica* serotype S1 is considered the predominant cause of bovine pneumonic pasteurellosis or BRD (Singh, et al. 2011). Virulence factors of the bacteria allow *Mannheimia haemolytica* to colonize lung tissues and establish infection. Virulence factors include leukotoxin (LKT), lipopolysaccharide, adhesions, capsular outer proteins, and proteases. The effects of LKT are species specific in ruminants (Sigh, et al., 2011). This is because of the interaction of LKT with the bovine β 2 receptor on the surface of bovine leukocytes. In low concentrations LKT can elicit leukocyte respiratory burst and degranulation with stimulation of cytokine release from macrophages and histamine release from mast cells resulting in severe inflammatory reaction within lung tissues (Sigh, et al., 2011). The activation of LKT at higher concentrations causes cell necrosis. Activation of LKT with leukocyte response by the immune system causes an interaction resulting in oxidative burst and the release of pro-inflammatory cytokines: interleukin 1, 6, and 8 and tumor necrosis factor α (Sigh, et al., 2011). This results in massive influx of leukocytes to the lung tissues. The release of oxidative burst chemicals give rise to additional tissue necrosis and resulting fibrinous

and lobular pneumonia. Additional lipopolysaccharides interacting with LKT give rise to a complex immune reaction of complement release and a coagulation cascade with cell cytolysis (Sigh, et al., 2011). Capsular outer membrane proteins, adhesions, and proteases assist in the colonization of *Mannheimia haemolytica* and severe infection.

Economic losses from morbidity and mortality associated with BRD in beef cattle continue to plague the beef cattle industry (Galvayan et al., 1999) with economical loss and treatment costs approaching \$3 billion annually (Radostits, 2007). BRD causes 25% of all calf deaths before weaning and is the primary cause of morbidity and mortality in the feedlot (USDA NAHMS, 2007). In the United States, 1.4% of all feedlot cattle perish before reaching harvest weight with the majority due to BRD. More feedlot cattle die from BRD than all other diseases combined (Van Eenennaam et al., 2011). BRD accounts for 28% of all US cattle industry deaths and causes annual losses of more than one million animals (USDA NAHMS, 2007).

Nutritional supplementation during pre-weaning has potential to reduce sickness and improve health in beef calf production (Fisher and Mac Pherson, 1990; Engle, 1999; Drake, 2000, Fernandes, 2003, Maas, 2005). Cobalt deficiencies affecting immune response in beef cattle have been studied recently. Deficiencies of Co in cattle reduce the ability of isolated neutrophils to kill yeast and /or bacteria. (Spears, 2000) Co deficiency has been associated with reduced resistance to parasitic infections. (Spears, 2000) Neutrophils isolated from calves deficient in Co had depressed ability to kill *C. albicans* (MacPherson et al. 1987; Paterson & MacPherson, 1990). Co-deficient calves had a decreased prepatent period and increased faecal egg output

following experimental infection with *Ostertagia ostertagi* (MacPherson et al. 1987). Higher faecal egg counts were also observed in Co-deficient lambs after natural infection with gastrointestinal nematodes (Vellema et al. 1996). Nutritional management efforts to minimize health problems affect production throughout life (Lofgreen, 1988; Engle, 1999; Smith, 2005). Sickness and decreased health have lasting effects on feedlot performance and carcass quality at slaughter (Mc Beth et al. 2001; Smith, R.A., 2009). Evaluation of data for comparison of objectives and goals for increased health during the feeding period and final carcass characteristics will be analyzed by Chi square. Results obtained in decreasing sickness and improving health in beef calves, would increase profitability in beef calf production. Calf morbidity and mortality will be evaluated the first two months after treatment and the first two months post weaning (during the feeding period) in the feedlot during this study.

OBJECTIVE OF STUDY

As BRD is still the number one problem in beef cattle production it is the goal to determine if a novel method of nutritional supplementation of Co will alter beef calf losses and improve health post weaning. This would result in decreased respiratory disease (BRD) and improve calf health. Increased beef calf health and increased performance in growth (from increased NRC Co supplementation during pre-weaning of beef calves) would be expected in this study from previous results.

The objective of this study is to evaluate increased NRC Co supplementation (approximately 2 X NRC) in pre-weaned beef calves affecting the humoral immune system. Expected outcomes would be increased humoral immune response with resulting decreased BRD pre-weaning. Expected results post weaning would be decreased sickness during the feeding period and improved feedlot performance. Expected final carcass characteristics are unknown. Cobalt is very safe to use and mix as oral ruminant intakes of greater than 1,000 times NRC have not created toxic clinical signs. The Co product is a 30 gram bolus (Cobalt oxide) dosed orally at preconditioning 21 days before weaning.

Timeline for objectives can be met in the six-eight months listed for the project as calves will be fed for approximately 140-165 days and then slaughtered. Treatment of Co during the pre-weaning period, feedlot health performance, and final carcass data will be evaluated during this time frame.

Objectives including: 1) pre-weaning- vaccinate all calves for *Mannheimia haemolytica* (Vista Once, Merck Animal Health, Milsboro, DE) 21 days pre-weaning. Blood collected for *Mannheimia haemolytica* antibody titers and vitamin B12 evaluation; 2) Cobalt supplement dosed orally at preconditioning 21 days before weaning. This is a booster dose; 3) post weaning blood samples collected for *Mannheimia haemolytica* titers and vitamin B12 serum evaluation at day 70 at the feedlot (Weber Feedlot, Sanborn, Minnesota); 4) Morbidity and mortality data (first 45 days) collection at the feedlot; and 5) final carcass characteristics measured at harvest (AP Packing, Wilmer, Minnesota).

MATERIALS AND METHODS

Two thousand six to eight month old beef calves with initial 220 kg BW (\pm 24 kg) were randomly selected for this study. Calves were from five ranches with the same forage, mineral, aquifer base and with similar genetics and age. Calves were experimental units and received the identical preconditioning health program, were weaned and shipped the same day to one Weber feedlot (Sanborn, MN), and were fed the same rations until harvest. Calves were vaccinated with *Mannheimia haemolytica* (Vista Once, Merck Animal Health, Millsboro, DE) twenty-one days before weaning. One hundred calves were randomly assigned and dosed orally with a sustained release Co bolus (30 g) at the time of vaccination (day1, September 21, 2013)) and one hundred calves were assigned as controls (received no Co bolus). At vaccination calves were bled via coccygeal vein to evaluate existing *Mannheimia haemolytica* leukotoxin antibody titers and again bled at day 70 at the feedlot to evaluate *Mannheimia haemolytica* leukotoxin antibody titer response. Care, handling, and sampling of the animals were done using recommendations by the Montana State University Animal Care Committee and utilizing BQA (Beef Quality Assurance) recommendations. At d 70 (December 17, 2013) calves were bled to evaluate *Mannheimia haemolytica* leukotoxin antibody response during Co supplementation. Blood was chilled in cold pack boxes to allow normal clotting and centrifuged at 24 hours at 2500 rpm for 20 minutes. Serum was collected and refrigerated until samples were mailed for *Mannheimia haemolytica* leukotoxin antibody analysis. Antibody response was

measured in leukotoxin antibody titers (as leukotoxin titers are preferred for evaluation of a *Mannheimia haemolytica* response) by Oklahoma State University Diagnostic Laboratory, Stillwater, OK). Calves were fed to grow approximately 1.4-1.8 kg / d and each calf was an experimental unit. *Mannheimia haemolytica* antibody data was analyzed by a Wilcoxon Rank Sum test with differences considered significant at $P < 0.05$. As calves are randomly assigned there are assumptions that normality does exist and calves were independently distributed with the mean and standard deviation (N, mean, and sd). Assumption of constant variance is made and independence is made as each calf is an experimental unit.

Antibodies to *Mannheimia. haemolytica* whole cells and to LKT were determined by enzyme-linked immunosorbent assays (ELISA; (Confer et al., 1997; Confer et al., 1998). The *P. haemolytica* A1 strain used for antigen preparation was originally isolated from a feedlot calf (Panciera and Corstvet, 1984). Formalinized *Mannheimia. haemolytica* was prepared from a washed 24-hour culture by suspending cells in 0.4% formalinized saline at a concentration determined spectrophotometrically to be 1.850 OD₆₅₀. LKT was prepared from culture supernatant from a 3-hour culture of *Mannheimia. haemolytica* A1 grown in RPMI-1640 medium at 37 ° C in a shaking incubator. The LKT was partially purified by precipitation with 40-60% ammonium sulfate as previously described (Clinkenbeard et al., 1994). The precipitate was resuspended in 3M guanidine containing 59 mM NaHPO₄ and 100 mM NaCl. By SDS-PAGE of the LKT preparation, one intensely staining band was identified at 105 kDa and confirmed to be LKT on a western blot using an anti-LKT monoclonal antibody

(Confer et al., 1998). Leukotoxic activity was 10^4 LKT Units per ml (Clinkenbeard et al., 1994). The 2-keto-3-deoxyoctonate concentration was 7.5 μg per mg of protein (Osborn, 1963). Wells of 96-well microtiter plates were coated with whole cells at an optical density reading equivalent to 10^8 CFU of a 24-hour culture or with LKT at 50 ng per well. Sera were diluted in PBS-Tween 20 containing 1% BSA and tested at dilutions of 1:800 for whole cells and 1:1600 for LKT. The extent of antibody binding was detected using a 1:400 dilution of horseradish peroxidase-conjugated, affinity purified rabbit anti-bovine IgG. Antibody responses are expressed mean $\text{OD}_{490} \pm \text{SD}$. Leukotoxin titers for *Mannheimia haemolytica* were completed by Oklahoma State University; Marie Montelongo, laboratory technician, under the direction of Anthony Confer PhD.

Serum sample size was not the same which required either a Wilcoxon Rank Sum test or a Welch Two Sample t test to be used. Analysis of both gave similar results with the Welch Two Sample t test resulting in a better P value ($P= 0.05868$) compared to the Wilcoxon Rank Sum test ($P= 0.0686$). Graph results are found on 165-169.

The objective of this study was to test the hypothesis that increased NRC recommendations of Co supplemented during pre-weaning would positively affect humoral immune function in beef calves by increasing antibody levels of *Mannheimia haemolytica* leukotoxin through immunization with Vista Once (Merck Animal Health, Milsboro, DE). Calves were evaluated by measuring humoral immune response (*Mannheimia haemolytica* leukotoxin antibody titers). Humoral immune response would result in increasing serum immunoglobulin G (IgG_1). In response to increased

antibody levels, beef calves would be more resistant to BRD and other health problems during the feeding period. *Mannheimia haemolytica* is an important factor in the pathogenicity of BRD and influencing mortality of young beef calves. Calf morbidity and mortality were evaluated for the first three weeks after treatment (records of morbidity, treatment, and necropsy examination by ranchers) and for the first three months post weaning during the feeding period in the feedlot. Final carcass characteristics (quality grade, yield grade and HCW) were evaluated at slaughter.

A similar study showed higher *Mannheimia haemolytica* titers in cows supplemented with cobalt (Grotelueschen et al., 2001). Recently completed work resulted in increased antibody response to a single antigen (*Brucella abortus*) using increased NRC Co supplementation (Sager, 2011). It was the goal of this study to duplicate results in a natural range condition, evaluate feedlot health (morbidity and mortality), and evaluate final carcass characteristics with increased supplemented Co levels fed during the pre-weaning period.

RESULTS

Results obtained are very close to expected and goals of the study. As discussed the main objective was to improve feedlot health because of pre-weaned supplementation Co above NRC recommendations. This was completed by showing a difference in feedlot health between treatment and control calves (P=0.007).

Results from *Mannheimia haemolytica* leukotoxin antibody results reflected a 42% increase in the treatment group (1.682 picograms / ml serum compared to 1.168

picogram / ml of serum in the control group ($P=0.05868$). This result was slightly less than results obtained in the first study ($P=0.004$) and is addressed in the discussion section. Final carcass characteristics were completed in late April or early May, 2014.

Yield, Grade and HCW traits were analyzed and compared between treatment and control groups of calves. Yield and Grade characteristics did not differ statistically between groups but HCW did differ as previously reported by Tiffany (Tiffany and Spears, 2005). Hot carcass weight was numerically increased by 14 kg in the treatment group compared to the control group ($P= 0.3714$).

DISCUSSION

Results were analyzed and compared to objectives of the study. Incidence of sickness and prevalence of sick days were measured in calves as daily animal health was tracked and recorded during the trial. Sick calves were not excluded from the study as natural infection should provide a more robust antibody response to natural infection with *Mannheimia haemolytica*. Records on treatment duration, dosage, and product used were recorded during the study by Weber Feedlot management. *Mannheimia haemolytica* leukotoxin antibody results for the treatment calves at day 70 showed an increase of 42 % ($P=0.05868$) compared to control calves. Statistical results were somewhat discouraging but the main objective in this study was decreased BRD and improved health during the feeding period. The statistical analysis was completed using a Welch Two Sample t test as calf treatment and control sample size were not equal. This gave a mean analysis instead of a median analysis. Results using the Wilcoxon

Rank Sum test showed similar but not as ideal results ($P=0.0686$). Serum analysis was completed using $\frac{1}{2}$ the total samples collected because of costs of the serum leukotoxin antibody test with a non-funded study. Consideration of evaluating the entire sample size collected from calves could lead to a P value closer to the objective. P value was only 0.00868 (increase of 17%) higher than objective P values. Increased *Mannheimia haemolytica* leukotoxin antibody titers do not directly correlate with the same proportional decrease in morbidity. The study design used a sustained Co bolus that has an average life of 120 days but individual calf rumen microbiome status would determine the microbe digestion and life-time utilization of the sustained bolus. This would expect to vary between individual experimental units (calves). Dr. Confer's laboratory explained the variation of *Mannheimia haemolytica* leukotoxin antibody titers at day 70 (in the feedlot) was due to leukotoxin antibody titer levels start to drop after day 60. Original plans were to bleed calves at day 60 but weather and coordination to bleed at the same time calves were being processed (implanted). This was the main factor in the timing date of the bleeding. Knowledge of *Mannheimia haemolytica* leukotoxin antibody titers dropping after day 60 was not known until leukotoxin analysis was completed in January, 2014. During the feeding period there was above usual weather stress with below average temperatures and increased wind days. This did not result in increased BRD in either group of calves.

The weather was an increased stress on beef calves during the first 45 days of the feeding period and could have increased BRD. In this study, foot rot infections were more common than usual because of ice and frozen ground conditions increasing foot

abrasions and cuts allowing *Fusobacterium* spp to increase virulence and morbidity rates from normal years. BRD cases were not as large as expected due to stresses of transportation (15 hours on truck) and above average weather stress. Humoral immune response is decreased by stresses of transportation and remains suppressed for several weeks (Mckenzie et al., 1997). Abrupt weaning does increase plasma cortisol and noradrenaline concentrations that decreases interferon- γ production up to 7 d after weaning (Hickley et al., 2008). Abrupt weaning with transportation has additive effects on humoral immune response (Mckenzie et al., 1997).

Results could be improved with an additional study if calves were bled within the 60 day post vaccination period and a larger sample size analysis was completed on *Mannheimia haemolytica* leukotoxin antibody titers. Both should be considered to improve probability ($P < 0.05$) with additional studies.

Health results during the 70 d study reflect decreased sickness ($P < 0.021$). Results are described on page 184-187 in chart form. This statistical result met objectives and goals of the study and could indicate above NRC Co levels are needed for improved beef calf health and decreased BRD problems in newly arrived beef calves.

Improved antibody production in weaned beef calves fed supplemented Co above a NRC requirement is most probable a direct result of increased vitamin B₁₂ production by rumen microbial synthesis. Decreased sickness and treatment between treatment and control calves showed statistical differences and resulted in improved health post weaning. This improved health did correlate to improved carcass

characteristics at harvest in April and May of 2014. Improved feedlot health and decreased sickness has lasting effects and improves carcass quality (Stovall et al. 2000; McBeth et al. 2001, Smith, R.A., 2009).

The statistical P value was larger than expected and can be explained because of the large variation in HCW's in the small number of samples collected at harvest. Pyatt et al. (2005) showed carcass weight was the most critical factor contributing to carcass value, whereas BW and carcass quality were the primary factors affecting steer profitability. Tiffany showed that steers supplemented with increased NRC Co increased dressing percent ($P < 0.10$) and HCW ($P < 0.01$) at slaughter.

Co supplementation above NRC recommendations resulted in improved health during the feeding period and resulted in achieving the objective and primary goal of this study.

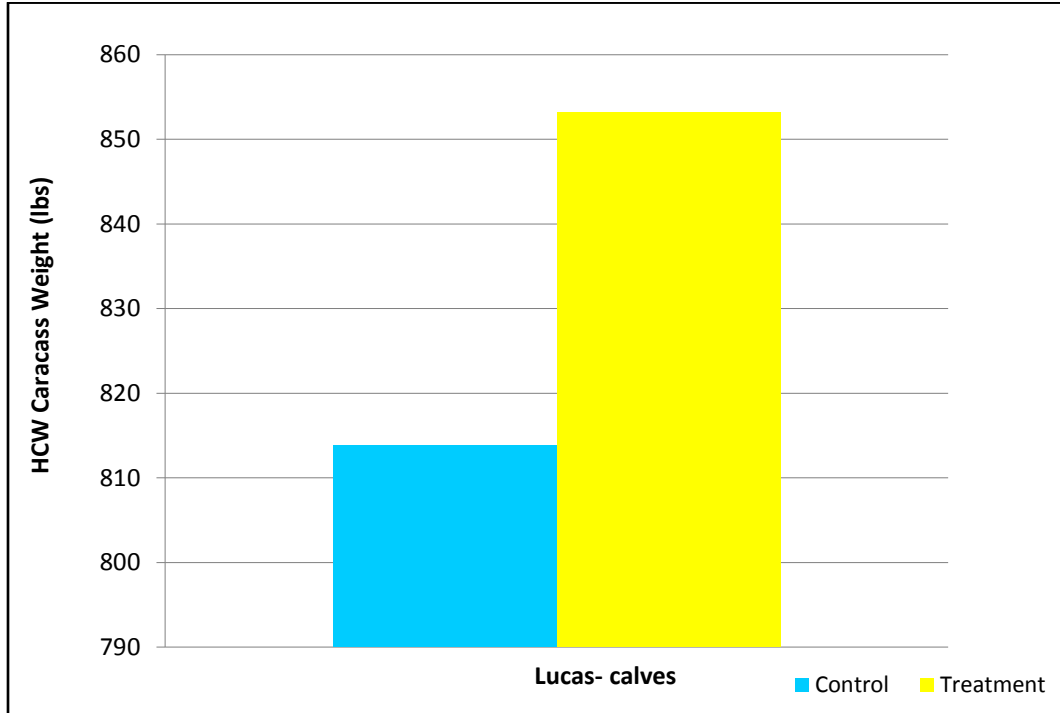


Figure 5.1. Final carcass weights of control and treatment calves (P=0.3714)..

IMPLICATIONS

The focus of this study was to improve animal health and decreased livestock losses through nutritional supplementation. Further rationale of this study was to determine nutritional supplementation during pre-weaning would positively affect post weaning health and performance.

This study rationale showed potential implications of decreasing BRD and improving weaned beef calf health. This was completed through nutritional supplementation of above NRC recommendations of Co. Expected results did result in decreased beef calf morbidity and improved economic performance in beef production. As BRD is still the number one problem in beef cattle production the goal of this study

was to determine a novel method to supplement Co that would result in decreasing BRD losses and improve beef calf health post weaning. This study was designed to test the hypothesis that supplemented Co (above NRC requirement) fed to pre-weaned beef calves will positively affect humoral immune response and thus decrease BRD. We believe this study accomplished set goals and results were as successful as anticipated. Results in increased NRC Co did show increased humoral immune response with decreased BRD.

The study trial may be a novel fit for nutrition supplementation to improve health, decrease BRD, and improve carcass characteristics in beef calves. Results determined by this study may also add to improved beef calf welfare and improve efficiency in beef production medicine through increased immune system response. Results determined did decrease BRD morbidity and economical loss, promoted increased efficiency during the feeding period, and improved profitability in beef cattle production for Weber Feedlot.

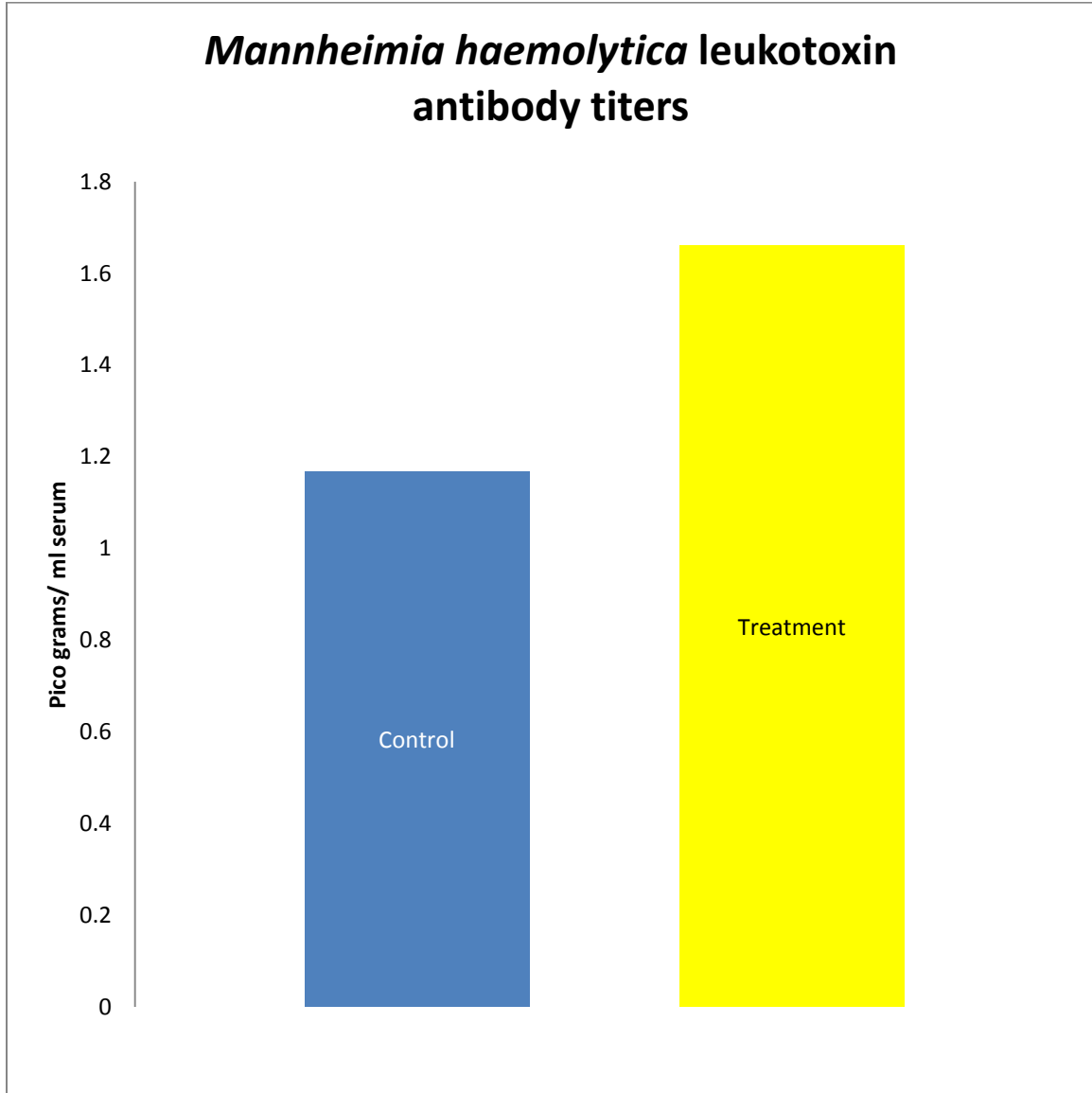


Figure 5.2. Comparison of treatment versus control calves *Mannheimia haemolytica* (picograms / ml serum).

Calf ID	Initial leukotoxin serum titer	Final leukotoxin serum titer	Difference	Calf ID	Initial leukotoxin serum titer	Final leukotoxin serum titer	Difference
756	0.569	2.086	1.517	64	0.265	2.342	2.077
50	0.344	2.14	1.796	14	0.165	0.227	0.062
708	0	0.541	0.541	926	0.498	2.653	2.155
412	0	2.31	2.31	951	0.835	1.27	0.435
975	0.503	2.196	1.693	979	0.008	2.285	2.277
849	0	1.573	1.573	165	0.348	1.373	1.025
772	0.146	2.785	2.639	883	1.698	2.487	0.789
136	1.291	2.291	1.000	115	0	1.731	1.731
51	1.682	2.785	1.103	658	0.008	2.425	2.417
968	0	0.132	0.132	83	0.908	1.236	0.328
84		1.781	1.781	31			1.1682
111	1.166	2.704	1.538	322			
907	0.5	2.062	1.562	304			
792	0.569	2.637	2.068	316			
82	0.389	2.587	2.198	30			
4	0.974	1.935	0.961	327			
133	0.144	1.542	1.398	334			
655	0.429	1.59	1.161	341			
948	0	2.677	2.677	384			
42	0	1.251	1.251	395			
934	0.268	1.605	1.337	396			
968r	0.521	2.123	1.602				
85	0.487	2.432	1.945				
mean			1.671924			mean	1.1682

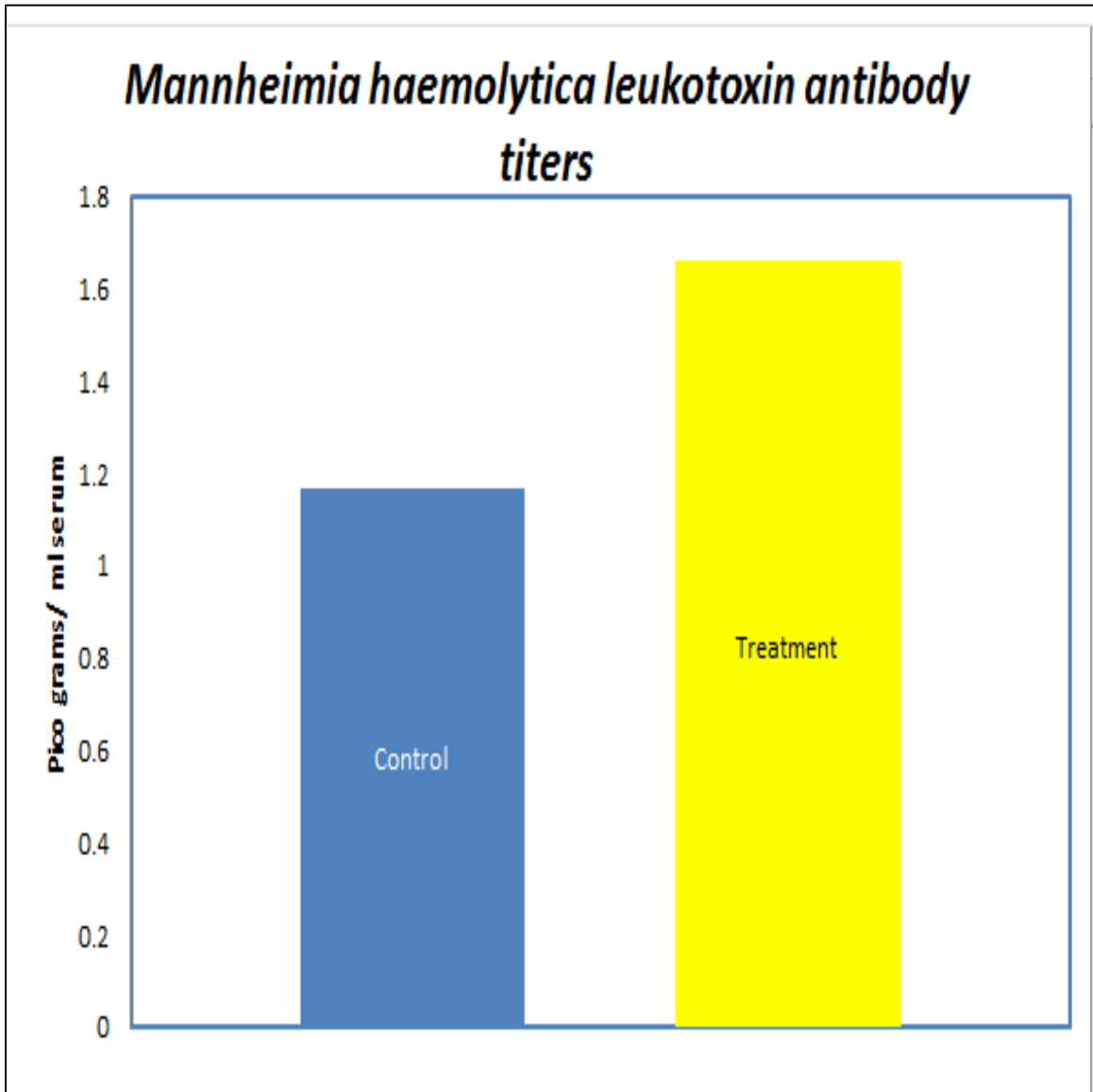


Figure 52. Cobalt control calves (mean 1.168 pg / ml) and Cobalt treatment calves (with mean titer 1.667 pg / ml) *Mannheimia haemolytica* leukotoxin antibody titer (P= 0.05868).

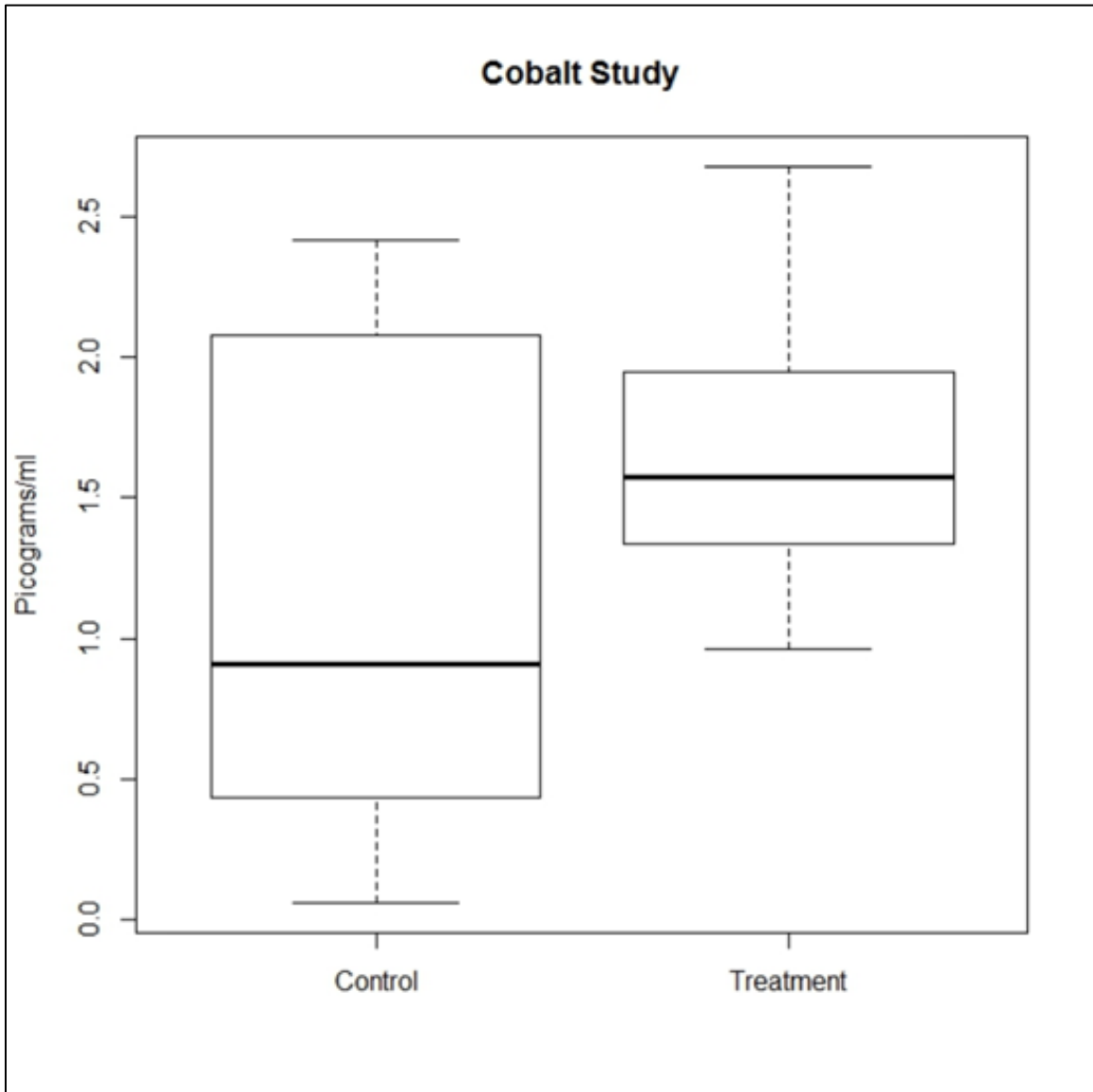


Figure 5.4. Box plot of the statistical analysis of the third calf study of *Mannheimia haemolytica* leukotoxin antibody response is shown with the dark lines are representative of the median of each group (Wilcoxon Rank Sum test).

Table 5.2 Lucas Calf Treatment and Health Records Weber Feedlot Sanborn, Minnesota			
Calf ID#	Treatment code	CalfID#	Treatment Code
756	0	64	1
50	0	14	0
708	0	182	2
412	0	926	0
975	0	951	0
849	0	979	1
772	1	165	0
136	0	883	0
51	0	115	0
968	0	658	0
84	0	83	1
111	0	995	0
907	0	882	0
792	0	31	0
82	0	30	0
4	1	304	0
133	0	316	1
655	0	322	0
948	0	327	0
42	0	334	1
934	0	341	0
304	0	384	0
325	0	395	0
330	0	396	0
337	0	399	0
361	0	305	0
348	0	307	1
364	0	308	0
368	0	400	0
386	1	408	1
393	0	503	0
403	0	520	0
404	0	635	3
413	0	703	0
488	0	720	0
A326	1	733	0

Calf ID#	Treatment code	Calf ID#	Treatment Code
A331	0	857	2
A337	0	903	0
491	0	909	0
A404	0	919	0
988	0	947	0
867	0	958	0
854	0	994	0
818	0	991	1
808	0	182	2
70-77	0	979	0
777	0	953	1
70-55	1	937	0
70-47	0	778	0
849	0	764	0
mean	0.10	mean	0.36

	Sick	No	TOTAL
Treatment	a	b	a+b
Control	c	d	c+d
TOTAL	a+c	b+d	a+b+c+d

In this table, **a** for instance is the number of animals of the treatment that got sick. Determine the values **a,b,c** and **d** and fill the rest of table with the sums.

Using this table the formula for the test is:

$$\text{Chi } 2 = \frac{(ad-bc)^2}{(a+b)(c+d)(b+d)(a+c)}$$

Then you look up this value on this table (your degrees of freedom are 1)

Df	0.5	0.1	0.05	0.02	0.01	0.001
1	0.455	2.706	3.841	5.412	6.635	10.827

	Sick	No	TOTAL
Treat	5	45	50
Control	13	37	50

So the calculations are going to be:

$$\text{Chi squared} = \frac{(5 \cdot 37 - 13 \cdot 45)^2 \cdot (100)}{(50) \cdot (50) \cdot (82) \cdot (18)} = \mathbf{4.336}$$

$$(50) \cdot (50) \cdot (82) \cdot (18)$$

Looking to the chi-squared table, the value is between 6.635 and 10.827, so the pvalue will be between 0.01 and 0.001.

The real p-value is **0.0213 ~ 0.021**

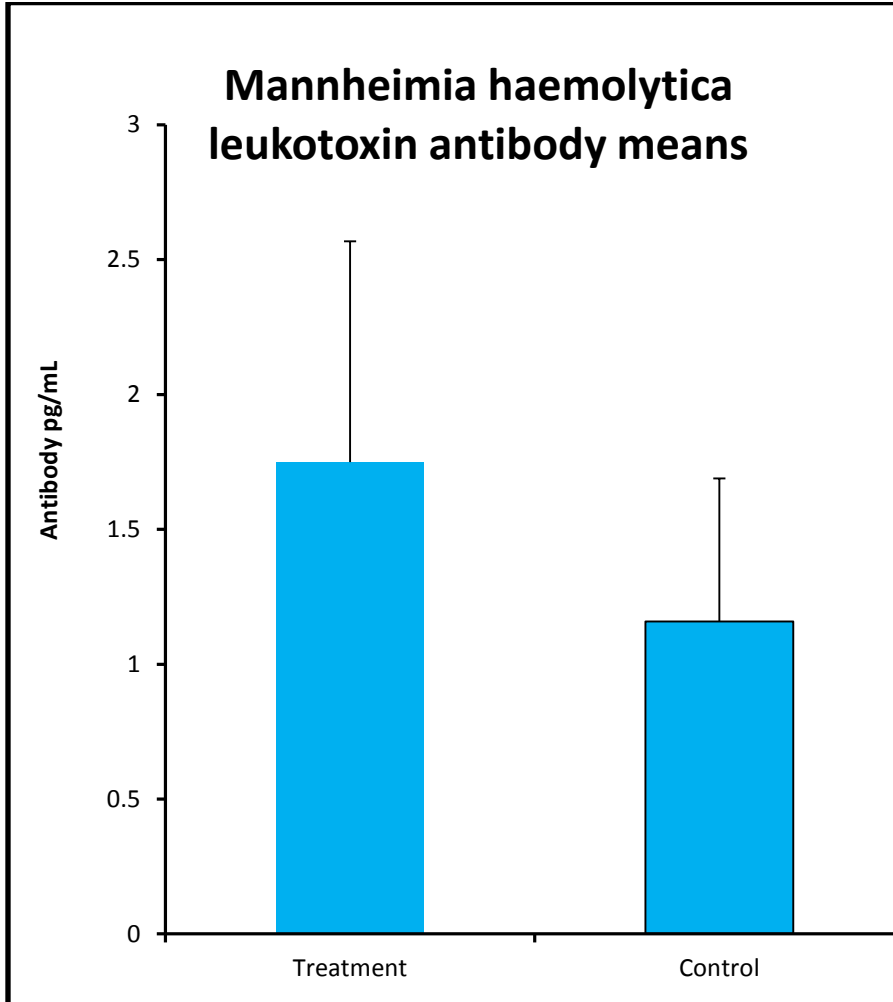


Figure 53. Mean *Mannheimia haemolytica* leukotoxin serum titers (pg/ml) in control versus Co treatment calves.

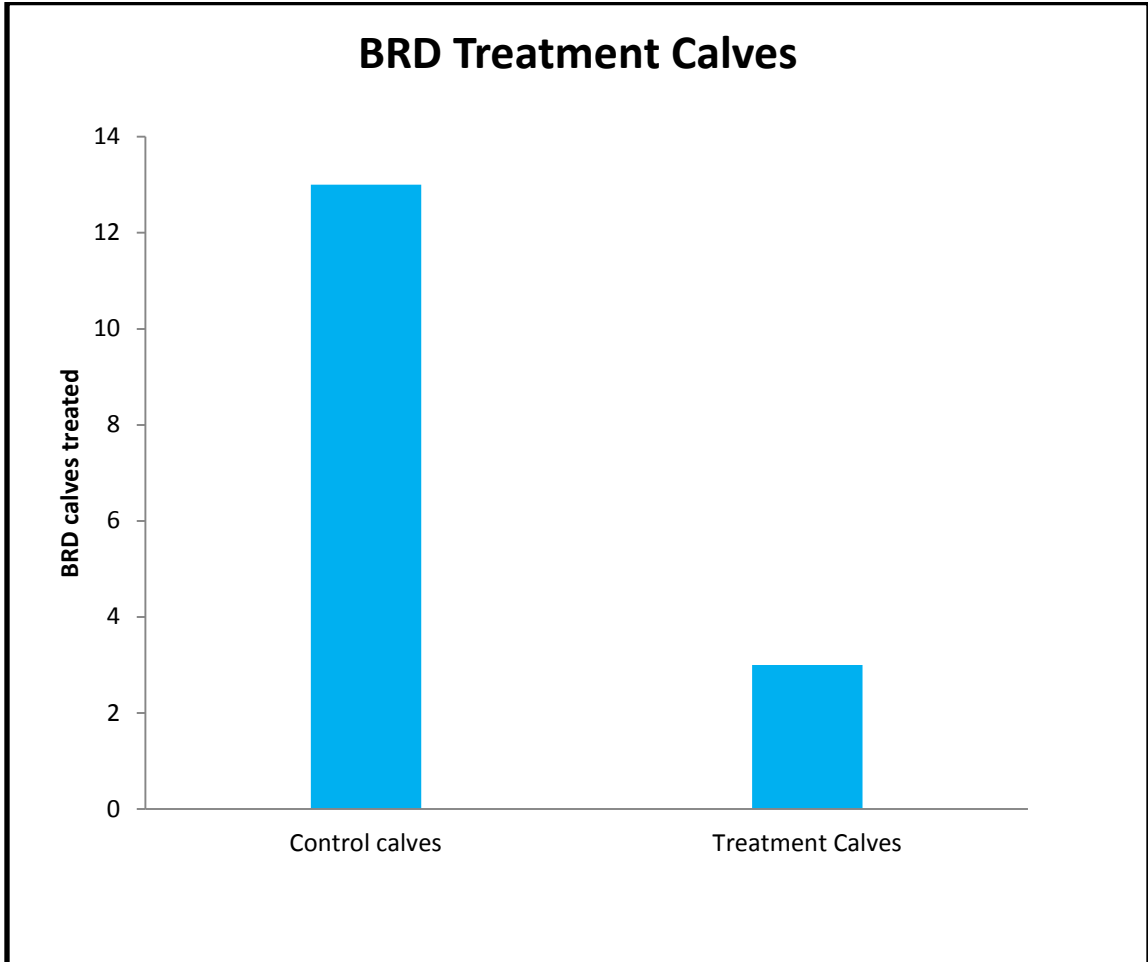


Figure 5.6. Number of cases of BRD reported during the feeding period in control versus Co treatment calves.

KILL NUMBER	EAR TAG #	Hot Weight	Grade	Yield	Co treatment	Yield	Grade	HCW	Controls	Yield	Grade	HCW		
1	905	758	Choice	3	655		3	2	680	905	3	2	758	
2	655	680	Choice	3	756		2	2	867	786	3	2	801	
3	756	867	Choice	2	792		3	3	820	926	4	2	879	
4	792	820	Select	3	51		4	2	832	83	3	2	900	
5	51	832	Choice	4	968		4	2	1001	64	3	2	732	
6	786	801	Choice	3	934		3	2	933	115	3	2	785	
7	PURPLE 968	1001	Choice	4	84		3	2	881	833	3	2	897	
8	926	879	Choice	4	133		2	2	801	979	2	2	759	
9	934	933	Choice	3	849		4	2	1036		3			
10	83	900	Choice	3	50		3	2	757					
11	84	881	Choice	3	111		3	2	777					
12	133	801	Choice	2										
13	64	732	Choice	3										
14	PURPLE 849	1036	Choice	4			3.090909	2.090909	853.1818	Means	3	2	813.875	
15	50	757	Choice	3										
16	115	785	Choice	3	Lucas carcass data on Co treated calves									
17	833	897	Choice	3										
18	979	759	Choice	2										
19	111	777	Choice	3										

Figure 5.7. Lucas calves final carcass measurements with HCW, Yield, and Grade per ID of calf.

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CHAPTER SIX
CONCLUSIONS

Three studies were conducted to test the hypothesis that cobalt concentration above the National Research Council recommendations affect immune response in cattle. In Experiment 1, 27 beef steers were fed treatment levels of cobalt at NRC recommendations 0.139 ppm (NRC), 0.489 ppm (4X NRC) , or 0.889 ppm (10 X NRC). Overall Co supplementation resulted in increased antibody response ($P < 0.004$) to RB 51 *Brucella abortus*. In Experiment 2, 15 Holstein steers were supplemented Co treatment rates of 0.10 ppm, 0.20 ppm, and 0.40 ppm in milk replacer fed twice daily. Increased immune response to *Mannheimia haemolytica* in the treatment groups resulted, but was not statistically significant. In Experiment 3, 268 beef steers received a Co (30 g of cobalt oxide) sustained release bolus with 100 steers randomly selected as controls (no cobalt bolus administered). Antibody response to *Mannheimia haemolytica* leukotoxin was analyzed by a Welch Two Sample t test with differences considered significant at $P < 0.05$. Differences between treatment and control calves showed treatment calves had a 42% increase to *Mannheimia haemolytica* leukotoxin antibody ($P = 0.0586$) compared to control calves. Feedlot health (morbidity and mortality) was evaluated pre-weaning and during the feeding period. Differences between treatment and control groups showed decreased BRD in the Co treatment group ($P = 0.0217$) compared to the control group.

Improved humoral immune function has potential to reduce sickness, improve health and increase profits in beef calf post-weaning production. Nutritional management efforts to minimize health problems affect production throughout life. Results of these

experiments suggest that increasing cobalt supplementation increases beef calf health through improved immune response. Bovine respiratory disease continually costs the beef industry over \$2 billion annually because of affected animals that increase cost of production, have decreased performance, and reduced carcass quality in spite of improved health programs, improved BRD treatment products and drugs, and increased knowledge.

Additional studies using increased number of calves (and improved replication) and serum sampling for *Mannheimia haemolytica* leukotoxin within the 60 d post-vaccination period would improve our results and understanding of the effects of Co supplementation in these beef calves. Cobalt supplementation above NRC recommendations resulted in improved health during the feeding period. Our findings warrant further investigation and indicate that NRC-recommended Co levels may need to be increased for improved beef calf health and decreased BRD problems in newly arrived beef calves.

This study is relevant to the U.S. Beef Cattle Industry in that these results indicate that Co may decrease incidence of BRD and improve health in weaned beef calves. Previous NRC Co recommendations were derived from studies during the 1950's when beef cattle production goals were much different than today. Results from this study indicate that current NRC Co levels should be increased to improve post weaned health and performance in beef calves.

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