Effects of Serum Total Protein on Health Measures, Average Daily Gain, and Metabolites in the Holstein Dairy Calf

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Authorization to Submit Thesis

This Thesis of Benjamin J. Tverdy, submitted for the degree of Master of Science with Major in Animal Science and titled "Effects of Serum Total Protein on Health Measures, Average Daily Gain, and Metabolites in the Holstein Dairy Calf," has been reviewed in final form. Permission, as indicated by the signatures and dates below, is now granted to submit final copies to the College of Graduate Studies for approval.

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Abstract

An objective of this study was to determine the associations of passive transfer status, by assessment of serum total protein (TP), with serum metabolites, daily gain, morbidity, and mortality in neonatal male Holstein calves (n=1,631). Calves were purchased from dairy farms in the western United States and placed in a calf ranch as one day old. Calves were assigned an individual electronic identification and recorded in Feedlot Health Management Services proprietary software, iFHMS (Feedlot Health Management Services, Okotoks, Alberta). Cause-specific morbidity and mortality was recorded for each calf daily from entry to exiting or death. A 5-mL tube of blood was collected from each animal at 48 ± 6h post-arrival. Whole blood was centrifuged at 2000 *g* for 10 min and serum was stored at -20°C. Serum TP was measured using a digital refractometer. Calves were categorized based on proposed USDA serum TP guidelines into poor (TP < 5.1 g/dL, n=159, mean ± SD 4.68 ± 0.31 g/dL), fair (5.1 < TP ≤ 5.7 g/dL, n=399, 5.45 ± 0.19 g/dL), good (5.8 ≤ TP ≤ 6.1 g/dL, n=322, 5.96 ± 0.11 g/dL) and excellent (TP > 6.1 g/dL, n=751, 5.96 ± 0.11 g/dL). Samples were analyzed using a reverse-phase HPLC using a C18 column for vitamins and a colorimetric assay for glucose. Data were analyzed using generalized linear mixed models and logistic regression models with significance declared at $P \leq 0.05$. Significant differences between poor and excellent were observed in mortality and otitis disease treatments ($P < 0.05$ for both). Differences were observed when comparing poor and fair to good and excellent TP categories for serum glucose ($P < 0.05$). For serum retinol, comparison of the poor protein category with all other groups indicated significant differences ($P = 0.001$). Serum β-carotene and α-tocopherol were different when comparing all TP categories against the excellent ($P < 0.05$ for both). Average daily gain at 90 d and for the overall feeding period was not statistically different among TP categories. Generally, serum metabolites showed differences among TP categories, suggesting a potential associative relationship with health and the immune system.

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Dedication

I dedicate this project to my beautiful wife and children; without their examples of selflessness, innocence, joy, and acceptance this project would lack depth and meaning.

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Literature Review

Introduction

Often the calf grower stage of production is overlooked, especially when considering the male dairy breeds. Most research and literature is focused on the female dairy calf for obvious reasons (Maunsell & Donovan, 2008). Calf grower operations do not "fit" in a traditional silo; feedlot or a dairy production setting and are overlooked and underreported as the resource they are. The primary challenges the calf grower facilities, raising large populations of male calves, face is morbidity and mortality. High morbidity and mortality are a result of failure of passive transfer of antibodies (Robison et al., 1988). Male dairy breed calves traditionally have been viewed as a byproduct as the focus is placed, by most dairy operations, on heifer rearing (Renaud et al., 2017; Holden & Butler, 2018). In recent years more and more dairy influenced cattle are being reared for beef production across North America. The focus of passive transfer of maternal antibodies in the dairy bull calf has gained attention, and warrants further examination (Fairbairn & Felix, 2020; Rusche, 2020; Rutherford, 2020).

Passive transfer of antibodies is vital to the overall health of the bovine species. Failure to ensure adequate transfer of maternal antibodies to the neonatal bovine, in the form of colostrum, negatively impacts health and growth performance throughout life (Robison et al., 1988; Pare et al., 1993; Donovan et al., 1998; Atkinson et al., 2017). The neonate leaves the sterile environment of the uterus and enters a nonsterile environment upon birth. Neonatal ruminants can mount an immune response. The calf's immune system is functional; however, it is naïve and immature, increasing the susceptibility to adverse health events such as diarrhea, septicemia, enteritis, omphalophlebitis, failure to thrive, and death (Barrington & Parish, 2001). The immune system immaturity in regards, to protective mechanisms, such as timed delay, antigen recognition, and robustness of immune response increases the likelihood of the calf succumbing to infections considered innocuous to the adult bovine (Barrington & Parish, 2001). Morbidity and mortality susceptibility negatively impact economics and perception of the industry. Neonatal calves are considered protected from diseases by the passive transfer of maternal immunity; otherwise, known as successful passive transfer of maternal antibodies. Successful passive transfer of maternal antibodies does not guarantee

absence of disease; however, it primes the immune system for future challenges. Failure of passive transfer (FPT) is a condition not a disease, this condition predisposes neonatal calves to disease. Calves with FPT may remain healthy in a sterile environment; conversely, calves with adequate passive (APT) transfer of maternal antibodies may be susceptible to disease if the environmental pathogen load is high enough. The term failure of passive transfer has been used widely to describe the lack of absorption of adequate amounts of immunoglobulins. Immunoglobulins are not the only constituent of colostrum; however, they are the most investigated and understood component. Colostrum is a complex fluid that contains: immune cells, cytokines, vitamins, carbohydrates, amino acids, fat, and minerals. (Barrington & Parish, 2001; Kehoe, 2006).

Immune development, modulation, and functionality are critical in mounting an appropriate immune response to a given stimulus. Glucose and lipid soluble vitamins are critical components of immune cell function, development and signaling. It has been suggested that deficiency of lipid soluble vitamins is a result of low colostral concentrations, dam's diet, and a reduction in placental transfer *in utero* (Loosli, 1949). The newborn calf born with low levels of tocopherols relies on colostrum to replenish lipid stores (Hidiroglou et al., 1992; Zanker et al., 2013). A direct correlation between fat content in colostrum and concentrations of lipid-soluble vitamins has been established (Weiss et al., 1990). After supplementation with alpha-tocopherol cows demonstrated unexplained increased levels of beta-carotene in colostrum (Weiss et al., 1990). An increase in serum vitamin A was noted in calves born to cows that were supplemented prepartum with vitamin A or carotene (Spielman et al., 1946). A synergistic relationship exists among adequate passive transfer of maternal antibodies, serum metabolites and overall health in the neonate bovine. There are two distinct processes that must occur to insure adequate passive transfer of maternal antibodies; the first is colostrogenesis in the Dam, the second is absorption of colostrum by the neonate.

Synthesis and Deposition of Immunoglobulins "Colostrogenesis"

Colostrum is the first milk secreted at parturition and can last up to 4 days after lactation begins (Godhia & Patel, 2013). Colostrum is vital in development of humoral, adaptive, and passive immunity in the bovine neonate (Barrington & Parish, 2001; Heinrichs, 2017).

Colostrogenesis is the process of transporting immunoglobulins from maternal circulation (specifically serum) into mammary gland tissues, thus synthesizing colostrum. Colostrogenesis involves many cellular, biochemical, and physiological processes that encompass many important factors and components. Colostrum contains proteins, carbohydrates, fat, vitamins, minerals, and biologically active molecules that are required for specific immune functions (Aydogdu & Guzelbektes, 2018). The most important are immunoglobulins which are glycoproteins that are produced by plasma cells. These proteins are referred to as antibodies and function to neutralize pathogens. Large amounts of immunoglobulins, as much as 500 g/wk., accumulate in the mammary gland in the weeks prior to parturition (Baumrucker et al., 2010). In the last few weeks of gestation, circulating estradiol and progesterone hormones are elevated and are responsible for initiating transfer of IgG1 from blood to mammary secretions (Stark et al., 2013). High concentration of colostral immunoglobulin G1 (IgG1) is derived from the circulating immunoglobulin pool facilitated by active, receptor mediated, specific transport by mammary alveolar epithelial cells (Barrington et al., 1997). It is hypothesized that IgG1 is transported across mammary epithelial cells by Fc receptors of mammary gland tissue (bFcRn) by a process termed transcytosis (Ghetie & Ward, 2000). While immunoglobulin G1 is not the only subclass of immunoglobulins transported, there is evidence that IgG1 is dominant in a ratio of 7:1 relative to IgG2 transport (Sasaki et al., 1976); therefore, the remaining immunoglobulin discussion herein will focus on IgG1.

The IgG1 antibody is transported via endosomes from blood through the polarized mammary epithelial cells into colostrum (Sasaki et al., 1976). On the basal side, cells enclose IgG1 forming another endosome where it is either transferred to the apical side of the cell (transcytoses) for antigen presentation or recycled (Stark et al., 2013). Endosomes containing IgG1 are regulated by small GTPases that are found intracellularly (Stark et al., 2013). The transcytotic functionality and capacity of mammary gland FcRn receptors seem to only be viable in the 3-4 wk prior to parturition (Barrington et al., 2001). Endocrine regulation induces changes in FcRn receptors which down regulates them as mature milk production occurs in the lactating cow (Akers, 1985). Experiments conducted *in vivo* two weeks prior to parturition, demonstrated and increase in estradiol concentration while progesterone decreased in blood, further demonstrating endocrine regulation of colostrogenesis (Tucker,

1981). The mass transcytosis of IgG1 in bovine mammary secretions occurs weeks prior to parturition and the induction of colostrogenesis is bimodal in nature (Baumrucker et al., 2010).

Absorption of Maternal Antibodies

It is understood that the neonatal bovine is void of circulating, functional immunoglobulins at birth. The transfer of immunoglobulins and other immunologically active cells in colostrum is termed passive transfer. Passive transfer of immunity in neonates varies among species. Maternal antibody transfer in the bovine is based solely upon ingestion of colostrum (Baumrucker et al., 2010). The placenta of the domestic bovine prevents transfer of immunoglobulins from the dam to the fetus in utero (Weaver et al., 2000). The synepitheliochorial classification of the placenta isolates maternal blood supply from fetal blood supply; therefore, calves are agammaglobulinemic at birth (Haeger et al., 2016). The term immuno-naïve has been used to describe the neonatal bovine's immune system and subsequent response to pathogens at birth. The immune response is functional, but it is considered naïve and immature in the newborn bovine. Failure of passive transfer is caused by poor quality colostrum, inadequate enteral uptake of IgG, and, or poor timing (Hedegaard & Heegaard, 2016).

Absorption of colostral antibodies from the gut lumen into the neonate's circulatory system leads to priming of the immune system. Ingestion of colostral immunoglobulins, within the first 24-hours of life, is vital in establishing passive immunity and aids in prevention of disease and promotion of growth performance outcomes in the bovine (Pierce & Feinstein, 1965). Adequate quality colostrum must be provided within twenty-four h of life to facilitate proper absorption of necessary levels of immunoglobulins (Weaver et al., 2000). The absorptive capacity by the intestines in the newborn calf is non-selective (Bush & Staley, 1980). Absorption is not regulated by molecular weight or size; however, some suggest it is regulated intracellularly by receptors that account for some selective transmission of proteins into the neonates circulation (Bush & Staley, 1980). The neonatal bovine is considered to have an open gut (Godden, 2008). This allows Fc-receptor-mediated transfer of IgG1 across the lumen to the circulation for the first 24 hrs. after birth (Hedegaard & Heegaard, 2016).

This assures rapid establishment of necessary circulating levels of maternal antibodies via ingestion of colostrum (Hedegaard & Heegaard, 2016).

Failure of Passive Transfer, and Morbidity and Mortality in the US Dairy Herd

Elevated neonatal bovine mortality is a common problem in modern animal agriculture in terms of health, economics, and welfare of the industry. Poels, at the request of the Dutch Minister of Internal Affairs, began investigating calf mortality as early as 1897 (Lateur-Rowet & Breukink, 1983). Literature suggests that failure of adequate passive transfer (FPT) increases morbidity, mortality by reduction in calf growth performance and decreased milk production in first and second lactations (Wells et al., 1998). Failure of passive transfer (FPT) is a condition that predisposes the bovine to disease; because of reduced humoral immunity and delayed or retarded gastrointestinal development (Weaver et al., 2000; Ballou et al., 2018). Colostrum has demonstrated protection against *Escherichia coli* O157:H7 challenge studies as evidenced by severity of disease demonstrated in trial (Dean-Nystrom et al., 1997). The condition FPT has major negative economic, growth performance, and health performance impacts on animal agriculture (Weaver et al., 2000). According to the USDA-Animal and Plant Health Inspection Services published in 2011, 19.2% of heifer calves reported FPT. In the most recent National Animal Health Monitoring System report published in 2016, 64% of dairy farms reported calves with FPT. Colostrum contains vital immunoglobulins, namely IgG, vitamins, and maternal cells that provide imunoprotection to the neonate and assist in programing the immune system. In a report titled Dairy Heifer Raiser, 2011 drafted by the USDA, “digestive problems and pneumonia were the most common diseases or disorders affecting pre-weaned heifers.” This same report identified respiratory disease as the most common disorder affecting weaned heifers and diarrhea as second most common malady; of which, both were treated with antibiotics on greater than 80% of operations costing the industry millions of dollars annually (USDA-Animal and Plant Health Inspection Service, 2011). Many academic studies fault FPT as the major risk factor in the incidence and severity of enteric, and respiratory calf diseases (Wells et al., 1996; Donovan et al., 1998; Tyler et al., 1999; Godden, 2008; Maunsell & Donovan, 2008). Failure of passive transfer calves have reduced average daily gain and are at an increased risk of dying in the first 90 days of life (Robison et al., 1988).

As previously mentioned, colostrum contains many macro and micronutrients that are crucial in immune development, function, and programming. Lipid soluble vitamins namely vitamin A and E play a critical role's in growth, reproduction, differentiation of epithelial cells, quenching free radicals and terminating lipid peroxidation respectively. Deficiencies in vitamins A, D and E translates to retardation of the immune-suppressing capabilities of the body (Puppel et al., 2019).

Glucose in the Neonatal Bovine

Glucose is an essential fuel source for life in the mammal. Colostrum ingestion doesn't directly affect endogenous glucose production; however, it stimulates intestinal growth and development thus promoting glucose absorption, hepatic glycogen storage and improving glucose status (Hammon et al., 2012). Accelerated maturation of somatotropic axis that is noted in colostrum-fed calves leads to enhanced production of insulin-like growth factor-1 further improving the energetic status of the neonate bovine (Hammon et al., 2012). Insulin-like growth factor-1 increases proliferation of epithelial cells of the intestinal crypts (Xu, 1996). Serum glucose concentrations are lower at birth than at 2 days of age in the newborn calf because of endogenous glucose production (Blum et al., 1997). Providing colostrum at birth clearly increases glucose absorption, gut maturation and immunity (Blum et al., 1997; Hammon et al., 2019). It has been demonstrated that enhanced milk ration feeding programs stimulate long non-coding RNA, which is involved in regulation of tight-junction protein synthesis (Weikard et al., 2018). Early diet affects tight-junctions protein synthesis and the development of intestinal epithelium, thus the intestinal mucous barrier (Malmuthuge & Guan, 2017; Steele et al., 2016). Defense against pathogens is dependent on maturation of the intestinal immune system that is obtained by the calf through maintaining an adequate plane of nutrition (Khan et al., 2011; Hammon et al., 2019). It has been demonstrated that calves with higher plane of nutrition had faster resolution from *Cryptosporidium parvum* and had higher resistances against *Salmonella typhimurium* (Ollivett et al., 2012; Ballou et al., 2018). In the diseased state, nutritional and energy stores are shifted away from anabolic and maintenance to metabolic processes that drive the immune system leading to an anorexic state (Lochmiller & Deerenberg, 2000). It has been established that whole-body glucose utilization can increase as much as 68% during the acute-phase of the immune response (Klasing, 1988).

This preponderance of data suggests synergy among colostrum, gut development, plane of nutrition, and the ability of calf immune system to mount an appropriate response to common gastrointestinal diseases.

Acquisition and Sequestration of Lipid Soluble Vitamins

Lipid soluble vitamins are absorbed into plasma and deposited in various organs and tissues. For the purposes of this work the discussion will focus on lipid soluble vitamins specifically vitamin A and E and relevant precursors. There are several major factors influencing an animal's usable source of lipid soluble vitamins; namely, dietary supply, intestinal absorption and metabolic ability to convert precursors to usable forms (Green & Fascetti, 2016). The pre-ruminant bovine serves as a model for beta-carotene absorption and metabolism in humans. Deposition of beta-carotene from plasma occurs and is deposited in various organs as well as adipose tissue (Poor et al., 1993). Vitamin A is not found in plants but carotenes, the precursors, are and the primary source of conversion is the intestinal mucosa (*DSM*, 2020). The preruminant calf readily absorbs beta-carotene, which leads to vitamin A stores (Hoppe et al., 1996). The neonatal calf is dependent on colostrum for its initial source of vitamin E (Millar et al., 1973). Calves that receive colostrum after 12 h of life have lower plasma concentrations of beta-carotene, retinol, and alpha-tocopherol lasting up to a month after birth (Zanker et al., 2013). The following sections will focus on absorption, transport and storage of retinol, beta-carotene and alpha-tocopherol.

Retinol and Beta-carotene Absorption and Storage

Vitamin A is essential for all vertebrate life. Retinoids and carotenoids are required for maintaining many essential physiological processes in the body, including normal growth and bone development, vision, a healthy immune system, normal reproduction, and healthy skin and barrier functions. It has been shown that as many as 500 genes are thought to be responsible for the metabolism of retinoids (D'Ambrosio et al., 2011). The precursors to vitamin A are obtained in the diet of man and animals. Vitamin A is essential to maintaining structure and function of specialized epithelial, glandular tissues and the visual functions of the retina and growth of the body (Jolliffe et al., 1950). Absorption of retinol and beta-carotene occurs in the gastrointestinal tract with the primary source of absorption being the proximal jejunum (*DSM*, 2020). Stallcup & Herman (1950) demonstrated the conversion of

carotene to vitamin A occurs in the small intestine. Erwin & Varnell (1959) found carotene and vitamin A to be associated with specific serum proteins, albumen and alpha globulin (D'Ambrosio et al., 2011). Metabolism of retinoids occurs in the lumen as well as enterocytes of the gut (D'Ambrosio et al., 2011).

> "Normal pancreatic, liver and bilary function and adequate fat intake are required for absorption of vitamin A and its precursors. Dietary retinyl esters are hydrolyzed to retinol by pancreatic esterase in the small intestine. They are absorbed as the free alcohol in association with lipid micelles and then re-esterified to form retinyl palmitate in the mucosa. The retinyl esters are transported via the lymphatic system, mainly in association with chylomicrons, to the liver where they are hydrolyzed to retinol and re-esterified for storage in parenchymal cell. Hydrolysis of the stored retinyl esters liberates retinol that is combined with retinol-binding protein for secretion into the bloodstream. The retinol binding protein is associated with a thyroid hormone; therefore, metabolic interdependence between vitamin A and thyroid function. Iodine deficiency impairs vitamin A metabolism, and vitamin A deficiency impairs thyroid function (*DSM*, 2020)."

A key point to beta-carotene and retinol absorption is normal physiologic activity of the mammal. It is also understood that dietary antioxidants such as vitamin E affect the utilization and perhaps the absorption of carotenoids (*DSM*, 2020).

Alpha-tocopherol Absorption and Storage

Vitamin E is a potent lipid soluble antioxidant. Absorption takes place synergistically with fat digestion and absorption and requires pancreatic lipase, esterase enzymes and bile salts (Sitrin et al., 1987). The efficiency with which vitamin E is digested and absorbed varies with dietary inclusion levels (Leeson & Summers, 2001). The proximal two-thirds of the small intestine is the site of absorption (Bjorneboe et al., 1990). Whether presented as free alcohol or esters vitamin E is absorbed in the alcohol form without being re-esterified (*DSM*, 2020). Enterocytes absorb vitamin E where it is incorporated into chylomicrons (Bjorneboe et al., 1990). These chylomicrons are then absorbed into the lymphatic system, hydrolyzed and finally absorbed by tissues (Bjorneboe et al., 1990). All tissues and organs accumulate vitamin E with largest accumulation being in adipose tissue, skeletal muscle and the liver

(Bjorneboe et al., 1990). Immune cells such as lymphocytes, neutrophils, and macrophages contain very high levels of vitamin E (*DSM*, 2020). There have been several trials conducted at the cellular and live animal levels that have found higher concentration of vitamin E in immune cells compared to blood cells. (Lewis et al., 2019). Vitamin E is known to be very effective at modulating immune function. The main functions of alpha-tocopherol are modulation of the immune system and providing protection against oxidation of immune cell membranes that are enriched with polyunsaturated fatty acids (Lee & Han, 2018). A preponderance of evidence suggests selenium and vitamin E function together to protect cell membranes from oxidative damage (Underwood et al., 2015). An examination of lipid soluble vitamins and the critical role they play in immune system and disease modulation is necessary.

Immune Function of Lipid Soluble Vitamins

The immune function of vitamins A and E have been well established. They are essential nutrients and serve several critical functions in leukocyte biology (Eicher et al., 1994). Neutrophil and macrophage chemotactic activity and bactericidal activity varies with supplementation of vitamin A and vitamin E *in-vitro* (Eicher et al., 1994). It has been hypothesized that micronutrient deficiency may relate to infectious diseases in five ways: decreased feed intake, nutrient absorption, nutrient loss, catabolic loss, and utilization impairment (Stephensen, 2001). In humans and animals, one of the most common signs of illness is decreased feed and water intake. For example, children with measles consumed 75% fewer calories when ill compared with their caloric intake after recovery. Certainly, scouring calves would have a direct loss of micronutrient namely lipid soluble vitamins through increased fecal output as well as malabsorptive losses based on disruption of gut lumen and enterocyte integrity (Naylor, 2009). Serum retinol is decreased in the acute phase response to trauma, and infection. The decrease in serum retinol is correlated to the severity of the insult (Stephensen, 2001). Neonatal calves are able to mobilize fat and provide NEFA for energy supply when milk intake is inadequate, suggestive of utilization of lipid soluble vitamins in immune function somewhat independent of nutritional status (Hammon et al., 2012). Neonatal calf diarrhea affects calves most severely in the first 30 days of life (Naylor, 2009). There are

several relevant etiologic agents that cause disease in the calf. These infectious substances are often classified into two general categories, viral and bacterial.

The innate immune system is made up of epithelial barriers (skin), phagocytes (neutrophils and macrophages) and natural killer cells, complement proteins and acute phase proteins that are regulated by proinflammatory cytokines (Stephensen, 2001). A preponderance of evidence suggests a deficiency in vitamin A compromises mucosal epithelial barriers of the eye, respiratory, gastrointestinal and urogenital tracts. Loss of mucus-producing goblet cells and; therefore, reducing the cleansing flow of mucous from the body has been shown in vitamin A deficiency (Eicher et al., 1994; Franklin et al., 1998; Stephensen, 2001; Spears & Weiss, 2014; Weiss, 2017; Puppel et al., 2019). Vitamin A regulates the speed of gene transcription, synthesis mucopolysaccharides co-creating connective tissue and glycoproteins, which are a constituent of body fluids and membrane proteins (Puppel et al., 2019). Deficiency of retinol lowers the protective capability of the epithelium and increases the chance for diarrhea caused by *Escherichia coli*, and viral pathogens (Lopez et al., 1988; Puppel et al., 2019). Hypovitaminosis A was demonstrated to be coupled with secondary *Escherichia coli* infections in beef calves (He et al., 2012). During disease, the immune system is altered; therefore, neutrophil function, lymphocyte responsiveness, antibody response and cellular cytokine production is affected (Mallard et al., 1998). Fully oxidized beta-carotene has been show to modulate the innate immune system in several ways including limiting inflammation (Burton et al., 2014). Further examination of all-trans retinoic acid and fully oxidized beta-carotene demonstrated increased neutrophil apoptosis and indicated the anti-inflammatory properties of these compounds (Duquette et al., 2014). Studies have shown promise as a nutraceutical strategy for treatment in cattle with respiratory disease (Duquette et al., 2014). Severe inflammation is a major component regarding the respiratory diseases caused. *Mannheima haemolytica* is the primary bacterial species associated with bovine respiratory disease (Cozens et al., 2019). Economic losses of 1 to 3 billion dollars from bovine respiratory disease annually are reported in the United States (Cozens et al., 2019). Inflammation becomes self-perpetuating and in this case is a result of extensive activation, and migration of neutrophils (Gompertz & Stockley, 2000). Retinoids have anti-inflammatory properties and show promise in the treatment of certain skin diseases and reduction in pulmonary infiltration in the rodent (Redlich et al., 1998; Swamidas et al.,

1999). The complete effects of retinoids and relationship, in neutrophilic function in the bovine is not currently understood.

Alpha-tocopherol plasma concentrations of less than 15-200 micrograms per deciliter are considered diagnostically deficient in cattle (Radostits et al., 2007). Higher levels of vitamin E have been found to influence humoral and cellular immune responses (Cipriano et al., 1982; Reddy et al., 1987). Vitamin E is a potent antioxidant scavenging peroxyl radicals and terminates the oxidation of polyunsaturated fatty acids (Lee & Han, 2018). Data suggest conventionally fed calves were considered deficient in vitamin E leading to oxidative damage of white blood cell membranes (Reddy et al., 1987). Reddy et al. suggest a serum concentration of alpha-tocopherol be 200-250 µg/dL to maximize performance by 4 weeks of life.

Failure of Passive Transfer and Lipid Soluble Vitamins

Failure of passive transfer of maternal antibodies in the form of colostrum causes immunodeficiency and immune dysfunction in the bovine. It has been well established that colostrum contains significant amounts of lipid soluble vitamins (Jones et al., 1962; Kehoe, 2006; Kehoe et al., 2007; Puvogel et al., 2008; Godhia & Patel, 2013). Neonatal calves can mobilize fat and provide non-esterified fatty acids (NEFA) for energy supply when milk intake is inadequate (Hammon et al., 2012). This mobilization suggests utilization of lipid soluble vitamins in immune function somewhat independent of nutritional status. Without ingestion of adequate amounts of colostrum the neonatal bovine has a reduced lipid soluble vitamin body stores; therefore, reduced functionality of the immune system (Eicher et al., 1994; Lewis et al., 2019; Samanta et al., 2006; Zanker et al., 2013). It has been reported that vitamin A and E increased phagocytosis by neutrophils through membrane stabilization (Eicher et al., 1994). Beta-carotene is taken up by lymphocytes, which is suggestive of a functional role of this vitamin A precursor (Chew, 1993). Researchers could hypothesis that FPT in the neonate bovine is multifactorial, which involves local and cell-mediated immunity, immunoglobulins, immune cells and various cytokines that are supported by glucose metabolism, and lipid soluble vitamins. Inadequate intake of good quality colostrum in the first 24 hours of life results in immune system dysfunction in the neonatal bovine. Researchers have demonstrated that colostrum quality varies among breed, and individual

cows (Pritchett et al., 1991; Maunsell et al., 1999; Gulliksen et al., 2008). It is understood that season and parity effects colostrum IgG quality (Gay et al., 1983; Yayalak et al., 2016). IgG levels of colostrum increase in the spring likely due to consumption of fresh green forages further bolstering the hypothesis that lipid soluble vitamins, especially A and E, play a major role in immune development and function (Gay et al., 1983; Gulliksen et al., 2008).

Research

Hypothesis

Calves with failure of passive transfer (FPT) of maternal antibodies will have poor health and growth performance outcomes compared to those for adequate passive transfer calves. Different levels of serum total proteins affect lipid soluble vitamins and immune development and function of the neonate calf.

Objectives

The first objective of the present study was to investigate the association of serum total protein levels and overall mortality. The second objective was to investigate any relationship between serum total protein levels and average daily gain, and clinically relevant health outcomes. The third objective was to assess the association of differing levels of serum metabolites (alpha-tocopherol, beta-carotene, retinol, and glucose).

Materials and Methods

Animals, Treatments, and Experimental Design

All animals and procedures were approved by the University of Idaho Animal Care and Use Committee (2018-20).

Animals:

This study was conducted at a commercial contract calf grower facility in the Western United States. A total of one thousand, six hundred thirty-one, day-old Holstein male calves were allocated in two different timeframes. Eight hundred eighty-one, study animals were enrolled from July 7th to July 11th, 2018 and the remaining seven hundred fifty, were enrolled from November 3rd to November 8th, 2018. An observational prospective cohort trial design was chosen to test associations.

Criteria for enrollment were that calves must appear bright alert and responsive and void of physical, birth, or congenital abnormalities. Study animals were weighed, then placed in individual 0.91 meter by 1.52 meter covered, wooden hutches that were set on a slate floor. The November enrolled animals were weighed, then placed in a hutch with exact dimensions; however, the flooring was dirt covered with wheat or barley straw that was replaced bi-weekly.

Water and standard mixed complete liquid and complete feeds were formulated to meet or exceed National Research Council nutritional requirements for calves were offered, throughout the study period. Calves were fed 1.89 L a commercially available liquid milk replacer that contained 28% protein and 28% fat (Table 1). Study animals were fed this twice a day for the first 4 weeks of life. Then calves were fed 2.84 L of a commercially available liquid milk replacer that contained 25% protein and 28% fat (Table 2). Study animals were fed this twice a day until weaning from milk. A timed weaning from milk approach was initiated at day 56 ± 3.5 days, at which time the animal received a single 2.84 L feeding of liquid milk replacer once a day for a week then weaned completely from milk.

Calves were provided starter grain that consisted primarily of corn and soybean ad libitum beginning day 4 of life through day 28 of life (Table 3). Study animals were offered

ad libitum grower total mixed ration (TMR) that consisted of corn, distillers' grains, alfalfa hay, soybean, and hay or corn silages, beginning at day 29 and were fed this diet throughout the calf's time at the calf grower operation. Milk and feed analysis were conducted at Washington State University (Pullman, WA. 99163) and Dairy One forage laboratory (Ithaca, New York, 14850), respectively.

Data Collection

The method for data collection was Feedlot Health Management Services (Okotoks, Alberta Canada), proprietary software iFHMS system. Over the observational period, health outcomes (treatments) and average daily gain were recorded and entered into iFHMS on an individual animal level from arrival to, or until, shipping, culling or death.

Experienced animal health personnel evaluated and observed study animals twice daily for signs of disease. Animals deemed ill based on subjective criteria; general appearance, attitude, gauntness, reluctance to move, were diagnosed and treated per standard calf grower protocols that were provided by the consulting veterinarian(s). The case definition for respiratory disease was a lack of abnormal signs attributable to any other body system. Treatment of respiratory disease was based on signs of increased resting respiratory rate, sound, or effort, with one or more additional signs such as coughing, nasal discharge, depressed appetite, or rough hair coat. The case definition for gastrointestinal disease was a lack of abnormal signs attributable to any other body system. Treatment of gastrointestinal disease was based on signs of manure of a looser consistency than deemed normal with one or more additional signs such as dehydration (increased skin tent time, sunken eyes) depression or inability to rise. The case definition for otitis disease was unilateral or bilateral ear droop. The animal may or may not have respiratory comorbidity. Otitis disease treatments was based on drooping of one or both ears with or without head tilt and lethargy.

Sample Collection

Composite samples of starter milk liquid rations were obtained beginning seven days post-allocation for each respective allocation periods (Table 1). For example, the first starter milk composite sampling began on the 18th of July 2018. A 0.16L aliquot of batch milk sample was added to a sterile container every day for 7 days totaling a 1.14L sample. This

same procedure was completed for starter milk ration fed to study animals allocated in November. A composite sample of grower milk samples were obtained in the same manner beginning 29 days after allocation and lasting for 7 days (Table 2).

Three starter grain composite samples were taken over a 7-day period. The first sample began on the 12th of July, the second began on the 19th of August, the final composite starter grain ration began on the 9th of November 2018 (Table 3). Three grower TMR composite samples were taken over a 7-day period beginning at weaning for each allocated block of calves respectively. The first sampling began on the 30th of August the second sample began the 1st of October and the final sample began the 3rd of December 2018 (Table 4).

An oxalated blood sample was collected via jugular venipuncture from each calf at 48 ± 6 h post-arrival (Wilm et al., 2018). The sample was collected using a 20-gauge, 1-inch hypodermic needle (BD Vacutainer Precision Glide, Becton Dickinson Co., Franklin Lakes, NJ.), into a sterile, plastic, Vacutainer tube without anticoagulant (BD Vacutainer, Becton Dickinson and Co.) and a clot was allowed to form. Whole blood samples were centrifuged at 2000 × *g* for ten minutes at room temperature after which serum was collected and stored at -22°C until analyzed. Serum total protein (TP) was measured using a digital refractometer (Weaver et al., 2000). Calves were categorized based on proposed USDA serum TP guidelines into Poor TP < 5.1 g/dL, Fair TP ≥5.1 - 5.7 g/dL, Good TP 5.8 - 6.1 g/dL, and Excellent ≥ 6.2 g/dL. The remaining serum was stored in an ultraviolet light proof container at – 20 °C until they analyzed further for serum metabolites.

Lipid-Soluble Vitamin Analysis

Serum samples were analyzed for lipid-soluble vitamins including α-tocopherol, β-carotene, and retinol. The serum was acidified with 20 µL 2 N acetic acid, denatured by adding 420 µL acetonitrile, and extracted by 1.5 mL organic solvent mixture (hexane: 2-propanol. 6.5:1.5; v/v). after one-minute vortex and centrifugation at 1000 × *g* for 3 minutes, the organic layer was transferred to another tube, and dried by nitrogen stream then reconstituted in a mobile phase (78.2% Acetonitrile, 13.0% Dichloromethane, 8.7% Methanol, and 0.1% η-butanol) for injection. The HPLC instrument included a Separation Module 2695 with a Symmetry C_{18} separation column (4.6 X 150 mm. 3.5 µm particle size). And Photodiode Array Detector (PDA) 2998. Waters 2659 and PDA 2998 were controlled by

Empower 3 software (Waters®, Milford, MA). Alpha-tocopherol, β-carotene, and retinol were detected at wavelengths on 290 nm, 450 nm, and 325 nm, with retention time of 3.10, 5.56, and 1.57 minutes, respectively. The temperature of auto sampler room set was 4 °C, and the column temperature was 50 °C maximum. The mobile phase flow rate was maintained at 1.5 mL/min for a total of 6.5 min for detection (Tsai et al., 2017).

Glucose Analysis

A capsule of 500 IU of glucose oxidase (*Aspergillus niger*), 100 purpurogallin units of 56 peroxidase (horseradish), and buffer salts (PGO) Enzyme Preparation (Solution A, Sigma Cal. No. P7119-10CAP) was dissolved in 50 mL of dH_20 into an amber flask. Using a squirt bottle, 20g$\pm$ 0.01g of d H_20 was added into the vial of dianisidine dihydrochloride (solution B; Sigma Cal. No. F5803-50MG). A 1.6 mL of solution B was added to 100 mL of A (solution A+B). A standard glucose curve was prepared in test tubes using the stock solution in the glucose kit. A plate with flat-bottom wells was used (Costar 3635). Using plate wells A1 and H1, A2 and H2, etc., through A6 and H6, transferred a 5 µL aliquot of each standard curve solution. Two samples, of each standard were read by a plate reader. A duplicate plate was made for each series of sample and duplicate samples of starch standard and in-house standard were added to each plate. Then a 5 µL aliquot of each sample was pipetted into the remaining wells. A 150 µl solution was added to each well with a multi-channel micropipette, the plate reader was turned on and the plates were shaken for 10 s. Plates remained at room temperature for 45 minutes covered with tin foil. The absorbance of the samples was read at 450 nm, then a regression equation was calculated. The regression equation was used to calculate milligrams of glucose per 100 mL for all samples.

Data Analysis

All population and descriptive statistics were generated using Microsoft Office Excel (Microsoft Corporation, 2016).

This experiment was an observational study; and the experimental unit was the individual animal (calf). All statistical analyses were performed with SAS software v9.4 (SAS Inst. Inc., Cary, NC). Generalized linear mixed models (PROC GLIMMIX) were fit to test the response of lipid soluble vitamins, glucose assay results, and average daily gain. In all cases a

normal distribution was assumed for these responses. The fixed effect in these models was serum total protein category, while calf was considered a random effect

Logistic regression for binary response data with fisher scoring algorithm was used to assess the odds of receiving treatment (yes or no) for respiratory, gastrointestinal, and otitis disease as a function of the predefined TP categories. Significance for all analyses was declared at $P \leq 0.05$.

Results

Descriptive Statistics

Table 5 shows least square means of serum total protein distribution. The poor category had 159 animals with a mean value of 4.68 g/dL with a SE of ± 0.024 g/dL; Fair 399 animals with a mean value of 5.45 with a SE of ± 0.009 g/dL; Good 322 animals with a mean value of 5.96 with a SE of ± 0.006 g/dL and Excellent category had 751 animals with a mean value of 6.90 g/dL and a SE of ± 0.021. These numbers will vary for lipid soluble vitamin HPLC analysis response variable. The researchers decided a representative sample 30% n = 491 serum samples were to be used in High Performance Liquid Chromatography analysis for retinol, beta-carotene, and alpha tocopherol.

Respiratory Disease

In Figure 2, the incidence of respiratory disease treatments by serum total protein is given: Poor 84.3% (n =159), Fair 84.7% (n =399), Good 83.9% (n =322), and Excellent 81.1% (n =751).

There were no significant effects of the serum total protein strata detected for respiratory disease treatments ($P = 0.69$). For example, when comparing Poor vs. Excellent serum total protein categories, the odds ratio (OR) was 1.213 (0.814, 1.805); thus, odds of receiving treatment for respiratory disease were not associated with the total protein strata. Similarly, comparison of Fair vs. Good categories resulted in an OR of 0.948 (0.704, 1.276) and Good vs. Excellent categories gave an OR of 0.948 (0.688, 1.305) further demonstrating treatment for respiratory disease showed no discernable relationship to the total protein strata.

Gastrointestinal Disease

As shown in Figure 3, the incidence of gastrointestinal disease treatments by serum total protein strata was: Poor 25.8% (n =159), Fair 21.37% (n =399), Good 17.4% (n =322), and Excellent 20.6% (n =751).

There were no differences detected when comparing gastrointestinal disease treatments by serum total protein strata via logistic regression ($P = 0.19$; Table 7). The OR for Poor vs. Excellent total protein categories 0.748 (0.503, 1.113) while Fair vs. Excellent

categories had an OR of 0.961 (0.713, 1.294) and Good vs. Excellent categories had an OR of 1.235 (0.881, 1.732). All strata compared to the Excellent serum total protein category were considered to have equal odds of receiving treatment for gastrointestinal disease.

Otitis Disease

As shown in Figure 4, the incidence of otitis disease treatments by serum total protein strata was: Poor 1.9% (n =159), Fair 4.6% (n =399), Good 3.7% (n =322), and Excellent 6.1% (n =751).

Statistically significant differences were recorded in the comparison of otitis disease treatments by serum total protein categories via logistic regression ($P = 0.03$; Table 8). Comparing Poor vs. Excellent serum total protein categories, an OR of 0.569 (0.363, 0.892) was observed. The odds of being treated for otitis disease in the Poor total protein category were less when compared to the excellent category. The OR for Fair vs. Excellent total protein categories was 0.671 (0.477, 0.944) while Good vs. Excellent categories had an OR of 0.700 (0.485, 1.010). Poor serum total protein category had less odds for being treated for otitis disease when compared to the excellent total protein category.

Mortality

As shown in Figure 1, crude mortality by serum total protein strata was: Poor 9.4% (n =159), Fair 8.0% (n =399), Good 9.6%% (n =322), and Excellent 6.5%% (n =751).

Statistically significant differences were recorded when comparing crude mortality by serum total protein strata ($P = 0.03$; Table 9.). Poor vs. Excellent serum total protein category comparison demonstrated an OR of 0.56 (0.372, 0.843). The odds of death in the Poor total protein category were greater when compared to the excellent total protein category. These results support the conclusion that < 5.1 g/dL serum total protein is associated with mortality risk in the dairy calf. Comparing Fair vs. Excellent serum total protein categories OR was 0.901 (0.651, 1.246) while Good vs. Excellent serum total protein categories had an OR of 0.759 (0.542, 1.063). These data suggest uncertainty in the odds of death for either Fair or Good serum total protein categories when compared to the Excellent category.

Growth Performance Data

There were no significant differences reported in average daily gain in the first 90 days on feed or for the overall feeding period (P = 0.35 and 0.98, respectively, (Figures 5 and 6). These results demonstrate growth performance is independent of serum total protein strata when average daily gain was measured at 90 days on feed and for the overall feeding period at the calf grower operation.

Retinol

Analysis of variance showed that serum alpha-tocopherol differed by serum total protein strata, having differences between Poor vs. Good or Excellent categories (P = 0.0006, Figure 7). Differences were also found when comparing the Fair and Good categories with Excellent. These results demonstrate a significant positive relationship between serum total protein levels and serum retinol.

Beta-carotene

Analysis of variance showed that serum beta-carotene differed by serum total protein strata, demonstrating differences comparing Poor, Fair, and Good with Excellent category (P = 0.04, Figure 8). These results demonstrate a significant positive relationship between serum total protein levels and serum beta-carotene.

Alpha-tocopherol

Analysis of variance showed that serum alpha-tocopherol differed by serum total protein strata, demonstrating differences comparing Poor, Fair, and Good with Excellent categories (P = 0.0006, Figure 9). These results demonstrate a significant increase in serum alpha-tocopherol concentration dependent on serum total protein strata.

Glucose

Serum glucose differed by serum total protein strata demonstrating differences comparing Poor and Fair vs. Excellent categories ($P = 0.02$, Figure 10). These results demonstrate an increase of serum glucose concentration is dependent on serum total protein values.

Discussion

Greater than 21% of calves (*n = 351*) on trial had a serum total protein value of less than 5.5 g/dL and were considered complete or partial FPT of maternal antibodies (Table 5). This observation is consistent with previous heifer data reported in the US dairy cow herd (USDA, 2016). As a result, morbidity and mortality outcomes (Figure 1-5) were less than optimal in FPT calves; however, this was not unexpected. The present study demonstrated statistically significant differences in mortality exists when comparing poor versus excellent serum total protein concentrations. These findings are supported by previous work that demonstrated serum total protein concentration, as measured by refractometry, is associated with mortality risk in the dairy calf when measured 48 ± 6 h post-arrival to the calf grower facility (Donovan et al., 1998) . Robinson et al. (1988) also noted that FPT calves are at an increased risk of mortality. The increase in mortality in FPT calves noted in our study is likely attributable to the lack of passive transfer of maternal antibodies from dam to calf via colostrum as would be supported by others (Donovan et al., 1998; Godden, 2008; Maunsell & Donovan, 2008; Tyler et al., 1999; Wells et al., 1996).

High morbidity incidence demonstrated in all categorical diagnosis independent of total protein strata is similar to previously reported data in a USDA report title “Dairy Heifer Raiser” published in 2011. In this report 71-90% of affected pre-weaned heifers were treated for diseases such as diarrhea, bloat, pneumonia, navel infection and lameness or injury. The lack of significant differences in respiratory disease treatments (Figure 2) may be explained by the high incidence ~80%, independent of total protein strata (*P* = 0.69, Table 6). In the production setting, individual animal treatments are subjective. The decision to administer a treatment to an animal is based on the experience and training of an individual employee. Rarely, are objective measures utilized in the detection and diagnosis of disease in the production setting; as a result, high treatment rates are recorded. This scenario also explains the lack of differences noted in gastrointestinal disease treatments (*P* = 0.19, Figure 3, Table 7). Donovan et al. 1998, demonstrated that calves dying from pneumonia, diarrhea, or septicemia by 14 weeks of age had lower than average serum protein concentrations. The present study did demonstrate significant differences among total protein strata for otitis disease treatment differences (*P* = 0.03, Figure 4, Table 8). Conflicting results were reported in a prospective cohort study involving 561 Holstein calves that reported calves with FPT

were more than twice as likely to suffer from otitis media in the preweaning period when compared to herd-mates with adequate passive transfer (Pithua & Aly, 2013). Teixeira et al. in 2017 reported approximately 30% incidence of otitis, in which antibiotic mass medication had no effect. In the present study the odds of being treated for otitis disease in the Poor total protein category were less when compared to the excellent category. It is entirely possible that unidentified factors unrelated to failure of passive transfer have contributed to the additional risk of otitis disease.

The present study suggests that growth performance is independent of serum total protein strata. The lack of average daily gain difference has been demonstrated in other reports. Nocek et al. (1984) found calves fed poor quality colostrum gained almost twice as much weight in the first 45 days of life when compared to those that received adequate quality colostrum. Calves that have concomitant respiratory and otitis disease had lower average daily gain during the pre-weaning period as shown by Teixeira et al. (2017). Other studies have demonstrated mean daily gain may be affected by serum IgG levels from birth to 13-16 months of age (Moraes et al., 2000). The present study did not measure serum IgG levels nor did the researchers tract animals to 13-16 months of age.

Statistically significant differences were recorded comparing poor vs. excellent serum total protein categories for all lipid soluble vitamins and serum glucose concentrations (Figures 7-9). The reduction in lipid soluble vitamins in FPT calves is supported by reduction of colostral intake (Eicher et al., 1994; Lewis et al., 2019; Samanta et al., 2006). One could hypothesize that the decrease in serum concentration of lipid soluble vitamins in the Poor and Fair serum TP categories may be related to severity of disease supported by numerically greater incidence of respiratory, and gastrointestinal disease treatments (Figures 2 and 3). A significant reduction in serum glucose was reported in FPT calves supported by previous reports that demonstrated providing colostrum at birth would increase glucose absorption leading to gut maturation and development of immunity (Blum et al., 1997; Hammon et al., 2019).

Conclusion

This study describes calves raised in a large-scale production setting; the findings of such are consistent with previously reported data. Calves that were considered as having FPT of maternal antibodies died at a much greater rate than their adequate passive transfer cohorts. A concerted effort in maternity and neonatal management practices can help mitigate health challenges and improves health outcomes in the male Holstein calf. Monitoring of colostrum collection ensures adequate amounts of high-quality colostrum is available on farm. The use of technology such as digital refractometers and thermometers are rapid, cost-effective solutions to monitoring and confirming high-quality colostrum is fed. Otitis disease treatments were significantly greater in calves with high serum total protein values, whereas no differences were detected in respiratory and gastrointestinal disease treatments. No differences in average daily gain were recording in the first 90 days on feed or for the overall feeding period. Lipid soluble vitamins were used as a proxy for immune system development and function and were significantly lower in FPT calves. Veterinarians and nutritionist working together is vital to confirm adequate lipid soluble vitamins are provided in the dry cow ration, colostrum, as well as calf milk replacers and starter grain rations. Serum glucose levels were different in calves with FPT as well. Based on these findings, the authors conclude that low serum total protein values can impact morbidity, mortality, and, serum metabolites. Additional factors need to be considered when assessing risk of disease occurrence such as hygiene, pathogen virulence, physical environment, nutritional status, and stress. These data demonstrate that failure of passive transfer in the bovine present an opportunity in modern agriculture. Further research is necessary to better understand the relationship among specific immunoglobulins, lipid soluble vitamins, and how the immune system develops and functions.

Tables

Table 1. Starter Milk Replacer analysis. Milk ration fed during the first 4 weeks of life.

	%Butter Fat	%Protein	%Lactose	%Solids Non-Fat	Somatic Cell Count
Sample 1	4.56	4.34	7.73	13.47	250
Sample 2	4.60	4.34	7.77	13.51	258
Mean	4.58	4.34	7.75	13.49	254
SEm	0.02	0	0.02	0.02	4

Table 2. Grower Milk Replacer analysis. Milk ration fed from 29 days of age to weaning 56 ± 3.5 days.

	%Butter Fat	%Protein	%Lactose	%Solids Non-Fat	Somatic Cell Count
Sample 1	2.29	2.66	5.40	9.03	78
Sample 2	2.27	2.66	5.44	9.06	84
Mean	2.28	2.66	5.42	9.05	81
SEm	0.01	0	0.02	0.015	3

Table 3. Starter grain ration analysis fed for the first 28 days of life. (dry matter basis).

	% Crude Protein	% ADF	% aNDF	%TDN	NE_L Mcal/kg	NE_M (Mcal/kg	NE_G (Mcal/kg
Sample 1	17.3%	5.4%	10.1%	84%	1.99	2.10	1.43
Sample 2	17.3%	5.0%	8.2%	85%	2.01	2.12	1.45
Sample 3	17.5%	5.1%	8.8%	84%	2.00	2.12	1.44
Mean	17.37%	5.17%	9.03%	84.33%	2.00	2.11	1.44
SEm	0.001	0.001	0.006	0.003	0.006	0.007	0.006

Table 4. Grower total mixed ration fed from 29 days until shipment (dry matter basis).

	%Crude Protein	%ADF	%aNDF	%TDN	NEL Mcal/kg	NEM (Mcal/kg	NEG (Mcal/kg
Sample 1	19.2%	9.3%	18.2%	82%	1.98	2.09	1.43
Sample 2	20.0%	9.7%	17.5%	83%	2.02	2.14	1.46
Sample 3	19.2%	9.4%	18.3%	82%	1.98	2.09	1.43
Mean	19.47%	9.47%	18.00%	82.33%.	1.99	2.11	1.44
SEm	0.003	0.001	0.003	0.003	0.013	0.017	0.010

Table 5. Least square means (± standard error of the means, SE) of serum total protein distribution.

Total Protein Category	n	Mean (g/dL)	SEm
Poor	159	4.68	0.024
Fair	399	5.45	0.009
Good	322	5.96	0.006
Excellent	751	6.90	0.021

Table 6. Comparison of respiratory disease treatments by serum total protein categories via logistic regression (model $P = 0.69$).

	Total Respiratory Disease Treatments Among Serum [TP] Classes			
Comparison	Odds Ratio	Lower CL*	Upper CL	*P* value
Poor vs. Excellent	1.213	0.814	1.805	0.24
Fair vs. Excellent	0.948	0.704	1.276	0.50
Good vs Excellent	0.948	0.688	1.305	0.53

*CL: 95% Confidence Level

Table 7. Comparison of gastrointestinal disease treatments by serum total protein categories via logistic regression (model $P = 0.19$).

Total Gastrointestinal Disease Treatments Among Serum [TP] Classes				
Comparison	Odds Ratio	Lower CL*	Upper CL	*P* value
Poor vs. Excellent	0.748	0.503	1.113	0.075
Fair vs. Excellent	0.961	0.713	1.294	0.93
Good vs. Excellent	1.235	0.881	1.732	0.054

*CL: 95% Confidence Level

Table 8. Comparison of otitis disease treatments by serum total protein categories via logistic regression (model $P = 0.03$).

Total Otitis Disease Treatments Among Serum [TP] Classes				
Comparison	Odds Ratio	Lower CL*	Upper CL	*P* value
Poor vs. Excellent	0.569	0.363	0.892	0.15
Fair vs. Excellent	0.671	0.477	0.944	0.57
Good vs. Excellent	0.700	0.485	1.010	0.84

*CL: 95% Confidence Level

Table 9. Comparison of crude mortality by serum total protein categories via logistic regression (model $P = 0.03$).

Total Mortality Among Serum [TP] Classes				
Comparison	Odds Ratio	Lower CL*	Upper CL	*P* value
Poor vs. Excellent	0.56	0.372	0.843	0.02
Fair vs. Excellent	0.901	0.651	1.246	0.25
Good vs. Excellent	0.759	0.542	1.063	0.77

*CL: 95% Confidence Level

Figures

Figure 1. Crude mortality by serum [total protein] categories (n = 1,631).

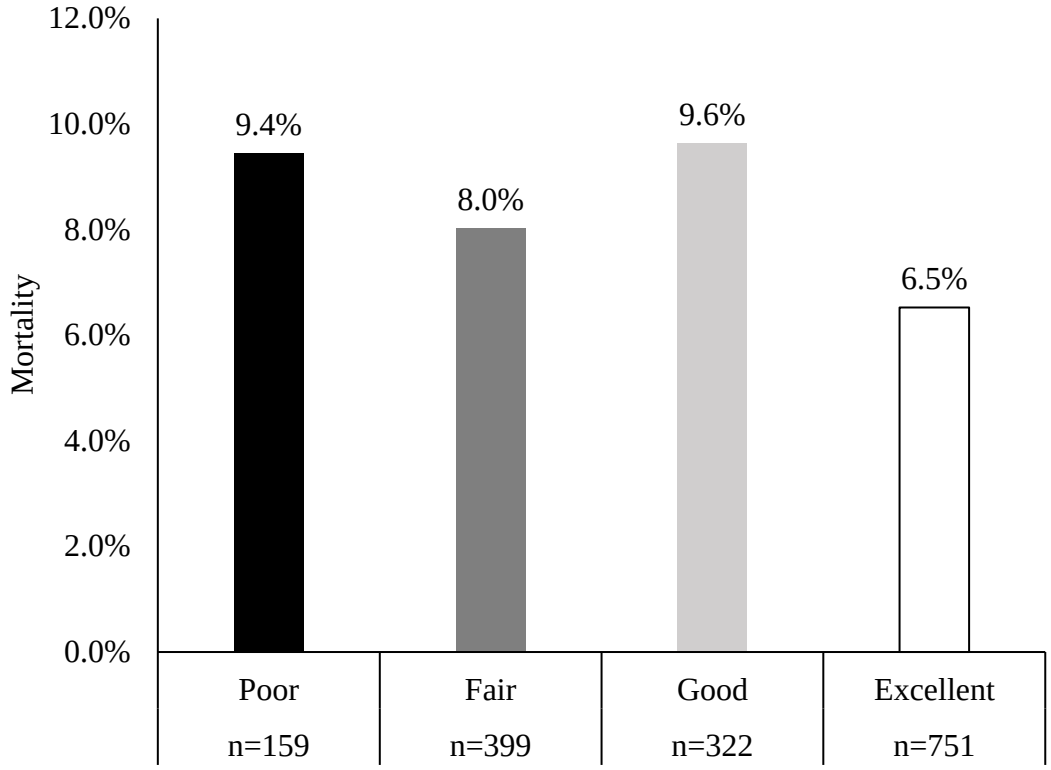


Figure 2. Incidence of respiratory disease treatments by serum [total protein] categories (n = 1,631).

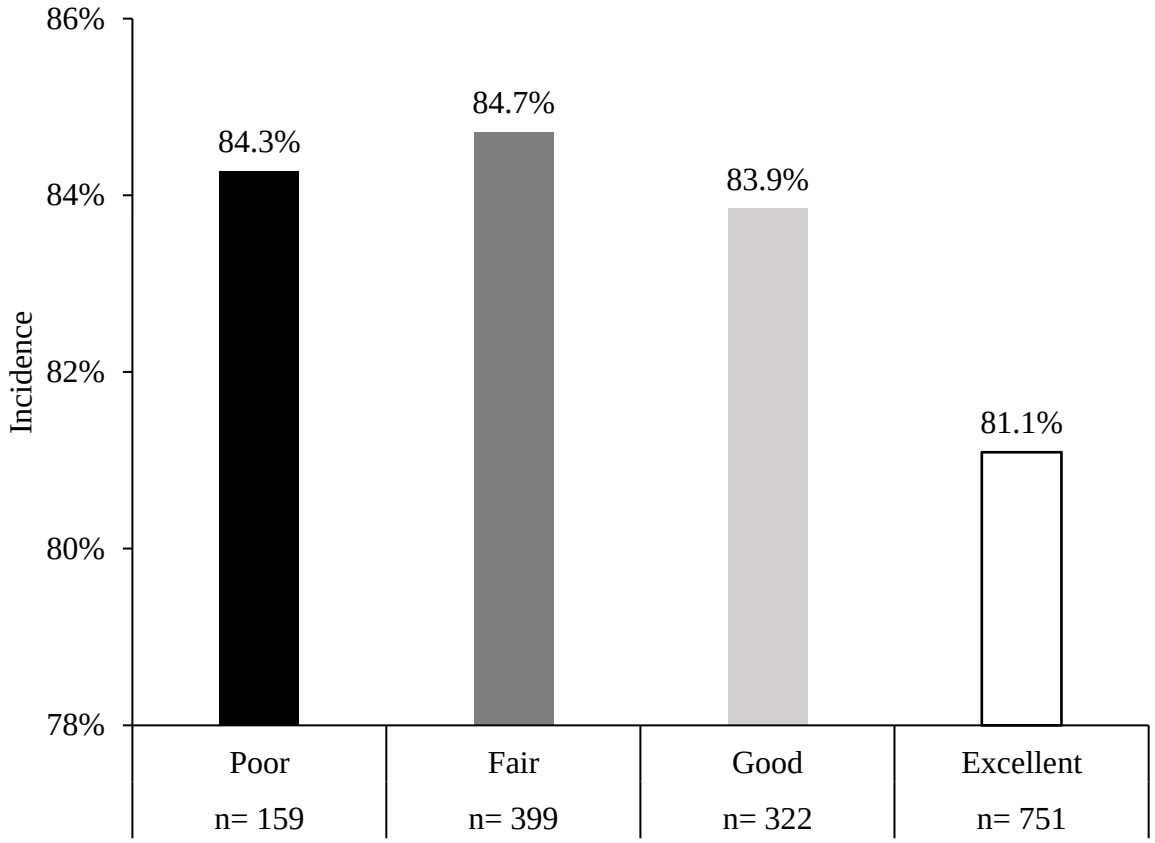


Figure 3. Incidence of gastrointestinal disease treatments by serum [total protein] categories (n = 1,631).

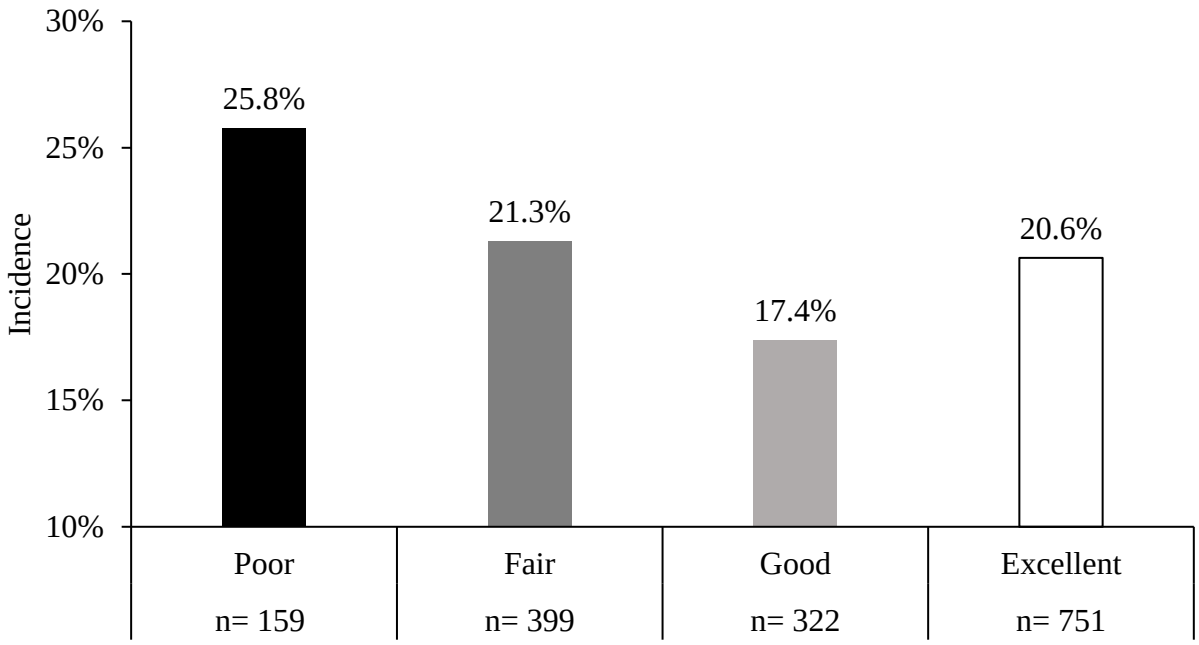


Figure 4. Incidence of otitis disease treatments by serum [total protein] categories (n=1,631).

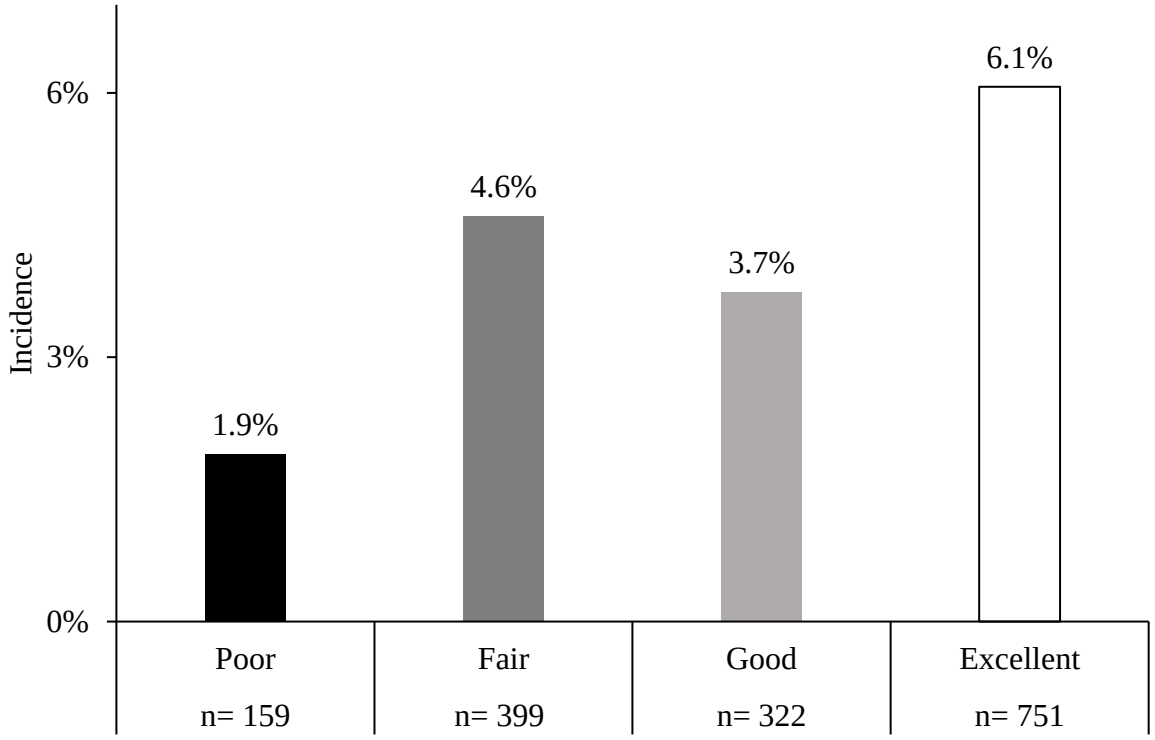


Figure 5. Least square means (± SEm) of average daily gain of 0-90 days on feed by serum [total protein] categories.

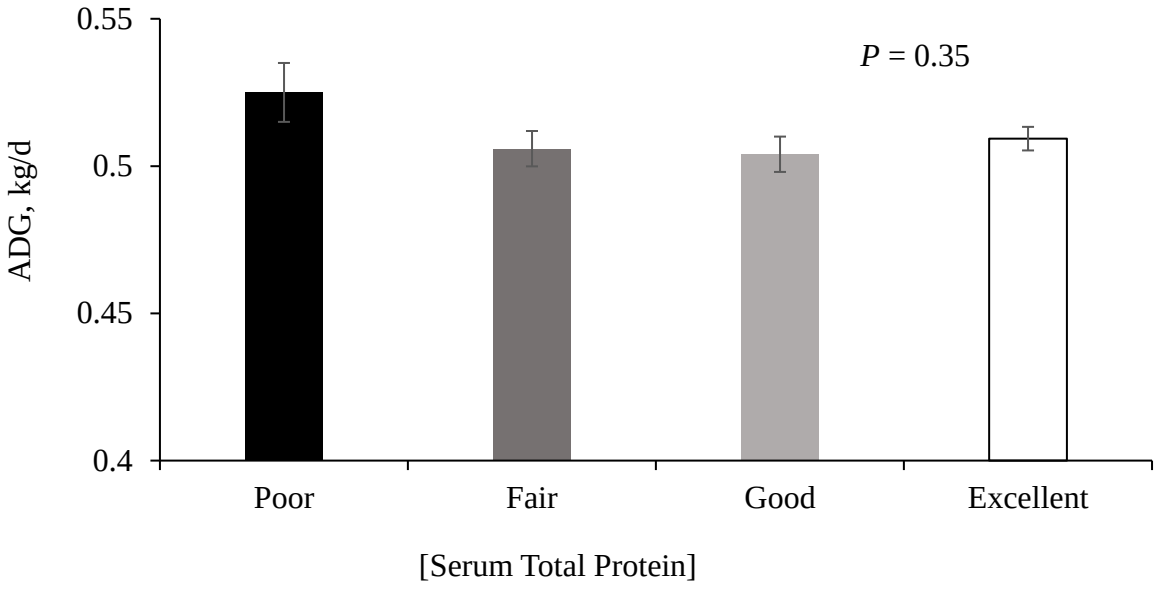


Figure 6. Least square means (± SEm) of average daily gain of overall days on feed by serum [total protein] categories.

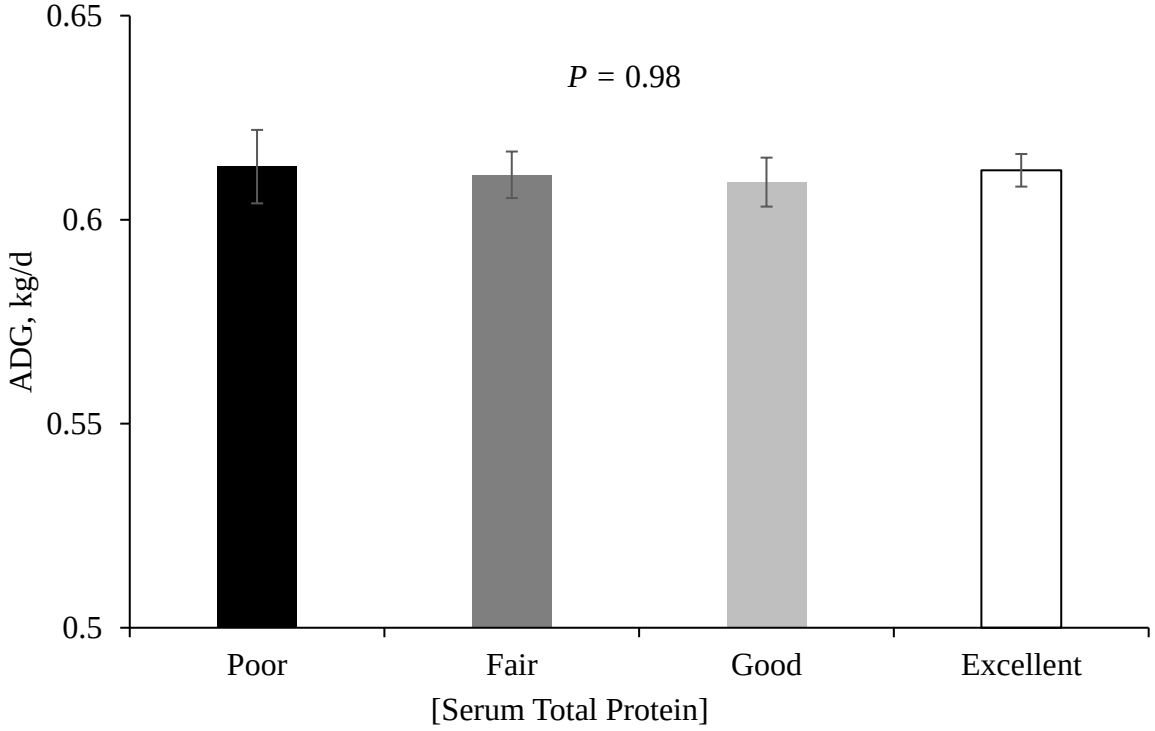


Figure 7. Least square means (± SEm) of serum retinol concentration (µg/mL) by serum [total protein] categories. Different superscripts show significant difference at $P \leq 0.05$.

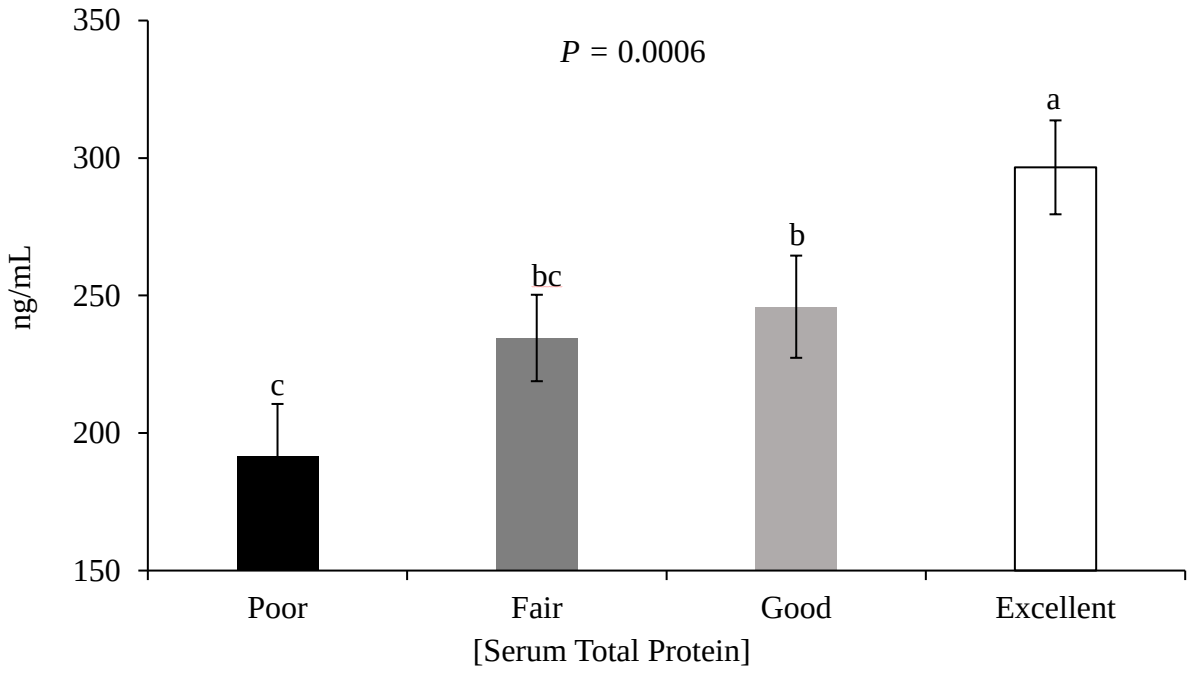


Figure 8. Least square means (± SEm) of serum β-carotene concentration (µg/mL) by serum [total protein] categories. Different superscripts show significant difference at $P \leq 0.05$.

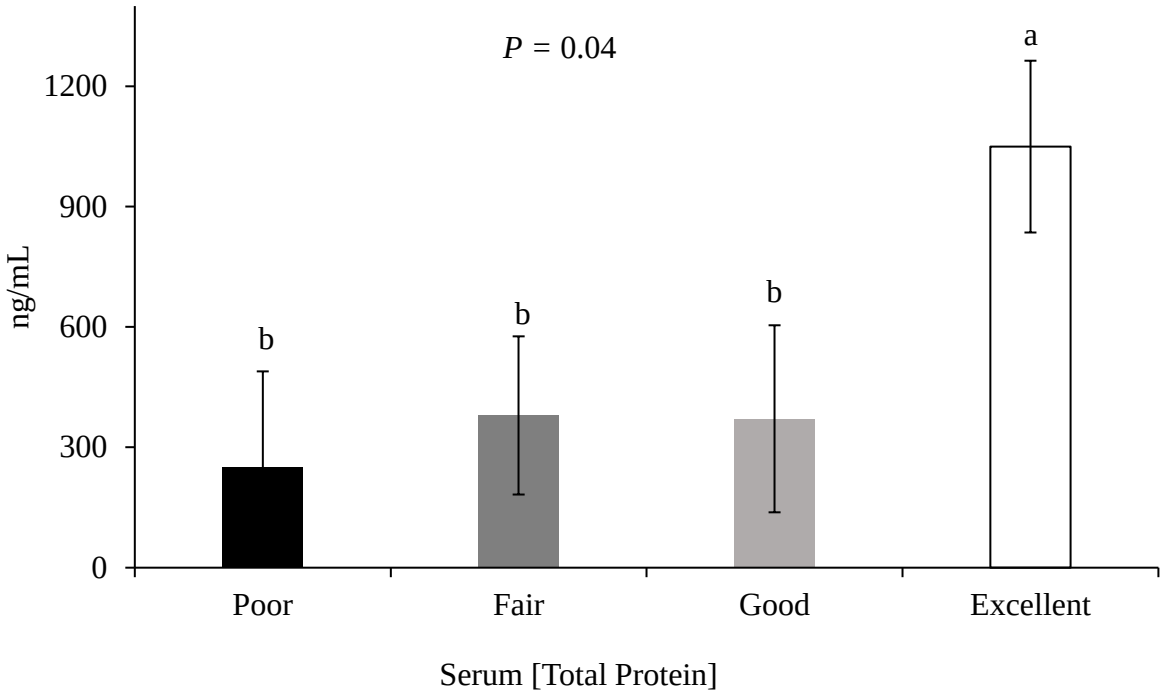


Figure 9. Least square means (± SEm) of serum α-tocopherol concentration (ng/mL) by serum [total protein] categories. Different superscripts show significant difference at $P \leq 0.05$.

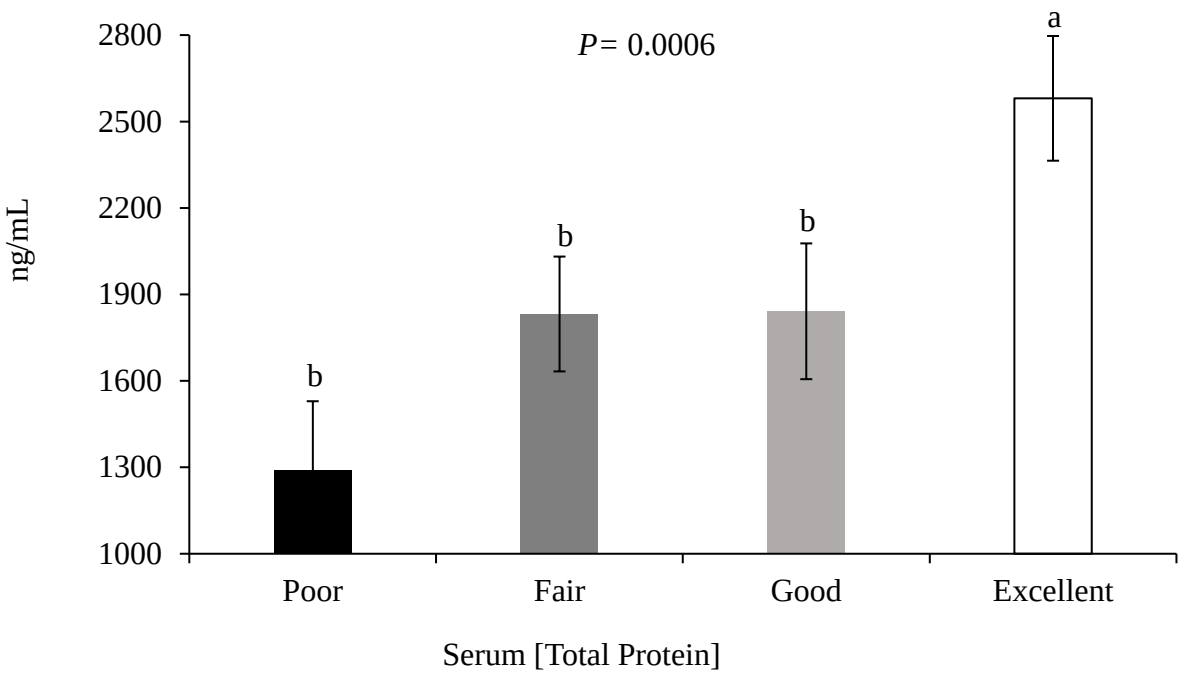
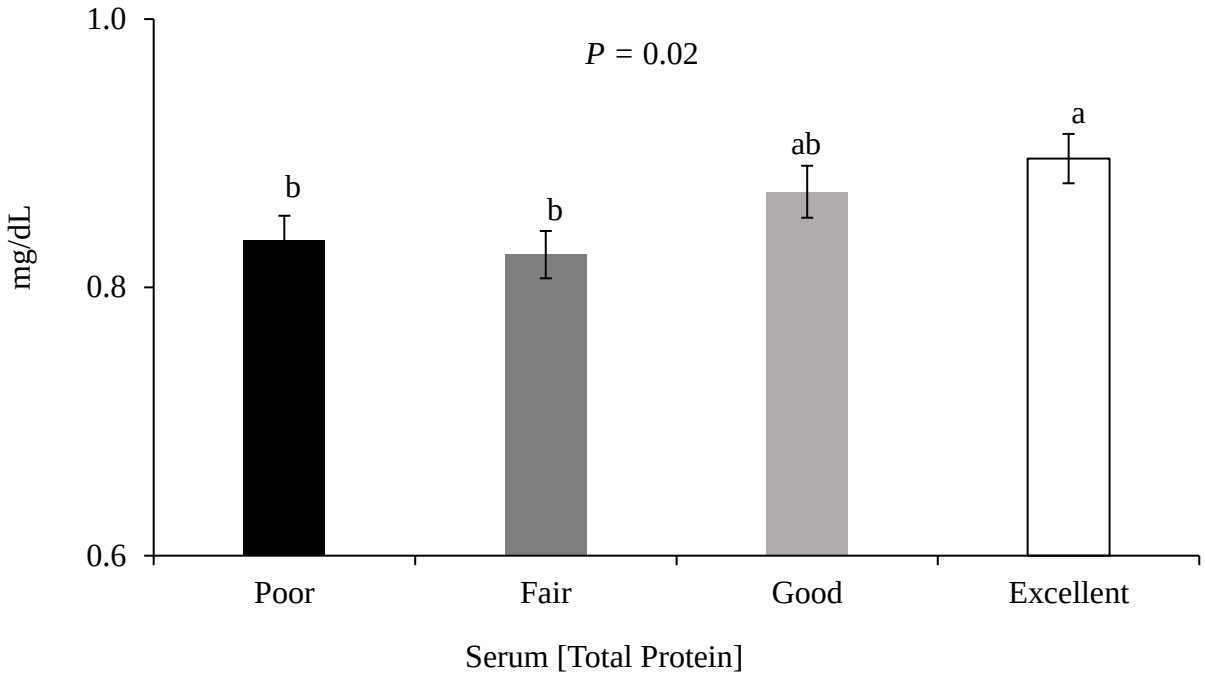


Figure 10. Least square means (± SEm) of serum glucose (mg/dL) by serum [total protein] categories. Different superscripts show significant difference at $P \leq 0.05$.



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Appendix 1

University of Idaho
Institutional Animal Care and Use Committee

Date: April 15, 2020

To: Pedram Rezamand

From: University of Idaho Institutional Animal Care and Use Committee

Re: Protocol IACUC-2018-20 *An epidemiological study of morbidity and mortality relationship among lipid soluble vitamins and failure of passive transfer in neonatal dairy calves*

Your requested renewal of the animal care and use protocol listed above was reviewed and approved by the Institutional Animal Care and Use Committee on 04/15/2020.

This renewal was originally submitted for review on: 03/24/2020

The original approval date for this protocol was: 09/04/2018
This approval will remain in effect until: 04/14/2021
The protocol may be continued by annual updates until: 09/03/2021

PLEASE NOTE: As the Principal Investigator, it is your responsibility to provide relevant information of this approved protocol to your collaborators and ensure that all work done complies with what was approved.

Federal laws and guidelines require that institutional animal care and use committees review ongoing projects annually. For the first two years after initial approval of the protocol you will be asked to submit an annual update form describing any changes in procedures or personnel. The committee may, at its discretion, extend approval for the project in yearly increments until the third anniversary of the original approval of the project. At that time, the protocol must be replaced by an entirely new submission.

Janet Rachlow, IACUC Chair

Appendix 2

University of Idaho
Institutional Biosafety Committee

To: Dr. Pedram Rezamand, Associate Professor, Department of Animal & Veterinary Science

From: Dr. Gulhan Unlu, Associate Professor, School of Food Science and Chair, University of Idaho Institutional Biosafety Committee (IBC)

Date: November 16, 2018

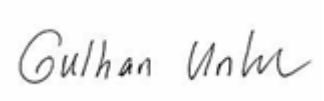
Subject: Amendment approval of B-019-18, "An epidemiological study of morbidity and mortality relationship among lipid soluble vitamins and failure of passive in neonatal dairy calves"

The IBC reviewed your amendment submitted on September 6, 2018, requesting to add a LPS challenge to the above-named protocol. The IBC agreed the proposed work, as described in the amendment, can be safely conducted at biosafety level 2 (BSL-2). The amendment was approved on 11/16/2018. **Please note: This does not change your protocol expiration date of 09/03/2021.**

If the scope of the research changes it may be necessary to submit an amended biosafety form to the IBC. Consult with the biosafety officer (biosafety@uidaho.edu or 208-885- 4054) prior to implementing any changes. Substantive changes that may require an amendment include, but are not limited to, using a microorganism or genetic tool other than what is listed in the experiments described, changes in procedures that may influence exposure potential, or changes in equipment, facilities, or personnel.

Please contact the IBC coordinator (ibc@uidaho.edu or 208-885-7258) or the biosafety officer with any questions.

Sincerely,



Dr. Gulhan Unlu
Chair, Institutional Biosafety Committee