The role of calcidiol supplementation in optimizing vitamin D status, growth performance, and immune function in beef cattle

by

Macie Clara Weigand

A.S., Hutchinson Community College, 2021
B.S., Kansas State University, 2023

A THESIS

submitted in partial fulfillment of the requirements for the degree

MASTER OF SCIENCE

Department of Animal Sciences and Industry
College of Agriculture

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2025

Approved by:

Co-Major Professor
Dr. Dale Blasi

Approved by:

Co-Major Professor
Dr. KC Olson

Copyright

Abstract

A series of experiments were conducted to evaluate vitamin D status in beef cattle and the effects of supplemental calcidiol (HyD®; DSM Nutritional Products, Plainsboro, NJ) on serum 25-hydroxyvitamin D_3 [25(OH)D_3] concentrations on performance and health. In experiment 1, 96 high-risk, crossbred heifer calves (initial BW = 222 ± 17.0 kg) were sourced from auction markets around Dickson, TN, and transported 1,086 km to Manhattan, KS. Upon arrival, Calves were allocated randomly to one of four dietary treatments: neither supplemental vitamin D_3 nor calcidiol (CON); 0.075 mg (3,000 IU) per head per day of vitamin D_3 (D3); 0.5 mg calcidiol per head per day (HyD Low); or 1.0 mg calcidiol per head per day (HyD High). Cattle were fed once daily, and treatments were top-dressed onto feed. Blood samples were collected on days -1 (prior to transport), 0 (on arrival), and at days 14, 31, and 60 of dietary treatment period and analyzed for serum 25(OH)D_3 concentration (TMAS, Belvidere, NJ). HyD High heifers had greater ($P < 0.001$) serum 25(OH)D_3 concentrations compared to all other treatments at days 14, 31, and 60. HyD Low heifers had greater ($P < 0.001$) serum 25(OH)D_3 than CON and D3 heifers at days 14, 31, and 60. In experiment 2, 603 crossbred steers and heifers were evaluated at four seasonal time points (spring, summer, fall, and winter) at a commercial feedlot in western Kansas to investigate vitamin D status. Cattle were housed in outdoor pens without shade and fed a common corn-based finishing diet supplemented with 275 IU per kg of vitamin D_3. Cattle were stratified by lot number; odd lot numbers received 1.0 mg per head per day HyD® (HyD) while even lot numbers did not receive HyD® (CON). Serum 25(OH)D_3 concentrations in CON cattle were greatest in summer (108 ± 22.6 ng/mL), intermediate in spring and fall (61 ± 15.3 and 59 ± 24.5 ng/mL), and least in winter (35 ± 6.4 ng/mL; $P < 0.001$). Cattle supplemented with HyD maintained significantly greater ($P < 0.01$)

serum 25(OH)D_3 across all seasons compared with non-supplemented cattle: 196 ± 58.0, 258 ± 54.3, 183 ± 40.4, and 184 ± 30.7 ng/mL in spring, summer, fall, and winter, respectively. In addition, hide pigmentation influenced vitamin D status. Red-hided cattle had greater ($P < 0.01$) serum 25(OH)D_3 than black and white-hided cattle. No effect of sex was observed ($P = 0.40$).

In experiment 3, eight cannulated, crossbred heifers (initial BW = 289 ± 44.9 kg) were randomly assigned to receive a single pulse dose of calcidiol solution via ruminal fistula at 3 or 5 mg per 272 kg BW. The elimination half-life ($t_{1/2}$) of calcidiol was 7.1 days. Calcidiol remained circulating in serum above baseline for an average of 32.6 days. Multiple predictive modeling simulations were analyzed. According to the model simulations performed for this experiment, the combination of two oral doses of 5 mg calcidiol 14 days apart with daily supplementation of 1 mg has the potential to sustain elevated serum 25(OH)D_3 concentrations above 100 ng/mg during a 56 day period; however, this should be confirmed *in vivo.* In experiment 4, 480 market-sourced, high-risk, crossbred heifer calves (226 ± 16.9 kg), were used to evaluate the effects of calcidiol supplementation on health and growth performance. Heifers were blocked by truck load on arrival and assigned randomly to one of four dietary treatments for 56 days: 0.075 mg (3,000 IU) vitamin D_3 per head per day (D3), 0.5 mg calcidiol per head per day (HyD Low), 1.0 mg calcidiol per head per day (HyD High), or 1.0 mg calcidiol + 100 mg beta-carotene per head per day (HyD + BC). All treatments were top-dressed onto the daily ration. Individual BW was recorded on days 0, 14, 28, and 56. There were no significant differences ($P = 0.36$) in final BW, average daily gain, dry matter intake, or feed efficiency across treatments. Respiratory-related morbidity and mortality was not different between treatments ($P = 0.16$). Serum 25(OH)D_3 concentrations were greater ($P < 0.001$) in HyD-supplemented groups compared to D3 on d 14, 28, and 56. Dietary treatment did not influence antibody titer levels or haptoglobin

concentrations ($P > 0.10$). Antibody titers for bovine viral diarrhea type 1 and type 2 increased linearly ($P < 0.01$) over time, with the greatest levels on d 56. Titers for infectious bovine rhinotracheitis increased linearly ($P < 0.01$) until d 28. In summary, calcidiol supplementation increased and maintained serum 25(OH)D_3 concentrations in beef cattle under various management and environmental conditions. Although growth performance and health outcomes in high-risk cattle were unaffected in the short term, the consistent elevation in serum 25(OH)D_3 concentrations suggests that calcidiol was absorbed and metabolically circulating potentially supporting vitamin D-dependent functions not directly measured in this study. It was observed that seasonal variation in vitamin D status existed, vitamin D supplementation may be beneficial during periods of limited sunlight exposure. Further research is warranted to determine the optimal serum 25(OH)D_3 concentration for achieving optimal health and performance outcomes in beef cattle.

Keywords

25-hydroxyvitamin D_3, high-risk cattle, immune function, seasonal variation, vitamin D_3

Table of Contents

List of Figures

List of Tables

Acknowledgements

First and foremost, I would like to thank my parents for their unwavering support, constant guidance, and, most importantly, their endless love and encouragement to chase my dreams. I would not be where I am today without them.

To my brother, Tristan, and sister, Amelya, thank you for always pushing me to be the best big sister I can be. Your love and support have kept me grounded. To my Grandma Witthar and Grandpa Weigand, thank you for your love and for the values you've instilled in our family that continue to guide me.

I am deeply grateful to my co-advisors, Dr. Dale Blasi and Dr. KC Olson. Dr. Blasi, thank you for being an incredible mentor and for creating a home away from home during graduate school. You and Roxann were always there with a helping hand and kind words, and that support meant more than I can ever fully express. Dr. Olson, thank you for sharing your knowledge so generously and for challenging me to grow as a scientist and thinker.

I am very appreciative for my committee members, Dr. AJ Tarpoff and Dr. Evan Titgemeyer. Dr. Tarpoff, thank you for deepening my understanding and appreciation of the beef industry. Your energy and dedication to teaching are contagious and inspiring. Dr. Evan Titgemeyer, thank you for the many thoughtful conversations and for your guidance with research design and data analysis. Your insight and encouragement helped shape my academic approach.

To Dr. Karol Fike, thank you for being both a mentor and a friend. Your support during moments of doubt and your belief in my potential were instrumental in my decision to pursue graduate school. It was an honor to learn and teach alongside you.

A special thank you to Ashley Hartman, friend, mentor, and one of my greatest inspirations. Your passion for teaching, your curiosity, and your unwavering support have shaped both my academic and personal journey. I truly don't know where I'd be without you.

To Barb McMurray, thank you for always being there to listen and offer honest, thoughtful feedback. Your friendship has been a steady source of strength and clarity.

To Erin Schwandt, thank you for being a genuine friend and mentor. Whether helping with writing, connecting me to others in the field, or simply being someone to talk to, your impact on my journey has been immense.

To my lab mates, Dr. Zach Duncan, Cole Ellis and Colton Weir, thank you for your help, patience, and the many laughs we've shared. Working alongside you all has been both educational and enjoyable.

To my ride or die, Tag, my dog, your loyal companionship, boundless energy, and joyful spirit brought light to even the hardest days. Whether it was a long walk or your hugs waiting for me at the end of the day, you reminded me to keep moving forward, find joy in the simple moments, and always be ready for the next adventure.

To the many friends, leaders, and mentors whose names aren't listed here but whose presence has been deeply felt—thank you. Your support, encouragement, and belief in me have been a foundational part of this journey. This chapter of my life has been full of growth, challenges, and unforgettable moments. I am so thankful to have walked it with the support of such an incredible group of people.

Dedication

This thesis is dedicated to the memory of…

My Grandma Weigand. Her love and unwavering support continue to inspire me every day. Though she is no longer here, her influence and values remain a guiding force in my life.

My Grandpa Witthar. Although we had only met a few times, I believe his values and legacy helped shape who I am today in ways I'm still discovering.

This accomplishment is, in many ways, a reflection of the foundation they helped build in my life.

Chapter 1 - Review of Literature

Introduction

The health and management of newly received cattle remains a significant challenge for the beef industry, impacting both economic performance and animal welfare (Galyean et al., 2022). Approximately 38.2% of feeder cattle entering feedyards are procured from auction markets, with an unknown health history (USDA APHIS, 2021). This presents significant management decision challenges for feedyard operators. According to data from the National Animal Health Monitoring System survey (NAHMS, 2013), approximately 16.2% of cattle placed in feedyards are affected by bovine respiratory disease complex (BRD) or "shipping fever," which is the leading cause of illness and death in feedyard cattle (Blakebrough-Hall et al., 2022).

Newly received cattle are typically categorized as high-risk or low-risk to help identify potential risks for health challenges mainly associated with BRD (Rojas et al., 2022). Low-risk cattle are often single-sourced, in good health, and have undergone a preconditioning program. Preconditioning program can be simple defined as a vaccination, nutritional, and management program design to help prepare cattle for stressful events (Lincoln and Hinman, 2003). High-risk cattle are often light-weight, commingled or combined from multiple sources, and have an unknown background, posing a greater risk to disease and death loss. These cattle are purchased due to their low initial cost; however, they can potentially have a greater return on investment when appropriately managed. High-risk cattle also tend to have greater stress levels during the receiving period, which is associated with commingling, long-haul transportation and new environments and diets. Based on that classification, many producers will administer antibiotic

metaphylaxis on arrival. Metaphylaxis has been shown to reduce morbidity up to 80-90% in high-risk cattle populations (Maples et al., 2022). New consumer concerns focused on antibiotic use in animals destined for the food supply have been gaining popularity. With the need for judicious antimicrobial stewardship, alternative nutritional strategies have been investigated to support cattle health.

In a survey of feedlot nutritionists, Samuelson et al. (2016) reported considerable variation in the level of vitamin D supplemented to beef cattle during both the receiving and finishing phases. Vitamin D_3 is formed through biosynthesis in the skin when the animal is exposed to ultraviolet light. It is assumed that beef cattle, which are most commonly housed outdoors, synthesize enough vitamin D_3 through ultraviolet exposure (Nelson et al., 2016). Vitamin D is essential for calcium homeostasis and bone metabolism (Hodnik et al., 2020; Poindexter et al., 2023a). New research has suggested that this fat-soluble vitamin could have an essential role in the immune systems of both dairy and beef cattle (Nelson et al., 2012) Poindexter et al., (2020) investigated the impacts of supplementing a vitamin D metabolite, calcidiol, on mammary immune function resulting in greater protection of mammary tissue when calcidiol supplementation was implemented.

Newly Received Stocker Cattle

Procuring lightweight cattle for backgrounding can be financially appealing; however, when feedlot inventory is low in cattle cycle phases such as contraction, lightweight cattle may also be placed directly into feedyards. This strategy for U.S. cattle feeders requires careful management to optimize performance while minimizing health issues, with the goal of maximizing economic returns. Newly received calves endure numerous sources of stress, or compounded stress, that may include handling, transportation, dehydration, temporal fasting,

temperature extremes, commingling at auction markets, and initial processing. Stress, which is defined as climatic, nutritional, social, or internal forces that disrupt homeostasis and compromise immune function, plays a critical role in calf health (Stott, 1981). Novel experiences exacerbate stress, triggering physiological and psychological changes (Fike and Spire, 2006). Given the multitude of stressors affecting newly received calves, proper evaluation of their nutritional and health status is essential to establishing an effective management program.

Preconditioning programs can also help to improve performance and feed intake while decreasing morbidity and health costs (Step et al., 2008). Preconditioning typically refers to a regime consisting of processing and diet acceptance that calves experience post-weaning to prepare them for value-added marketing strategies. Oftentimes, these programs consist of vaccinations, dehorning or castrations if needed, deworming, and a required weaning period prior to sale (Vining et al., 2024). Cattle that undergo a preconditioning program have greater performance and reduced need for antibiotic treatment during the receiving phase (Richeson et al., 2012). The feasibility of preconditioning varies, as it requires additional expenses for feed, labor, and facilities, with nutrition alone accounting for 45-60% of the total cost (Vining et al., 2024). Newly received cattle that enter backgrounding yards or feedlots with an unknown history undergo similar management strategies to preconditioning programs to help cattle be successful later in the feeding period. On arrival, cattle are typically given 12 to 24 hours to rest before they are processed (Samuelson et al., 2016). During the rest period, they are supplied with *ad libitum* access to clean water and long-stem grass hay. Subsequent processing typically includes animal identification, vaccination, implantation, bodyweight measurement, and health assessment before being assigned to a pen at the cattle feeding operation

Once in the feedyard, calves encounter additional stress from unfamiliar facilities such as feed bunks and water troughs, social hierarchy adjustments, environmental factors, and exposure to new pathogens. Vaccines are used to help control those pathogens especially microbes that are linked to BRD and other communicable diseases to improve cattle health and growth performance (Blakebrough-Hall et al., 2022). Vaccination protocols vary based on region, risk of pathogen exposure, and cattle health history; however, most target the following infectious diseases: infectious bovine rhinotracheitis (IBR), bovine viral diarrhea virus (BVD) types one and two, bovine respiratory syncytial virus (BRSV), parainfluenza-3 virus (PI3), and clostridial organisms (Terrell et al., 2011). Another common management practice for new cattle on arrival is metaphylactic antibiotic treatment to mitigate subclinical or anticipated disease outbreaks. According to NAHMS, (2013), 59% of U.S feedlots use metaphylaxis on 20.5% of cattle placed in the yard. Mass metaphylaxis and body temperature-based treatments can reduce respiratory disease incidence (Galyean et al., 1995). Effectively managing newly received cattle requires a comprehensive approach that accounts for stress management, nutritional adaptation, health interventions, and disease prevention. By addressing these challenges, producers can enhance growth and immune function, reduce morbidity and mortality, and ultimately improve the profitability of cattle entering feedlots.

High Health-Risk Cattle

Factors such as cattle prices, feed costs, and availability contribute to decisions regarding the timing and location of cattle purchases. Cattle prices are usually the most important of these transactional factors, and seeking discounted prices is commonplace. According to a survey of 24 consulting feedlot nutritionists, approximately 28% of receiving calves are classified as high-risk (Samuelson et al., 2016). Cattle considered to be "high-risk" are one such category of price

discounts. The term “high-risk” is a reference to anticipated health challenges with the cattle that arise from recent maternal separation, light body weight at the time of purchase, unfamiliar with concentrate feeds or automated water-delivery devices, have not been dehorned or castrated, no or limited vaccination, commingling with unfamiliar cattle, or have undergone transport stress. High-risk cattle are typically purchased at a discount due to the financial risks of disease and potential death loss that the buyer accepts. Although both feedyards and background yards use this practice, it is more common with backgrounding enterprises. Through effective management, the value of high-risk cattle can be significantly enhanced within 1 to 3 months. This improvement enables them to be sold at a premium or transitioned to a feedlot with an anticipated smooth finishing cycle. These management practices involve vaccination, gradual introduction of concentrate diets, and behavioral adaptation to minimize morbidity and mortality.

Another concern with the purchase of high-risk cattle is the presence of persistently infected (PI) calves, which contract bovine viral diarrhea (BVD) in utero and shed the virus throughout their lifetime, posing a significant threat to other immunocompromised, high-risk calves (Handel et al., 2011). Identifying and quantifying PI calves in real-time can be challenging due to the current diagnostic testing available. Approximately 0.1% to 1.7% of cattle are positive within a population (Campbell, 2005). The most common testing procedure for persistently infected calf identification is via an "ear-notch" skin sample (Nickell et al., 2011). Persistently infected cattle have low rates of gain and higher morbidity and mortality rates and pose a risk of infecting other calves. When purchasing high-risk cattle, it is important to identify and isolate PI calves as this could help to prevent additional disease outbreaks and financial losses.

Transportation

Due to the geographical widespread and segmented nature of the U.S. beef industry, transportation is a necessary part of the business. Transportation occurs during life events such as weaning, processing, and slaughter (Van Engen and Coetzee, 2018). Cow-calf production occurs in all 50 states, but grain production, feedyards, and abattoirs are most concentrated in the Great Plains region of the U.S. Therefore, the distance some calves must be transported to reach these operations can be substantial.

Many factors associated with transportation affect the amount of stress cattle experience. For instance, gathering, loading, transit time, and unloading are primary factors. Additionally, truck and trailer design, stocking density, driver experience, road quality, ventilation, and environmental conditions inside and outside the trailer are also significant elements (Broom, 2008). Early research assumed that long periods of transport without feed and water had adverse effects on the ruminal microbial population (Galyean et al., 1981; Cole and Hutcheson, 1985); however, Fluharty et al. (1996) reported that feed and water deprivation did not affect ruminal cellulolytic or total bacteria populations following a 72-h fast and an 8-h transportation event. Transportation stress has been associated with increased health challenges linked to the interaction between cattle stress load and impaired immune system response.

Oxidative stress occurs during transportation and poses concerns to calf health due to its negative effect on immune function (Deters and Hansen, 2020). Oxidative stress occurs when an animal experiences an imbalance in free radicals and antioxidants on a cellular level. This can ultimately lead to cell and tissue damage and disease (Tufarelli et al., 2023). Oxidative stress not only impacts the animal's health but also meat quality and performance in many species (Deters and Hansen, 2020). Cernicchiaro et al. (2012) conducted an observational study using data from

21 U.S. commercial feedlots collected between 1997 and 2009. Cattle were analyzed as cohorts in a multivariate, mixed-effects, negative binomial model to predict BRD morbidity, overall mortality, ADG, and HCW based on distance traveled (DTV), mean arrival BW, cohort size, and other demographic variables. It was reported that cohorts of cattle transported over 1,000 km had greater BRD morbidity and overall mortality than other cohorts. Across all cohort categories, ADG was lower in cattle transported distances > 250 km compared to those traveling ≤ 250 km. In addition, adverse effects on HCW were most pronounced in cattle transported between 501 to 750 km. When considering the interaction between DTV and BW, the most significant negative influences on health were observed in lighter-weight cattle traveling >250 km. It was also reported that most of the long-haul, light-weight cattle received antibiotic metaphylaxis on arrival due to the added health risk.

Feed Intake

Feed intake is the primary factor influencing feeder cattle performance, and predicting it remains one of the biggest challenges for researchers and cattle producers (Galyean and Hubbert, 1995). Low feed consumption, as a percentage of body weight, is common in newly received cattle as they transition to a new feeding facility and dietary changes, which can exacerbate health challenges. Hutcheson and Cole (1986) reported an anticipated voluntary dry matter intake of 0.5 to 1.5% of BW during the first 7 d after arrival can be expected. In addition, one way to manipulate dietary energy consumption is to increase the dietary energy density of the diet. Spore et al. (2019) conducted an experiment evaluating the effects of a high-energy, corn gluten feed diet in newly received beef cattle. Heifers were used to assess the impact of four diets formulated at various energy levels and intakes. The first diet was supplied at 0.99 Mcal NEg/kg DM and was fed *ad libitum*. The remaining diets were limit-fed and targeted at 95%, 90 %, and

85% of *ad libitum* intake and consisted of diet energy concentrations of 1.10, 1.21, and 1.32 Mcal of NE_g/kg of DM, respectively. After 55 days, there were no differences in morbidity or mortality among dietary treatments; however, gain:feed increased linearly as dry matter intake decreased. Researchers concluded that a limit-fed, high-energy receiving diet did not negatively impact health and resulted in more efficient BW gains.

The Interplay Between Stress and Immunity

Over the past 50 years, extensive research has been conducted to better understand the impacts of behavioral and physiological stress in cattle. Weaning, commingling, transportation, relocation, unfamiliar feeding environments, and dietary changes, including feed ingredients and concentration levels, can significantly influence an animal's performance and overall health during the receiving period. Among these, weaning is recognized as one of the most stressful events in a calf's life, with well-documented negative effects on health and growth performance (Taylor et al., 2020). Compound stress further exacerbates the negative interactions between stress and immune function which occur through the hypothalamic-pituitary-adrenal axis (Minton, 1994; Smith and Vale, 2006). This system plays a key role in maintaining homeostasis. Its function is largely mediated by a potent group of steroid hormones known as glucocorticoids (Munck et al., 1984; Bellavance and Rivest, 2014). Because glucocorticoid receptors are present in most immune cells, the secretion of these hormones from the adrenal glands during periods of stress directly influences immune function. The adrenal gland also produces cortisol, another common steroid hormone released when the body experiences stress to regulate metabolism, inflammation, and various bodily functions. Cortisol has also been used as a biomarker to measure stress (Minton, 1994).

The Immune System: Innate vs Adaptive Immune Response

The immune system acts as the body's primary defense network by protecting against external threats such as bacteria, viruses, fungi, toxins, and parasites, as well as internal challenges like cancer cells and autoimmune conditions (Tizard, 2018). The immune system comprises two main branches: innate immunity and adaptive immunity. These two branches work together to help maintain overall health. Factors, such as, the animal's nutritional status, vaccinations, and level of stress, can greatly influence the effectiveness of the immune responses (Vlasova and Saif, 2021). There are many commensal bacteria present in the upper respiratory tract of cattle and during elevated levels of stress or active infection, these bacteria become amensal as they move down the respiratory tract to the lungs, often causing BRD (Cowick et al., 2022). Maintaining a strong and robust immune system can help to combat infections that may arise (Tizard, 2018).

The innate immune system, which is developed at birth, is the body's first barrier against invading pathogens. This rapid and non-specific immune response relies on physical defenses such as the skin and mucous membranes to prevent pathogen entry (Tizard, 2018). Cellular components, including natural killer cells and phagocytes, which provide immediate defense by neutralizing threats. The innate system is also responsible for initiating inflammation, which increases blood flow and recruits immune cells to sites of infection. When pathogens breach these physical barriers, phagocytic cells like macrophages engulf the invaders and damaged tissue, detecting pathogen-associated molecular patterns and damage-associated molecular patterns (Jounai et al., 2013). Activating these pathways and toll-like receptors leads innate immune cells to secrete cytokines, key signaling molecules that help initiate and direct the adaptive immune response (Vivier et al., 2011).

The adaptive immune system is activated when the innate response is insufficient to eliminate a threat. This immune defense typically requires 4 to 7 days to fully engage (Janeway et al., 2001). Unlike the innate system, adaptive immunity is particular and develops memory, enabling it to recognize and respond more efficiently to previously encountered pathogens (Tizard, 2018). If the pathogen is novel, the adaptive system builds immunological memory for long-term protection. Adaptive immunity is divided into two types: humoral immunity and cell-mediated immunity. Humoral immunity is mediated by B-cells, which produce antibodies targeting specific antigens. In contrast, cell-mediated immunity involves T-cells that are activated by macrophages and other antigen-presenting cells to destroy infected cells directly (Janeway et al., 2001). Although the innate immune response is broad and non-specific, the adaptive immune system is finely tuned to detect and eliminate distinct pathogens. Furthermore, activated T-cells release cytokines that stimulate the proliferation of antigen-specific B-cells, ensuring a targeted and effective immune response. Antigens are any foreign substances capable of binding to lymphocyte receptors and provoking an immune reaction. Vaccination is based on improving the adaptive immune response by introducing these antigens. Innate and adaptive immunity form a sophisticated and tightly regulated system that defends against internal and external threats while minimizing harmful side effects (Hoebe et al., 2004). Without innate and adaptive immune systems, common practices today, such as vaccination, would not be possible (Clem, 2011).

Titer Levels and Cytokine Roles in Stress

There are various methods for analyzing cattle stress and immune status; antibody titer levels, acute-phase proteins, and cytokines can be used to quantify health status in cattle. Respiratory disease is a common cause of cattle health crises. When measuring antibody levels,

researchers often evaluate IBR, PI3, BRSV, BVD I, and BVD II because this pathogens are often found in the vaccination protocol used in newly received cattle. Acute phase proteins, haptoglobin or serum amyloid A, are good indicators of inflammation, and can also be used as markers to measure the presence of BRD infection (Svensson et al., 2007; Orro et al., 2011). Bovine antibody titer levels can help evaluate the animal's response to a vaccine or wild-type virus (McAllister et al., 2024). Little research has investigated antibody titer levels that are considered protective against disease in cattle. Antibody titer levels, therefore, are not indicative of the animal's ability to withstand diseases. Dahmer et al. (2022) investigated the effects of resting time after transportation on antibody titers by varying the length of time between arrival and processing after a 6 h trucking event. IBR antibody titers were evaluated in blood serum directly upon arrival at hour 0, or at 6, 24, and 48 h following arrival and on d 35 of the trial . Cattle that were processed at 24 h or 48 h had no difference in antibody titer concentration between d 0 and d 35, leading to the assumption that cattle were most likely exposed to wild-type virus and had time to seroconvert antibodies before vaccination. No differences were reported between morbidity or mortality during the receiving period, indicating antibody titer levels did not correlate with the animal health outcomes. Hudson et al. (2020) investigated the impacts of compound stress on modified-live (MLV) vs killed virus (KV) respiratory vaccine response. They measured that response through acute phase proteins, titer antibodies, and animal performance. All cattle tested seronegative for IBR, PI3, BVD, and bovine respiratory syncytial virus (BRSV) at d -37. Non-stressed control (C) cattle were weaned at the origin location on d -37 and held for 14 d before shipment to the study location on d -21 where they were allowed to acclimate before trial began. Stress induced (S) cattle were weaned on d -3 and shipped d -2 to research facility, rested for 24 h, and then shipped again on d -1 to the study location to mimic

beef cattle marketing. All vaccines were administered on d 0 and KV was boosted on d 14. Stress-induced cattle had lower DMI compared to C cattle for the first 7 d of the trial. Vaccine type had a significant influence on ADG, with KV cattle exhibiting greater weight gains compared to MLV. Non-stressed control cattle showed a greater initial response to vaccine through BRSV titer for the first 14 d, S cattle surpassed C cattle on d 21, 28, 36 and 42 after primary vaccine. This is most likely due to greater immunosuppression that resulted from the multiple stressors they experienced prior to vaccination. It is still unclear what level of antibody titer from the various pathogens are considered protective for cattle, and more research is needed to better understand bovine antibody titer levels.

A new avenue to understand the bovine immune system and how it functions is through cytokines. Cytokines are small proteins found in the body that help regulate the body's pro- and anti-inflammatory responses. In the last 20 years, significant advancements have been made to understand bovine cytokines. Bovine cytokines have many similarities to other mammal species' cytokines; however, exact mechanisms may differ, and receptor binding methods and signaling transduction pathways can respond differently. For example, the bovine interleukin-10 (IL-10) amino acid sequence is a 76.8% match to that of human IL-10. Den Hartog et al. (2011) investigated the immunomodulatory activities of bovine IL-10 on human monocytes and dendritic cells. Neonatal calf models are often used to help understand human respiratory disease challenges, specifically in infants and young children. A neonatal calf model was used to examine cytokine expression differences during respiratory syncytial virus (RSV) infection. Sacco et al. (2012) investigated how vitamin D status affected cytokine expression in lung tissue during RSV in calves. Calves were classified into two groups: high or low serum $25(OH)D_3$ status, which were 189.9 and 26.5 ng/mL, respectively. Calves were challenged with RSV,

observed for 7 days, and then euthanized and necropsied to collect lung tissue samples for cytokine gene expression analysis. TGF-ß and IL-10, anti-inflammatory cytokines, are present when inflammation arises in the body to reduce inflammation without overstimulating the immune system itself (Rubtsov et al., 2008). Sacco et al. (2012) reported no differences between vitamin D status and the presence of TGF-ß and IL-10 in the lung tissue. IL-8 and IL-12p40, pro-inflammatory cytokines, aim to trigger or intensify inflammation (Sacco et al., 2012). The high vitamin D treatment calves expressed greater IL-12p40 cytokine and tended to have greater IL-8 as well. Elevated levels of pro-inflammatory cytokines are generally considered harmful as they can lead to a heightened immune response. IFNy mRNA levels were greater in the lung lesions of RSV-infected calves than non-lesion lung across both treatment groups. Therefore, calves with high vitamin D status did not have a suppressed response in pro-inflammatory cytokine genes. More research is needed to better understand how vitamin D plays a role in bovine immune function.

Vitamin D and Immune Health

In ruminants, vitamins, minerals, and antioxidants are crucial for proper body function. Although these micronutrient organic compounds make up a small percentage of the diet, they are essential nutrients for maintaining life. Micronutrients aid in improving quality of life, overall health, and immune support (Wu, 2022). Vitamin nutrition is a complex and evolving field for ruminant nutritionists. Eight editions of "Nutrient Requirement of Beef Cattle" have been published by the National Research Council (NRC; 1944 to 2000) and the National Academies of Sciences, Engineering, and Medicine (NASEM; 2016). These publications aim to provide recommendations for vitamins and minerals to avoid deficiency and toxicity, but little of the supporting data has been collected during the last 50 years (Weiss, 1998). Recent reports

show vitamin D_3 inclusion levels are highly variable in both receiving and finishing beef cattle diets. Samuelson et al., (2016) reported in a nutritional survey that vitamin D_3 recommendations from consultants were 271 and 142 IU/kg in receiving and finishing diets, respectively. Both values fall below the NASEM (2016) recommendation of 275 IU/kg of diet dry matter for beef cattle. The mode in this survey was 0 IU per kg in receiving and finishing diets, indicating that vitamin D_3 was not included in most feedlot diets. Vitamins are classified by their solubility, either water-soluble or lipid-soluble. Water-soluble vitamins can be produced by ruminal microbes. Most lipid-soluble vitamins (i.e., A, D, and E) must be consumed in the diet to meet the growth or milk production requirement. Cattle typically receive adequate vitamin A, D, and E by consuming green, growing forages or synthesize it in the case of vitamin D. However, when forages mature and become dormant during certain times through the year, they become limited in nutritive value, specifically protein, energy, vitamin, and mineral content. Cattle in confinement often only have access to preserved forages with lower vitamin content and bioavailability. Vitamin E can be obtained from feed sources such as fresh growing forages and seed oils, like soybeans. Vitamin A precursor, ß-carotene, is present in many actively growing plants. Cattle can convert ß-carotene into an active form of vitamin A, retinol. To obtain vitamin D, cattle can naturally acquire it from two sources: vitamin D_2 which is found in plant sources like sun-cured forages or plants associated with fungi or vitamin D_3 which is synthesized in the skin through ultraviolet exposure from the sun. In the case of supplementation, vitamin D_3 is preferred to avoid conversion inefficiencies from vitamin D_2 (Nelson et al., 2016). Animals that are housed indoors require supplementation to meet requirements for vitamin D_3 because of limited sunlight exposure.

Vitamin D Metabolism and Status

Early research focused mainly on defining minimum requirements to avoid clinical diseases like rickets from vitamin D deficiency in cattle. Current recommendations today can be traced back to that early research. Due to technology advancements and genetic improvements, the modern beef animal has changed greatly over the last 50 years, and nutritional recommendations for vitamins have remained the same are based on research conducted decades ago. More recent research in vitamin D investigated the effects of supplementation on meat tenderness (Montgomery et al., 2000), immune function support (Nelson et al., 2012), and improved growth (Acedo et al., 2018). Newer data investigating calcidiol supplementation in dairy cattle also show positive results in mammary immune health (Poindexter et al., 2020). Vitamin D is essential for the proper growth and development of cattle (Bechdel et al., 1937). Typically, vitamin D is obtained from sun exposure of 7-dehydrocholesterol (7-DHC) (NRC, 2000; Vasconcelos and Galyean, 2007). 7-Dehydrocholesterol is present in the skin of all mammals. When ultraviolet rays strike the skin, 7-DHC is converted to pre-vitamin D_3 and then undergoes thermal isomerization to become vitamin D_3. Vitamin D_3 is then released from the skin and enters the extracellular fluid to become available in the blood for liver metabolism (Horst and Reinhardt, 1983). When vitamin D_3 is supplemented through the diet, metabolism starts in the rumen by present microbes. Although vitamin D_2 and D_3 can both elevate vitamin D status in cattle, vitamin D_3 is the predominant form (Montgomery et al., 2000). Although cattle can produce adequate levels of vitamin D_3 through the summer months (Hymøller and Jenson, 2012), it is possible that cattle experience suboptimal vitamin D status for immune support during the winter months.

For vitamin D_3 to attain its an active form, it must undergo two hydroxylation steps in the body. The first conversion takes place in the liver. The enzyme 25-hydroxylase (CYP2R1) converts vitamin D_3 to 25-hydroxyvitamin D_3 [25(OH)D_3; calcidiol]. The metabolite 25(OH)D_3 is considered an inactive form of vitamin D_3; therefore, it does not contribute to calcium absorption. It is, however, one of the most reliable indicators of animal vitamin D status. The second conversion takes place in the kidney. There, the enzyme 1-hydroxylase (CYP27B1) converts 25(OH)D_3 to 1,25-dihydroxyvitamin D_3 [1,25$(OH)_2D_3$], also known as calcitriol (Nelson et al., 2012). This biologically active form is the primary regulator of calcium / phosphorus homeostasis and bone remodeling (Sacco et al., 2012). The 1,25(OH)D_3 molecule is referred to as the circulating hormonal form of vitamin D.

Little research has been conducted in order to understand vitamin D status in beef cattle. The minimum threshold of vitamin D status to meet basic vitamin D metabolic functions of calcium and phosphorus homeostasis in cattle has been estimated to be around 30 ng/mL serum 25(OH)D_3 concentration (Nelson and Merriman, 2014). Seasonal variation, among other factors, makes it challenging to establish a benchmark for vitamin D status in beef cattle. Casas et al. (2015) evaluated the seasonal variation in vitamin D status of beef cattle reared in the central United States. They evaluated blood serum for 25(OH)D_3 concentration at different timepoints throughout the year to evaluate seasonal variation due to day length and sun exposure as well as evaluate different ages of cattle. Blood was collected on 2- to 3-month-old calves in June, 5- to 6-month-old calves in August, 6- to 7-month-old calves in September, and 10- to 12-month-old cattle in March. Vitamin D status during March was lower compared to all other time points and was at concentrations below that recommended level for optimal calcium and phosphorus homeostasis which is ~ 25 ng/mL (Nelson et al., 2012) Nelson et al. (2016) reported similar

results when they investigated the effects of geographical location and season on serum 25(OH)D_3 concentration in beef cows and calves. Blood samples were collected from herds that were participating in ongoing research trials across three different states (Florida, Idaho, and Minnesota) at three seasonal time points (winter/spring, summer, and late summer/fall). Hide color varied between herds; the Florida herd consisted of Angus and Brangus genetic lines, the Idaho herd consisted of purebred Charolais genetics, and the Minnesota herd consisted of purebred Angus genetics. The Florida herd was supplemented with a free-choice mineral during pregnancy that was formulated to provide 2,800 IU vitamin D_3 per day. The Idaho cattle were offered free-choice mineral during late gestation that was formulated to provide 5,000 IU vitamin D_3 per day. During the last 30 days of gestation, vitamin D_3 was removed from the mineral for Idaho cows. The Minnesota herd received approximately 12,500 IU of vitamin D_3 per day through free-choice mineral supplement during the winter months. In April, cows were switched to a free-choice mineral providing 3,750 IU vitamin D_3 per day. It was reported that cows and calves had greater serum concentrations during the summer, with cows ranging from 59 to 88 ng/mL and calves ranging from 36 to 62 ng/mL. No interaction was found between time and location.

Skin pigmentation is known to interfere with humans' ability to use UV exposure to convert 7-DHC to Vitamin D. (Sawicki et al., 2016; Wolf et al., 2022). People with darker pigmented skin needs greater UV exposure to reach a similar vitamin D status in comparison to people with lighter pigmented skin (Sawicki et al, 2016). Skin color in humans likely evolved to help with biological events that center around the need for greater vitamin D status (Jablonski and Chapin, 2000). Hodnik et al. (2025) investigated the impacts of hair color and black skin area on vitamin D concentration and milk production in Holstein cows. The first treatment group

was shaved 30cm from the midline between the withers and the hook bones and received a daily radiation dose of 80 J/m^2. The second group was unshaved and received daily radiation during milking time and did not exceed 360 J/m^2. The third group served as a control and was not shaved or irradiated. Serum 25(OH)D_3 was used to measure the vitamin D concentration of the animals in the 60-d trial. To quantify the amount of black hair, each animal was photographed from above and the picture was analyzed by imaging software to determine the percentage of black. They assumed that the percentage of black hair present was equal to the percentage of black skin. Hodnik et al. (2025) concluded that shaved cows had greater vitamin D status over time than unshaved and control cows. A high percentage of black skin or hair was negatively correlated to vitamin D status.

Calcidiol Supplementation

Calcidiol, the bioactive metabolite 25-hydroxyvitamin D_3, became commercially available for supplementation in the late 1990's and currently is used in swine, poultry, aquaculture, and ruminant species. Calcidiol is used in the poultry and swine industry due to indoor housing environments of those animals. In these industries, calcidiol supplementation improved bone health, calcium and phosphorus absorption, meat yield, and immune function. Research conducted in Brazil evaluated calcidiol supplementation to Nelore beef bulls and reported increased dressing percentage and HCW compared to cattle not fed HyD® (Acedo et al., 2018). In dairy cattle, calcidiol improved calcium metabolism through the transition period through lactation (Nelson and Merriman, 2014). Calcidiol elevates circulating levels of 25(OH)D_3 more efficiently compared to supplementation with vitamin D_3 (Poindexter et al., 2020; Poindexter et al., 2023b).

Vitamin D Within The Immune System

The active form of vitamin D, 1, 25$(OH)_2$D, helps to regulate both the innate and adaptive immune systems through vitamin D receptors (VDR). 25-hydroxyvitamin D_3 also positively influences the immune system through immune cells like macrophages and dendritic cells (Wimalawansa, 2023). These immune cells also contain the 1-α hydroxylase enzyme that can convert 25(OH)D to 1, 25$(OH)_2$D. Once vitamin D has reached its active form, it activates its own receptors (Wimalawansa, 2023). These VDR are nuclear, a ligand-dependent transcription factor that interacts with 1, 25$(OH)_2$D to activate many genes responsible for psychological functions (Kongsbak et al., 2013). Furthermore, 1, 25 -hydroxyvitamin D acts somewhat differently on the innate immune system of cattle when compared to humans; however, in the adaptive immune system, mice, humans, and cattle all have similar modes of action (Nelson et al., 2012). Once 1, 25$(OH)_2$D binds to VDR in the cell nucleus, VDR dimerizes with the retinoid X receptor (RXR). This complex then binds to vitamin D response elements in the promoter regions of various genes, regulating their transcription. This VDR-RXR complex influences genes involved in immune function. Specifically, it upregulates antimicrobial peptides (AMP), such as cathelicidin and defensins, which are essential for the innate immune responses against pathogens (Wimalawansa, 2023).

The activation of VDR enhances the pathogen-fighting ability of macrophages and monocytes. By increasing the production of AMP, it strengthens the first line of immune defense. Additionally, it modulates the release of pro-inflammatory cytokines, which can help prevent excessive inflammation that may lead to tissue damage. VDR activation by 1,25$(OH)_2$D influences T-cell differentiation, promoting regulatory T-cell formation, which dampens immune overactivation. It also reduces the differentiation of pro-inflammatory Th1 and Th17 cells,

curbing potential autoimmunity and excessive inflammation. Furthermore, it can suppress B-cell antibody production in certain contexts, contributing to a balanced immune response (Wimalawansa, 2023). Overall, through VDR activation, 25(OH)D modulates both the innate and adaptive immune responses, enhancing pathogen defense while promoting immune tolerance and reducing chronic inflammation. This dual action helps prevent excessive immune reactions and supports the resolution of infections without triggering autoimmunity.

Conclusion

Newly received cattle continue to face the same challenges year after year. From birth to slaughter, cattle experience elevated stress from weaning, transportation, dietary change and disease challenge. Good husbandry practices are crucial to minimize stress, but stressful events are sometimes unavoidable and can affect cattle health and performance. Finding ways to help prepare cattle for stress or unlocking defense mechanism to combat the effect of stress on the body could prove beneficial for animal welfare and economic performance of the cattle. Vitamin D research has shown very promising results in human medicine by improving immune function, mood, and overall nutritional balance. From basic calcium phosphorus homeostasis and muscle tenderness to improved immune function and enhanced growth performance, further investigation of defining optimal vitamin D requirements and other metabolic functions it can support is warranted. Advancing the understanding of the role calcidiol supplementation plays in the bovine immune system may improve the productivity of feeding lightweight, high-risk cattle, especially those exposed to early periods of stress during the feeding period.

Literature Cited

Acedo, T. S., V. Nunes de Gouvea, G. de Souza Floriano Machado de Vasconcellos, M. Arrigoni, C. Ludovico Martins, D. Millen, L. R. Muller, G. Fernandes de Melo, R. A. Rizzieri, C. Floret da Costa, and A. Domingues Sartor. 2018. Effect of 25-hydroxy-vitamin-D3 on feedlot cattle. J. Anim. Sci. 96(Suppl. 3):447–448. (Abstr.) doi:10.1093/jas/sky404.977

Bechdel, S. I., N. W. Hillston, and N. B. Guerrant. 1937. The vitamin D requirement (U.S.P. units) for growth and well-being of calves from birth to six months of age. J. Dairy Sci. 20:432–433.

Bellavance, M. and S. Rivest. 2014. The HPA-immune axis and the immunomodulatory actions of glucocorticoids in the brain. Front. Immunol. 5:136. doi:10.3389/fimmu.2014.00136

Blakebrough-Hall, C., P. Hick, T. J. Mahony, and L. A. González. 2022. Factors associated with bovine respiratory disease case fatality in feedyard cattle. J. Anim. Sci. 100:1–9. doi:10.1093/jas/skab361

Broom, D. 2008. The welfare of livestock during road transport. In: Long distance transport and welfare of farm animals. p. 157–181. doi:10.1079/9781845934033.0157

Campbell, J. R. 2005. Effects of bovine viral diarrhea virus in the feedlot. Vet. Clin. North Am. Food Anim. Pract. 20:39–50. doi:10.1016/j.cvfa.2003.11.003

Casas, E., J. D. Lippolis, L. A. Kuehn, and T. A. Reinhardt. 2015. Seasonal variation in vitamin D status of beef cattle reared in the central United States. Domest. Anim. Endocrinol. 52:71–74. doi:10.1016/j.domaniend.2015.03.003

Cernicchiaro, N., B. J. White, D. G. Renter, A. H. Babcock, L. Kelly, and R. Slattery. 2012. Associations between the distance traveled from sale barns to commercial feedlots in the

United States and overall performance, risk of respiratory disease, and cumulative mortality in feeder cattle during 1997 to 2009. J. Anim. Sci. 90:1929–1939. doi:10.2527/jas.2011-4599

Clem, A. S. 2011. Fundamentals of vaccine immunology. J. Glob. Infect. Dis. 3:73–78. doi:10.4103/0974-777X.77299

Cole, N. A., and D. P. Hutcheson. 1985. Influence of realimentation diet on recovery of rumen activity and feed intake in beef steers. J. Anim. Sci. 61:692–701. doi:10.2527/jas1985.613692x

Cowick, C. A., B. P. Russ, A. R. Bales, B. Nanduri, and F. Meyer. 2022. Mannheimia haemolytica negatively affects bovine herpesvirus type 1.1 replication capacity in vitro. Microorganisms. 10:2158. doi:10.3390/microorganisms10112158

Dahmer, P. L., C. A. Zumbaugh, M. E. Reeb, N. B. Stafford, Z. T. Buessing, K. G. Odde, J. S. Drouillard, A. J. Tarpoff, and C. K. Jones. 2022. Impacts of post-transport/pre-processing rest period on the growth performance, anthelmintics efficacy, and serum metabolites changes in cattle entering a feed yard. Transl. Anim. Sci. 6:1–7. doi:10.1093/tas/txac085

Den Hartog, G., H. F. Savelkoul, R. Schoemaker, E. Tijhaar, A. H. Westphal, T. de Ruiter, E. van de Weg-Schrijver, and R. J. van Neerven. 2011. Modulation of human immune responses by bovine interleukin-10. PLoS ONE. 6(3):e18188. doi:10.1371/journal.pone.0018188

Deters, E. L., and S. L. Hansen. 2020. Invited review: linking road transportation with oxidative stress in cattle and other species. Appl. Anim. Sci. 36:183–200. doi:10.15232/aas.2019-01956

Fike, K. E., and M. F. Spire. 2006. Transportation of cattle. Vet. Clin. North Am. Food Anim.

Pract. 22:305–320. doi:10.1016/j.cvfa.2006.03.012

Fluharty, F. L., S. C. Loerch, and B. A. Dehority. 1996. Effects of feed and water deprivation on ruminal characteristics and microbial population of newly weaned and feedyard-adapted calves. J. Anim. Sci. 74:465–474. doi:10.2527/1996.742465x

Galyean, M. L., R. W. Lee, and M. E. Hubbert. 1981. Influence of fasting and transit on ruminal and blood metabolites in beef steers. J. Anim. Sci. 53:7–18. doi:10.2527/jas1981.5317

Galyean, M. L., S. A. Gunter, and K. J. Malcolm-Callis. 1995. Effects of arrival medication with tilmicosin phosphate on health and performance of newly received beef cattle. J. Anim. Sci. 73:1219–1226. doi:10.2527/1995.7351219x

Galyean, M. L., and M. E. Hubbert. 1995. Effects of season, health, and management on feed intake by beef cattle. In: Symposium: Intake by Feedyard Cattle. F. N. Owens, ed. Oklahoma Agric. Exp. Stn. p. 226–234.

Galyean, M. L., G. C. Duff, and J. D. Rivera. 2022. Galyean appreciation club review: revisiting nutrition and health of newly received cattle—what have we learned in the last 15 years? J. Anim. Sci. 100(4):1–17. doi:10.1093/jas/skac067

Handel, I. G., K. Willoughby, F. Land, B. Koterwas, K. L. Morgan, V. N. Tanya, and B. M. deC. Bronsvoort. 2011. Seroepidemiology of bovine viral diarrhea virus (BVDV) in the adamawa region of cameroon and use of the SPOT test to identify herds with PI calves. PLoS ONE. 6(7):e21620. doi:10.1371/journal.pone.0021620

Hodnik, J. J., J. Ježek, and J. Starič. 2020. A review of vitamin D and its importance to the health of dairy cattle. J. Dairy Sci. 87:84–87. doi:10.1017/S002202992000042

Hodnik J.J., M. Jankovec, J. Ježek, Z. Krušič, S. Mitterhofer , and J Starič. 2025. Effects of UV-B light exposure during automatic milking on vitamin D levels in holstein friesian cows.

Front. Vet. Sci. 11:1433230. doi: 10.3389/fvets.2024.1433230

Hoebe, K., E. Janssen, and B. Beutler. 2004. The interface between innate and adaptive immunity. Nat. Immunol. 5:971–974. doi:10.1038/ni1004-971

Horst, R. L. and T. A. Reinhardt. 1983. Vitamin D metabolism in ruminants and its relevance to the periparturient cow. J. Dairy Sci. 66:661-678.doi:10.3168/jds.S0022-0302(83)81844-X

Hudson, R.E., D. J. Tomczak, E. L. Kaufman, A. M. Adams, J. A. Carroll, P. R. Broadway, M. A. Ballou, and J. T. Richeson. 2020. Immune responses and performance are influenced by respiratory vaccine antigen type and stress in beef calves. Animals (Basel). 30:1119. doi: 10.3390/ani10071119

Hutcheson, D. P., and N. A. Cole. 1986. Management of transit-stress syndrome in cattle: Nutritional and environmental effects. J. Anim. Sci. 62:555–560. doi:10.2527/jas1986.622555x

Hymøller, L., and S. K. Jensen. 2012. 25-hydroxycholecalciferol status in plasma is linearly correlated to daily summer pasture time in cattle at 56 degrees N. Br. J. Nutr. 108:666–671. doi:10.1017/S0007114511005964

Janeway, C., P. Travers, M. Walport, and M. Shlomchik. 2001. Immunobiology. 5th ed. Garland Science, New York, NY. ISBN: 9780815336426.

Jounai, N., K. Kobiyama, F. Takeshita, and K. J. Ishii. 2013. Recognition of damage-associated molecular patterns related to nucleic acids during inflammation and vaccination. Front. Cell. Infect. Microbiol. 2:168. doi:10.3389/fcimb.2012.00168

Kongsbak, M., T. B. Levring, C. Geisler, and M. R. von Essen. 2013. The vitamin D receptor and T cell function. Front. Immunol. 4:148. doi:10.3389/fimmu.2013.00148

Lincoln, S. D., and D. D. Hinman. 2003. Preconditioning of calves. Beef Cattle Handbook,

BCH-5475. Univ. of Idaho and Caldwell Veterinary Teaching Center, in cooperation with Ext. Beef Cattle Resource Comm. https://www.iowabeefcenter.org/bch/PreconditioningCalves.pdf (Accessed 20 July 2025).

Maples, W. E., B. W. Brorsen, D. Peel, and B. Hicks. 2022. Observational study of the effect of metaphylaxis treatment on feedlot cattle productivity and health. Front. Vet. Sci. 9:947585. doi:10.3389/fvets.2022.947585

McAllister, H. R., B. I. Ramirez, M. E. Crews, L. M. Rey, A. C. Thompson, S. F. Capik, and M. A. Scott. 2024. A systematic review on the impact of vaccination for respiratory disease on antibody titer responses, health, and performance in beef and dairy cattle. Vet. Sci. 11:599. doi:10.3390/vetsci11120599

Minton, J. E. 1994. Function of the hypothalamic-pituitary-adrenal axis and the sympathetic nervous system in models of acute stress in domestic farm animals. J. Anim. Sci. 72:1891–1898. doi:10.2527/1994.7271891x

Montgomery, J. L., F. C. Parrish, Jr., D. C. Beitz, R. L. Horst, E. J. Huff-Lonergan, and A. H. Trenkle. 2000. The use of vitamin D3 to improve beef tenderness. J. Anim. Sci. 78:2615–2621. doi:10.2527/2000.78102615x

Munck, A., P. M. Guyre, and N. J. Holbrook. 1984. Physiological functions of glucocorticoids in stress and their relation to pharmacological actions. Endocr. Rev. 5:25–44. doi:10.1210/edrv-5-1-25

NAHMS. 2013. National Animal Health Monitoring System. Feedlot 2011. Part IV: Health and health management on U.S. feedlots with a capacity of 1,000 or more head. USDA–APHIS–VS–CEAH–NAHMS, Fort Collins, CO.

NASEM. 2016. Nutrient requirements of beef cattle. 8th ed. Natl. Acad. Press, Washington, DC.

Nelson, C. D., T. A. Reinhardt, J. D. Lippolis, R. E. Sacco, and B. J. Nonnecke. 2012. Vitamin D signaling in the bovine immune system: A model for understanding human vitamin D requirements. Nutrients 4:181–196. doi:10.3390/nu4030181

Nelson, C. D. and K. E. Merriman. 2014. Vitamin D metabolism in dairy cattle and implications for dietary requirements. Proc. 25th Florida Ruminant Nutr. Symp. p. 78–90.

Nelson, C. D., J. L. Powell, D. M. Price, M. J. Hersom, J. V. Yelich, M. E. Drewnoski, S. L. Bird, and G. A. Bridges. 2016. Assessment of serum 25-hydroxyvitamin D concentrations of beef cows and calves across seasons and geographical locations. J. Anim. Sci. 94:3958–3965. doi:10.2527/jas.2016-0611

Nickell, J. S., B. J. White, R. L. Larson, D. G. Renter, J. Roque, R. Hesse, R. Oberst, L. Peddireddi, and G. Anderson. 2011. Onset and duration of transient infections among antibody-diverse beef calves exposed to a bovine viral diarrhea virus persistently infected calf. Int. J. Appl. Res. Vet. Med. 9:1.

NRC. 2000. Nutrient requirements of beef cattle. 7th rev. ed. Natl. Acad. Press, Washington, DC.

Orro, T., T. Pohjanvirta, U. Rikula, A. Huovilainen, S. Alasuutari, L. Sihvonen, S. Pelkonen, and T. Soveri. 2011. Acute phase protein changes in calves during an outbreak of respiratory disease caused by bovine respiratory syncytial virus. Comp. Immunol. Microbiol. Infect. Dis. 34:23–29. doi:10.1016/j.cimid.2009.10.005

Poindexter, M. B., M. F. Kweh, R. Zimpel, J. Zuniga, C. Lopera, M. G. Zenobi, Y. Jiang, M. Engstrom, P. Celi, J. E. P. Santos, and C. D. Nelson. 2020. Feeding supplemental 25-hydroxyvitamin D_3 increases serum mineral concentrations and alters mammary immunity of lactating dairy cows. J. Dairy Sci. 103:805–822. doi:10.3168/jds.2019-16999

Poindexter, M. B., R. Zimpel, A. Vieira-Neto, A. Husnain, A. C. M. Silva, A. Faccenda, A. S. de Avila, P. Celi, C. Cortinhas, J. E. P. Santos, and C. D. Nelson. 2023a. Effect of prepartum source and amount of vitamin D supplementation on lactation performance of dairy cows. J. Dairy Sci. 106:974–989. doi:10.3168/jds.2022-22388

Poindexter, M. B., R. Zimpel, A. Vieira-Neto, A. Husnain, A. C. M. Silva, A. Faccenda, A. S. de Avila, P. Celi, C. Cortinhas, J. E. P. Santos, and C. D. Nelson. 2023b. Effect of source and amount of vitamin D on serum concentrations and retention of calcium, magnesium, and phosphorus in dairy cows. J. Dairy Sci. 106:954–973. doi:10.3168/jds.2022-22386

Richeson, J. T., E. B. Kegley, J. G. Powell, P. A. Beck, B. L. Vander Ley, and J. F. Ridpath. 2012. Weaning management of newly received beef calves with or without continuous exposure to a persistently infected bovine viral diarrhea pen mate: Effects on health, performance, bovine diarrhea virus titers, and peripheral blood leukocytes. J. Anim. Sci. 90:1972–1985. doi:10.2527/jas.2011-4077

Rojas, H. A., B. J. White, D. E. Amrine, and R. L. Larson. 2022. Predicting bovine respiratory disease risk in feedyard cattle in the first 45 days post arrival. Pathogens. 11:442. doi:10.3390/pathogens11040442

Rubtsov, Y. P., J. P. Rasmussen, E. Y. Chi, J. Fontenot, L. Castelli, X. Ye, P. Treuting, L. Siewe, A. Roers, W. R. Henderson, W. Mueller, and A. Y. Rudensky. 2008. Regulatory T cell-derived interleukin-10 limits inflammation at environmental interfaces. Immunity. 28:546–558. doi:10.1016/j.immuni.2008.02.017

Sacco, R. E., B. J. Nonnecke, M. V. Palmer, W. R. Waters, J. D. Lippolis, and T. A. Reinhardt. 2012. Differential expression of cytokine in response to respiratory syncytial virus infection of calves with high and low circulating 25-hydroxyvitamin D_3. PLoS ONE.

7(3):e33074. doi:10.1371/journal.pone.0033074

Samuelson, K. L., M. E. Hubbert, M. L. Galyean, and C. A. Loest. 2016. Nutritional recommendations of feedlot consulting nutritionists: The 2015 New Mexico State and Texas Tech University survey. J. Anim. Sci. 94:2648–2668. doi:10.2527/jas.2016-0282

Sawicki, C. M., M. I. Van Rompay, L. E. Au, C. M. Gordon, and J. M. Sacheck. 2016. Sun-exposed skin color is associated with changes in serum 25-hydroxyvitamin D in racially/ethnically diverse children. J. Nutr. 146:751–757. doi:10.3945/jn.115.222505

Smith, S. M., and W. W. Vale. 2006. The role of the hypothalamic-pituitary-adrenal axis in neuroendocrine responses to stress. Dialogues Clin. Neurosci. 8:383–395.

Spore, T. J., S. P. Montgomery, E. C. Titgemeyer, G. A. Hanzlicek, C. I. Vahl, T. G. Nagaraja, K. T. Cavalli, W. R. Hollenbeck, R. W. Wahl, and D. A. Blasi. 2019. Effects of a high-energy programmed feeding protocol on nutrient digestibility, health, and performance of newly received growing beef cattle. Appl. Anim. Sci. 35:397–407.

Step, D. L., C. R. Krehbiel, H. A. DePra, J. J. Cranston, R. W. Fulton, J. G. Kirkpatrick, D. R. Gill, M. E. Payton, M. A. Montelongo, and A. W. Confer. 2008. Effects of commingling beef calves from different sources and weaning protocols during a forty-two-day receiving period on performance and bovine respiratory disease. J. Anim. Sci. 86:3146–3158. doi:10.2527/jas.2008-0883

Stott, G. H. 1981. What is animal stress and how is it measured? J. Anim. Sci. 52:150–153. doi:10.2527/jas1981.521150x

Svensson, C., P. Liberg, and J. Hultgren. 2007. Evaluating the efficacy of serum haptoglobin concentration as an indicator of respiratory-tract disease in dairy calves. Vet. J. 174:288–294. doi:10.1016/j.tvjl.2006.07.009

Taylor, J. D., J. N. Gilliam, G. Mourer, and C. Stansberry. 2020. Comparison of effects of four weaning methods on health and performance of beef calves. Animal. 14:161–170. doi:10.1017/S1751731119001228

Terrell, S. P., D. U. Thomson, B. W. Wileman, and M. D. Apley. 2011. A survey to describe current feeder cattle health and well-being program recommendation made by feedyard veterinary consultants in the United States and Canada. Bov. Pract. 45:140–148. doi:10.21423/bovine-vol45no2p140-148

Tizard, I. R. 2018. Veterinary Immunology. 10th ed. Elsevier/Saunders, Philadelphia, PA. ISBN: 9780323523493

Tufarelli, V., M. A. Colonna, C. Losacco, and N. Puvača. 2023. Biological health markers associated with oxidative stress in dairy cows during lactation period. Metabolites. 13:405. doi:10.3390/metabo13030405

USDA APHIS. 2021. Management practices on U.S. feedlot, 2021. United States Department of Agriculture, Animal and Plant Health Inspection Service. Available from: https://www.aphis.usda.gov/sites/default/files/feedlot-health-2021-mgmt-practice-dr1.pdf

Van Engen, N. K., and J. F. Coetzee. 2018. Effects of transportation on cattle health and production: A review. Anim. Health Res. Rev. 19:142–154. doi:10.1017/S1466252318000075

Vasconcelos, J. T., and M. L. Galyean. 2007. Nutritional recommendations of feedyard consulting nutritionists: The 2007 Texas Tech University survey. J. Anim. Sci. 85:2772–2781. doi:10.2527/jas.2007-0261

Vining, P., P. Beck, K. C. Raper, D. Lalman, B. Wilson, R. Biggs, and D. Peel. 2024. Calf preconditioning: A comprehensive overview. Oklahoma Cooperative Extension Services.

ID: AFS-3039. Available from: https://extension.okstate.edu/fact-sheets/calf-preconditioning-a-comprehensive-overview.html

Vivier, E., D. H. Raulet, A. Moretta, M. A. Caligiuri, L. Zitvogel, L. L. Lanier, W. M. Yokoyama, and S. Ugolini. 2011. Innate or adaptive immunity? The example of natural killer cells. Science. 331:44–49. doi:10.1126/science.1198687

Vlasova, A. N. and L. J. Saif. 2021. Bovine immunology: Implications for dairy cattle. Front. Immunol. 12:643206. doi:10.3389/fimmu.2021.643206

Weiss, W. P. 1998. Requirements of fat-soluble vitamins for dairy cows: A review. J. Dairy Sci. 81:2493–2501. doi:10.3168/jds.S0022-0302(98)70141-9

Wimalawansa, S. J. 2023. Infections and autoimmunity—The immune system and vitamin D: A systematic review. Nutrients. 15:3842. doi:10.3390/nu15173842

Wolf, S. T., G. A. Dillon, L. M. Alexander, N. G. Jablonski, and W. L. Kenney. 2022. Skin pigmentation is negatively associated with circulating vitamin D concentration and cutaneous microvascular endothelial function. Am J Physiol Heart Circ Physiol. 323:490-498.

Wu, G. 2022. Nutrition and metabolism: Foundations for animal growth, development, reproduction, and health. Adv. Exp. Med. Biol. 1354:1–24. doi:10.1007/978-3-030-85686-1_1

Chapter 2 - Calcidiol Supplementation, Seasonal Variation Influence on Vitamin D Status, and a Nutrikinetic Evaluation on Pulse-Dosing Calcidiol in Cattle

Introduction

Vitamin D plays a critical role in regulating the absorption of calcium and phosphorus, skeletal development, immune function, and overall cattle productivity. It is considered an essential nutrient for growth and development of cattle (Bechdel et al., 1937). Vitamin D can be synthesized in the skin with sufficient sun exposure, but the optimal vitamin D status in beef cattle remains unknown. Recent research reported a positive role for vitamin D on HCW of feedlot cattle and mammary immune function of dairy cattle (Acedo et al., 2018; Poindexter et al., 2020). Despite the roles of vitamin D in growth and health, it has been largely overlooked in beef cattle nutrition because it is commonly assumed that cattle acquire adequate vitamin D through the photoconversion of 7-dehydrocholesterol (7-DHC) to vitamin D_3 in the skin or the ingestion of vitamin D_2 from forages (Nelson et al., 2016). The 25-hydroxyvitamin D (**25(OH)D**) metabolites (which includes both 25-hydroxyvitamin D_2 [**25(OH)D_2**] and 25-hydroxyvitamin D_3 [**25(OH)D_3**]) are the best indicator of vitamin D status (Hollis and Horst, 2007; Nelson et al., 2012). Vitamin D is readily converted to 25(OH)D by 25-hydroxylases in the liver, and 25(OH)D serves as the precursor for the active vitamin D hormone, 1,25-dihydroxyvitamin D (**1,25(OH)$_2$D**). Serum 25(OH)D concentrations < 5 to 7 ng/mL are indicative of deficiency and put cattle at risk for rickets and weak bones. Serum 25(OH)D_3 concentrations between 10 and 20 ng/mL are insufficient for calcium-phosphorus homeostasis and jeopardize overall bone health. A serum 25(OH)D_3 concentration ≥ 30 ng/mL has been proposed as a lower threshold for

sufficiency, and thresholds to support an immune response in dairy cows have been estimated to be ≥100 ng/mL (Nelson and Merriman, 2014; Poindexter et al., 2019). In the current literature, supplementation with Vitamin D_2 or D_3 has not been shown to elevate serum 25(OH)D levels above 100 ng/mL. Therefore, we evaluated vitamin D status of cattle in trials that used 25(OH)D_3 supplementation. Experiment 1 explored serum 25(OH)D_3 concentrations in high-risk receiving beef heifers when 25(OH)D_3(HyD®; dsm-firmenich, Belvidere, NJ) supplementation was provided. High-risk heifers in exp. 1 were fed diets with and without 25(OH)D_3 supplementation via top-dress. Experiment 2 investigated vitamin D status across all four seasons in two commercial feedyards. One milligram per animal per day of HyD was supplemented to half of cattle within each yard for approximately the last 130 d on feed. The common corn-based finishing diet offered to steers and heifers in exp. 2 contained 275 IU of supplemental vitamin D_3 per kg of diet dry matter. Factors like day length, sex, 25(OH)D_3 supplementation, and hide color are all variables that can influence vitamin D status. Experiment 3 evaluated the nutrikinetic profile of a large single pulse dose of HyD® solution and then used these profiles to simulate the effects of different dosing regimens on serum 25(OH)D_3 concentrations. Experiment 3 aims to identify a quicker way to elevate vitamin D status. Eight cannulated beef heifers received an infusion with a HyD® solution through a ruminal fistula to evaluate the nutrikinetic profile of calcidiol. Results from nutrikinetic evaluation could be used for predictive modeling simulations with the aim to develop different drench application strategies.

Materials and Methods

All procedures involving the use of animals were approved by the Kansas State University Institutional Animal Care and Use Committee (IACUC # 4650).

Experiment 1. Calcidiol supplementation in high-risk receiving heifer calves

Ninety-six high-risk heifer calves (BW= 222 ± 17.0 kg) were purchased from auction markets and assembled at an order buying station before being transported to the study location. Heifers were used in a randomized complete-block design to evaluate vitamin D status in receiving diets containing varying amounts of supplemental vitamin D_3 or HyD® fed during a 60-d receiving period. Prior to transport, heifers were individually weighed and tagged with an individual animal identification ear tag; at the same time, a blood sample from each heifer was collected via the jugular vein and the serum was analyzed for 25(OH)D_3 concentration to establish a baseline for vitamin D status. Heifers were then transported 1,086 km from the order buying station in Dickson, TN to the Kansas State University Beef Stocker Unit in Manhattan, KS on March 10, 2023. Upon arrival, heifers were stratified by individual body weight and assigned to pens containing 12 heifers. Pens were randomly assigned to 1 of 4 treatments which resulted in 2 pens per treatment for a total of 8 pens. Dietary treatments consisted of these daily vitamin D_3 amounts: neither vitamin D_3 nor calcidiol (**CON**), 0.075 mg of vitamin D_3 (**D3**), 0.5 mg of calcidiol (**HyD Low**) or 1.0 mg calcidiol (**HyD High**). Treatments were applied starting d 0 until the completion of the study on d 60. Pens were soil-surfaced and of equal size (9.1 × 15.2 m). Concrete fence-line bunks were 9.1 m in length and set on a 3.6-m apron.

Experimental diets are presented in Table 2.1. Heifers were offered diets at 2.2% of BW daily (DM basis). Diets were formulated to supply 1.31 Mcal/kg net energy for gain (NEg) based on tabular values (NASEM, 2016). The diet included (DM basis) 39.5% dry-rolled corn, 40.0% wet corn gluten feed (Sweet Bran; Cargill Animal Nutrition, Blair, NE), 13.0% ground warm-season grass hay, and 7.5% pelleted supplement. The pelleted supplements provided the source of vitamin D_3 in this study; therefore, two distinct supplements were utilized in the trial. Dry-

rolled corn, wet-corn gluten feed, and warm-season grass hay were mixed and delivered to each pen at 0700h using a Roto-Mix feed wagon (Model #414-14B; Roto-Mix, Dodge City, KS). Due to the small quantity of supplement required, supplements for each pen were weighed and delivered separately to each pen as a top-dress. Dried distillers' grains with solubles (DDGS) were used as a carrier for treatments. Pens assigned to CON received 908 g/d of DDGS as a top-dress. For HyD Low, 43.58 g/d of HyD (6 mg of 25(OH)D_3 per 454 g) and 864.42 g of DDGS were mixed to make the supplement. Similarly, 87.17 g of HyD and 820.83 g of DDGS were combined to make the HyD High supplement. Treatment supplements were top-dressed immediately following feed delivery. All top-dress calculations were determined using 12 head pens and adjusted if heifers were removed from the experiment. On d 0, heifers were fed at 1.0 % of average pen BW (DM basis). The following day, feed bunks were assessed at 0630 h, and feed allocations were increased by 0.25% of BW daily (DM basis) if the bunk was slick, this strategy continued until a targeted feed intake of 2.2% of BW daily (DM basis) was reached. Pen weights were measured on d 0, 14, 21, 28, 35, 42, 49, and 56 using a pen scale (Rice Lake Weighing Systems; Rice Lake, WI) to calculate weekly feed calls. A sample of each top dress treatment and both supplements were analyzed for vitamin D_3 concentration before the start date of the trial. Analysis was performed by dsm-firmenich Technical Marketing Analytical Services laboratory (TMAS; Belvidere, NJ). Individual feed ingredient samples were collected weekly to determine DM and composition. A small aliquot of each ingredient sample was dried in a forced-air oven at 105°C for approximately 48 h to determine ingredient DM. The remaining sample was frozen at -20 °C. Following the completion of the experiment, feed ingredient samples were composited and sent to a commercial laboratory (SDK Laboratories, Hutchinson, KS) for nutrient analysis. Table 2.2 represents the experimental diet nutrient composition.

On arrival (d 0), all heifers were weighed (Silencer; Moly Manufacturing Inc., Lorraine, KS), tagged with a pen identifying number, vaccinated for respiratory (Titanium 5; Elanco, Greenfield, IN) and clostridial diseases (Ultrachoice 7; Zoetis, Parsippany, NJ), and treated for internal (Valbazan; Zoetis, Parsippany, NJ) and external parasites (Clean Up II; Elanco, Greenfield, IN). All heifers were bled via jugular puncture to assess vitamin D status after the trucking event took place. All whole blood samples that were collected from heifers via jugular puncture prior to transport (d -1), on arrival (d 0), and at d 14, 31, and 60 of the treatment periods were obtained using an 18-gauge needle (VACUETTE® Multiple Use Drawing Needle; Greiner Bio-One, Kremsmünster, Austria) and a 10-mL additive-free evacuated blood tube (Ideal® Blood Collection Tube, Plain; Neogen, Lansing, MI). Whole blood was allowed a minimum of 30 min to coagulate before it was centrifuged at 1,500 × *g* for 12 min. Serum was then transferred into 2-ml cryovials with a transfer pipette and frozen at -20 °C until analyzed. Analysis for 25(OH)D_3 was performed by dsm-firmenich TMAS using High Performance Liquid Chromatography (HPLC) coupled with tandem mass spectrometry (MS).

Experiment 2. Seasonal variation in vitamin D status in a commercial feedlot

Two commercial feedyards located in western Kansas participated in an experiment from June 2023 to April 2024 to evaluate vitamin D status of steers and heifers at four different seasonal timepoints. All cattle were housed in outdoor pens without shade and fed a common finishing diet comprised of steam-flaked and high-moisture corn. Liquid supplement was formulated to meet NRC requirements for finishing cattle and provided 275 IU of vitamin D_3 per kg dietary dry matter. Cattle were stratified by odd or even lot numbers and received 1 of 2 treatments. All even numbered cattle lots received 1 mg per animal per day of HyD **(HyD)** and

all odd numbered cattle lots received no supplemental HyD (**CON**). Treatments were applied for a minimum of 60 d prior to the first blood collection.

Whole blood samples (n = 603) were collected from the jugular vein at the time of terminal reimplant (approximately 70 d before slaughter) from 2 randomly selected pens per treatment (150 to 200 cattle per pen, with samples collected from ~10% of each pen) at each of the four time points: summer (June/July 2023), fall (October 2023), winter (January 2024), and spring (April 2024). Hide pigmentation data was recorded at the same time blood collection took place. Hide pigmentation was determined based upon hide hair color and nose pigmentation and was categorized as either black, red, or white. Whole blood samples were allowed to clot before being centrifuged at 1,500 x g for 12 min. Serum was decanted into 2-ml cryovials with transfer pipette, frozen at -20 °C, and shipped to TMAS for 25(OH)D_3 analysis. Serum samples were analyzed using an HPLC coupled with tandem MS.

Experiment 3. A nutrikinetic evaluation of pulse-dosed calcidiol

Eight cannulated, crossbred, yearling beef heifers (initial body weight (BW) 289 ± 44.5 kg) were used for the evaluation of a single pulse dose of calcidiol solution (HyD®). This trial took place from October 9, 2023, through December 18, 2023. Heifers were randomly assigned to 1 of 2 treatments: 3 mg calcidiol per 272 kg BW or 5 mg of calcidiol per 272 kg BW (n = 4 per treatment). Dose concentrations were based on safety trial data from Celi et al., (2018). Heifers were weighed on d 0 to calculate the calcidiol dose. At that time, jugular venipuncture with an 18-gauge blood-collection needle (VACUETTE® Multiple Use Drawing Needle; Greiner Bio-One) and 8.5 mL serum-separator blood tubes (BD Vacutainer SST; Becton Dickinson, Franklin Lake, NJ) was used to collect a blood sample for baseline analysis of circulating serum 25(OH)D_3 concentrations.

Following the initial blood collection, calcidiol treatments were administered via a ruminal cannula using a syringe and 12-gauge plastic tubing. Following the dose administration, the line was flushed with 35 mL of distilled water and 35 mL of air. To measure serum 25(OH)D_3 concentration for the nutrikinetic evaluation, serial blood samples were collected at 2, 4, 6, 8, 12, and 24 h and 2, 3, 5, 7, 10, 14, 21, 28, 35, 42, 49, 56, 63, and 70 d relative to dosing. In each case, serum was separated by centrifugation at 1,500 × g for 12 min and then decanted into 2-mL cryovials using a transfer pipette. Samples were frozen at -20 °C until analysis. Serum samples were analyzed by TMAS using an HPLC coupled with tandem MS to analyze the serum samples for 25(OH)D_3 concentration. Heifers received no added vitamin D_3 in the diet during the experiment.

Changes in serum 25(OH)D_3 concentrations from baseline over time after calcidiol administration for each animal were analyzed by noncompartmental analysis using Phoenix WinNonlin (version 8.3.5.340, Certara USA inc., Princeton, NJ). The maximum observed change in serum concentration from baseline reached (C_{Max}) and time to reach maximum observed change in serum concentration (T_{Max}) were determined directly from the change in serum 25(OH)D_3 versus time data. The terminal half-life ($t_{1/2}$) and terminal rate constant (λ_z) were determined with log-linear regression from the terminal portion of the concentration-versus-time plots using at least three time points. The area under the curve from time 0 to the last time point that measured above baseline concentration, post dose ($AUC_{0\text{-last}}$) was calculated using the log-linear trapezoidal method. The summary pharmacokinetic data for each dose group, except the time to reach maximum concentration (T_{Max}) are presented as geometric mean and range. Because T_{Max} is a categorical variable, it is presented as the median and range. The change

in serum 25(OH)D_3 concentration from baseline-versus-time profiles are presented as arithmetic mean ± SD.

Simulations of changes in 25(OH)D_3 serum concentration from baseline versus time profiles following various dosing scenarios were performed with the Nonparametric Superposition (NPS) feature within WinNonlin using the individual profiles from the single-dose study. The NPS tool assumes each drug dose acts independently, and that linear pharmacokinetics apply within multiple doses, meaning that the rate and extent of absorption as well as total drug clearance do not change with changes in dose or dosing interval. Simulations examined the change in steady state 25(OH)D_3 serum concentrations from baseline following one or two pulse-dose applications of calcidiol at 3 or 5 mg, in combination with or without daily dietary supplementation of calcidiol at 0.5 or 1 mg. These simulations were based on common management practices of high-risk receiving cattle. Simulations took place over a 56-d time frame to mimic a receiving period. Oral drenches were administered 14 d apart to mimic the timing of administration during initial processing and at revaccination.

Statistical analysis

In Exp. 1, data were analyzed using the MIXED procedure of SAS (version 9.4; SAS Inst. Inc., Cary, NC). Pen was the experimental unit, and animal was the observational unit. Fixed effects included day, treatment, and their interaction (day × treatment). Least square means were calculated, and pairwise t-tests were used to identify significant differences between treatment groups at each time point. Only animals that completed the trial were used in the serum analysis.

In Exp. 2, data were analyzed using the MIXED procedure of SAS. Animal was considered the experimental unit. The model included treatment, sex, season, and hide

pigmentation and all possible interactions as fixed effects, and pen was included in the model as a random effect.

In Exp. 3, to determine if dose proportionality (linear pharmacokinetics) existed between the two doses, dose-normalized $AUC_{0\text{-last}}$ and C_{Max} for the two doses were analyzed for linearity using the Sapiro-Wilk test and then two-tailed Student's t-tests using SigmaPlot (version 14.5, Grafiti LLC, Palo Alto, CA).

For all experiments, significance threshold was set at $P \leq 0.05$ and tendencies were considered at $P \leq 0.10$.

Results and Discussion

Experiment 1. Calcidiol supplementation in high-risk receiving heifer calves

Prior to transport (d -1), serum $25(OH)D_3$ concentration averaged 18 ± 10.9 ng/mL. Following transport (d 0), serum $25(OH)D_3$ concentration averaged 13 ± 9.7 ng/mL. Serum $25(OH)D_3$ concentration < 20 ng/mL was considered suboptimal for calcium and phosphorus homeostasis and overall bone health (Nelson and Merriman, 2014). In addition, serum $25(OH)D_3$ levels <5 ng/mL has been reported as deficient, and cattle are at risk of displaying clinical signs of vitamin D deficiency (Weiss, 1998). Interestingly, there was a 5 ng/mL difference in average heifer serum $25(OH)D_3$ level in pre-transport to post-transport, indicating that oxidative stress, feed and water withholding, and other factors that occur during transport may play a role in vitamin D depletion. Furthermore, consideration to the time of year when this study took place could be considered as a contributor towards low vitamin D status. According to Casas et al. (2015), calves in winter months had lower vitamin D status compared to calves in summer months.

On d 14, CON and D3 cattle averaged 13 ± 10.4 ng/mL and 16 ± 8.4 ng/mL, respectively, whereas HyD Low and HyD High cattle serum concentrations were 44 ± 19.7 ng/mL and 85 ± 28.4 ng/mL, respectively. Figure 2.1 represents the difference between heifers supplemented with HyD compared to CON and D3 cattle with HyD having greater ($P < 0.001$) serum 25(OH)D_3 concentrations at d 14, 31, and 60 compared with heifer fed CON or D3 treatments. HyD High cattle had greater ($P < 0.001$) serum 25(OH)D_3 compared to HyD Low at d 14, 31, and 60. Heifers on CON and D3 treatments were not different ($P = 0.82$) at d 14; however, at d 31, D3-fed heifers tended to have greater ($P = 0.10$) serum concentrations 25(OH)D_3 than CON. At d 60 sampling, serum 25(OH)D_3 concentrations for CON and D3 cattle were not different ($P = 0.85$). Exposure to UV radiation from sunlight increases during the spring season; therefore, it was presumed that vitamin D_3 supplementation had a similar effect to sunlight exposure alone. Overall, HyD elevated vitamin D status during a 60-d receiving period. Further research is needed to evaluate optimal vitamin D status and investigate effects of calcidiol supplementation on the health and growth performance of newly received calves.

Experiment 2. Seasonal variation of vitamin D status in a commercial feedlot

For CON cattle, serum 25(OH)D_3 was greatest during summer (108 ± 22.6 ng/mL; $P < 0.01$), intermediate during spring and fall (61 ± 15.3 and 59 ± 24.5 ng/mL, respectively; $P = 0.85$), and least during winter (35 ± 6.4 ng/mL; $P < 0.01$). In all seasons, HyD supplementation produced greater ($P < 0.01$) serum 25(OH)D_3 than non-supplemented cattle. For cattle receiving HyD, serum 25(OH)D_3 was 196 ± 58.0, 258 ± 54.3, 183 ± 40.4 and 184 ± 31.7 ng/mL in spring, summer, fall, and winter, respectively. Serum 25(OH)D_3 concentrations differed among the four seasonal time points ($P < 0.001$). Similar observations have been observed in the dairy population by Hymøller and Jensen (2012) and young beef population by Casa et al. (2015).

In Figure 2.2, average daylength during season of blood sample collection shows a similar pattern to vitamin D status of CON cattle. Sex did not affect ($P = 0.40$) serum 25(OH)D_3 nor did it interact with the seasonal response ($P = 0.42$); however, there was an interaction of sex, treatment, and season (Figure 2.3). Heifer serum levels were greater than those of steers in all seasons with the exception of fall; however, it is unclear why this response was observed. Table 2.3 shows the effects of hide pigmentations on serum 25(OH)D_3 concentration across all cattle and timepoints. Red-hided cattle had greater ($P < 0.01$) serum 25(OH)D_3 concentrations than black- and white-hided cattle. Hide pigmentation may influence cattle's ability to synthesize vitamin D from sun exposure. Skin pigmentation often affects the human population's vitamin D status; more UV exposure is needed for people with darker pigmented skin to reach serum 25(OH)D_3 concentrations similar to those of lighter pigmentated people (Sawicki et al., 2016). Dietary vitamin D supplementation may need to be adjusted throughout the year to maintain adequate circulating vitamin D concentrations, but more research is needed to determine the vitamin D status required for optimum health and performance of feedlot cattle.

Experiment 3. A nutrikinetic evaluation of pulse-dosed calcidiol

The data presented in Table 2.4 show that the maximum concentration following the calcidiol pulse dose was reached in 48 h. The half-life ($t_{1/2}$) of calcidiol averaged 7.1 d. Serum 25(OH)D_3 concentrations remained above baseline following calcidiol pulse dose for approximately 32 d for the 3-mg treatment and 40 d for the 5-mg treatment (Figure 2.4). The trial timeline, combined with the lack of supplemental vitamin D_3 in the diet, allowed vitamin D status to drop below baseline. Lack of sun exposure as the season moved through late fall during this experiment also likely contributed to the decline in vitamin D status of the cannulated heifers.

The half-life we determined from the single administration studies was used for the simulations. In Figure 2.5, a simulation of two 5.0 mg per 272 kg BW calcidiol pulse doses administered 14 days apart without additional dietary calcidiol supplementation indicated an initial change in serum 25(OH)D_3 concentration of 37.0 ng/mL at 47 h after administration. After the second pulse dose administration, a max change in serum 25(OH)D_3 concentration of 54.2 ng/mL was reached at 411 h post-initial dose. Serum levels were predicted to return to baseline by d 56. This simulation demonstrated not only the initial changes in serum concentrations of 25(OH)D_3, but also that to maintain a greater vitamin D status, daily supplementation is likely necessary.

Figure 2.6 demonstrates the predicted changes in serum 25(OH)D_3 when an oral administration of calcidiol is dosed at 5.0 mg per 272 kg BW followed by daily supplementation of 1.0 mg calcidiol per 272 kg BW through the feed. An initial increase in serum 25(OH)D_3 concentration of 47.5 ng/mL is predicted at 47 h after administration. Around h 336, serum 25(OH)D_3 concentrations reach 88.6 ng/mL. By h 871, serum levels are predicted to reach a steady state at ~108 ng/mL above baseline for the remainder of the 56-d simulation. Although change in serum level reached over 100 ng/mL, it took approximately 4 wk to achieve that status, which may be too long when trying to elevate vitamin D status for health-challenged cattle on arrival.

In Figure 2.7, simulations of two oral administrations of calcidiol dosed at 5.0 mg per 272 kg BW on d 0 and d 14 with daily supplementation of 1.0 mg calcidiol per 272 kg BW through the feed show the potential change in serum 25(OH)D_3 over a 56-d period. An initial change in serum 25(OH)D_3 concentration of 48.5 ng/mL is observed 48 h after administration. After the second pulse dose administration, a maximum change in serum 25(OH)D_3 concentration of 129.9

ng/mL is observed at 398 h. By h 750, serum levels reached a steady state at ~120 ng/mL above baseline for the remainder of the 56-d simulation. Administration of two oral doses of calcidiol at 5.0 mg with daily feeding of calcidiol at 1.0 mg resulted in a rapid and sustained increase in serum $25(OH)D_3$. This simulation projects an efficient way to achieve optimal vitamin D status to target an immune response.

These simulations do not account for many factors that can affect vitamin D status such as age, seasonal variations, and baseline $25(OH)D_3$ concentrations. These modeled responses should be confirmed *in vivo*, especially in the target population of high-risk cattle. Administering a high dose of calcidiol orally to high-risk receiving cattle after arrival would help boost their vitamin D status, with the ultimate goal of priming the immune system. The oral drench method is a reliable way to ensure cattle receive calcidiol supplementation promptly. Calcidiol supplementation through the diet may not allow cattle to obtain the necessary dose because of poor intakes that occur early in the receiving period.

Implications

These studies were designed to further understand factors influencing vitamin D status in beef cattle. Calcidiol supplementation is currently the most effective means to elevate circulating serum $25(OH)D_3$ concentrations in beef and dairy cattle. Because it was observed that vitamin D status changed across season and may be attributed to day length, a more defined calcidiol supplementation program may be beneficial to elevate vitamin D status of beef cattle during winter months. More research is needed to further define optimal vitamin D status in beef cattle and to better understand the role it plays in immune function, growth and development.

Acknowledgement

This research was funded by dsm-firmenich.

Literature Cited

Bechdel, S. I., N. W. Hillston, and N. B. Guerrant. 1937. The vitamin D requirement (U.S.P. units) for growth and well-being of calves from birth to six months of age. J. Dairy Sci. 20:432–433.

Casas, E., J. D. Lippolis, L. A. Kuehn, and T. A. Reinhardt. 2015. Seasonal variation in vitamin D status of beef cattle reared in the central United States. Domest. Anim. Endocrinol. 52:71–74. doi:10.1016/j.domaniend.2015.03.003

Celi, P., S. Williams, M. Engstrom, J. McGrath, and J. La Marta. 2018. Safety evaluation of dietary levels of 25-hydroxyvitamin D3 in growing calves. Food Chem. Toxicol. 111:641–649. doi:10.1016/j.fct.2017.11.053

Hollis, B. W., and R. L. Horst. 2007. The assessment of circulating 25(OH)D and 1,25(OH)2D: where we are and where we are going. J. Steroid Biochem. Mol. Biol. 103:473–476. doi:10.1016/j.jsbmb.2006.11.004

Hymøller, L., and S. K. Jensen. 2012. 25-hydroxycholecalciferol status in plasma is linearly correlated to daily summer pasture time in cattle at 56 degrees N. Br. J. Nutr. 108:666–671. doi:10.1017/S0007114511005964

NASEM. 2016. Nutrient requirements of beef cattle. 8th ed. Natl. Acad. Press, Washington, DC.

Nelson, C. D., T. A. Reinhardt, J. D. Lippolis, R. E. Sacco, and B. J. Nonnecke. 2012. Vitamin D signaling in the bovine immune system: A model for understanding human vitamin D requirements. Nutrients 4:181–196. doi:10.3390/nu4030181

Nelson, C. D., and K. E. Merriman. 2014. Vitamin D metabolism in dairy cattle and implications for dietary requirements. Proc. 25th Florida Ruminant Nutr. Symp. p. 78–90.

Nelson, C. D., J. L. Powell, D. M. Price, M. J. Hersom, J. V. Yelich, M. E. Drewnoski, S. L.

Bird, and G. A. Bridges. 2016. Assessment of serum 25-hydroxyvitamin D concentrations of beef cows and calves across seasons and geographical locations. J. Anim. Sci. 94:3958–3965. doi:10.2527/jas.2016-0611

Poindexter, M. B., M. F. Kweh, R. Zimpel, J. Zuniga, C. Lopera, M. G. Zenobi, Y. Jiang, M. Engstrom, P. Celi, J. E. P. Santos, and C. D. Nelson. 2020. Feeding supplemental 25-hydroxyvitamin D_3 increases serum mineral concentrations and alters mammary immunity of lactating dairy cows. J. Dairy Sci. 103:805–822. doi:10.3168/jds.2019-16999

Sawicki, C. M., M. I. Van Rompay, L. E. Au, C. M. Gordon, and J. M. Sacheck. 2016. Sun-exposed skin color is associated with changes in serum 25-hydroxyvitamin D in racially/ethnically diverse children. J. Nutr. 146:751–757. doi:10.3945/jn.115.222505

Figures

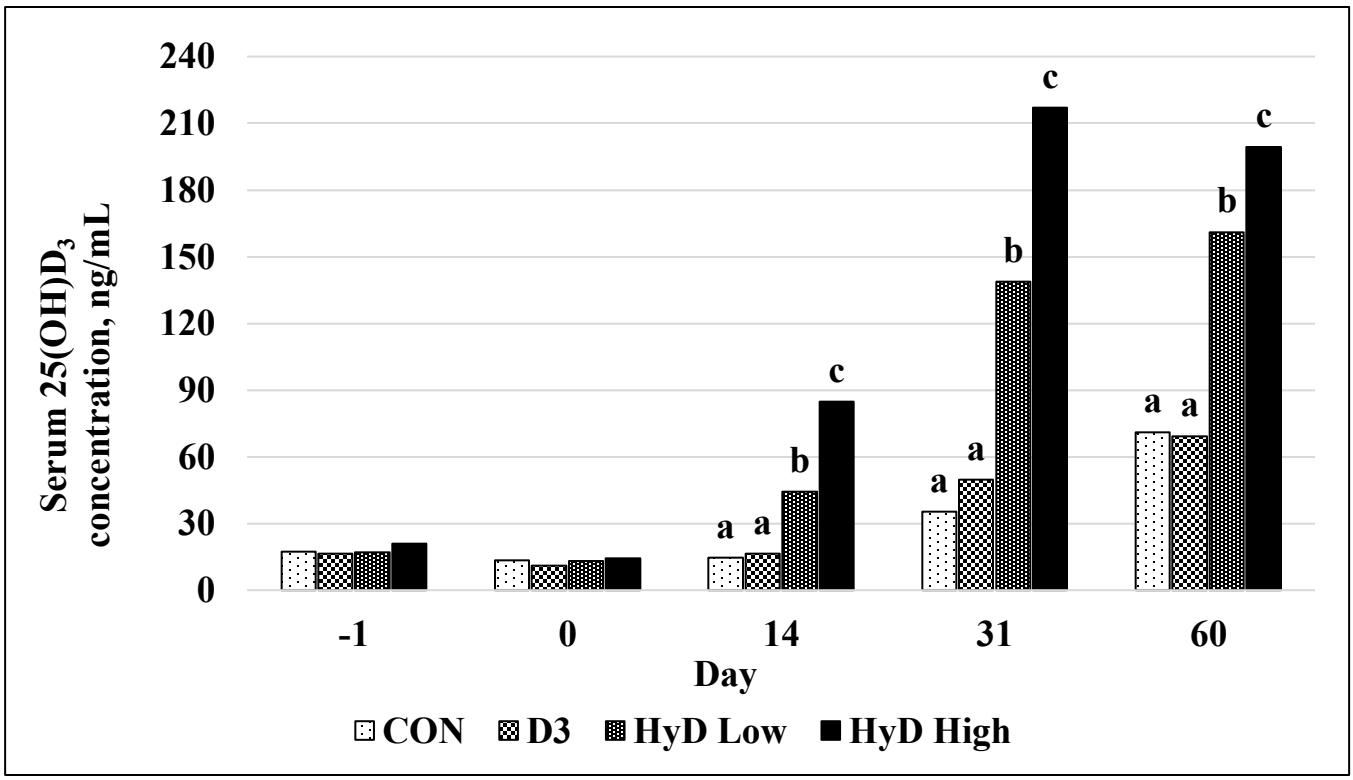

Figure 2.1. Effect of various vitamin D_3 and calcidiol supplementation strategies on serum 25(OH)D_3 concentration through a 60-d receiving period with high-risk heifer calves.
Treatment ($P > 0.001$); Day ($P > 0.001$); Treatment × Day ($P > 0.001$); SEM = 6.39.

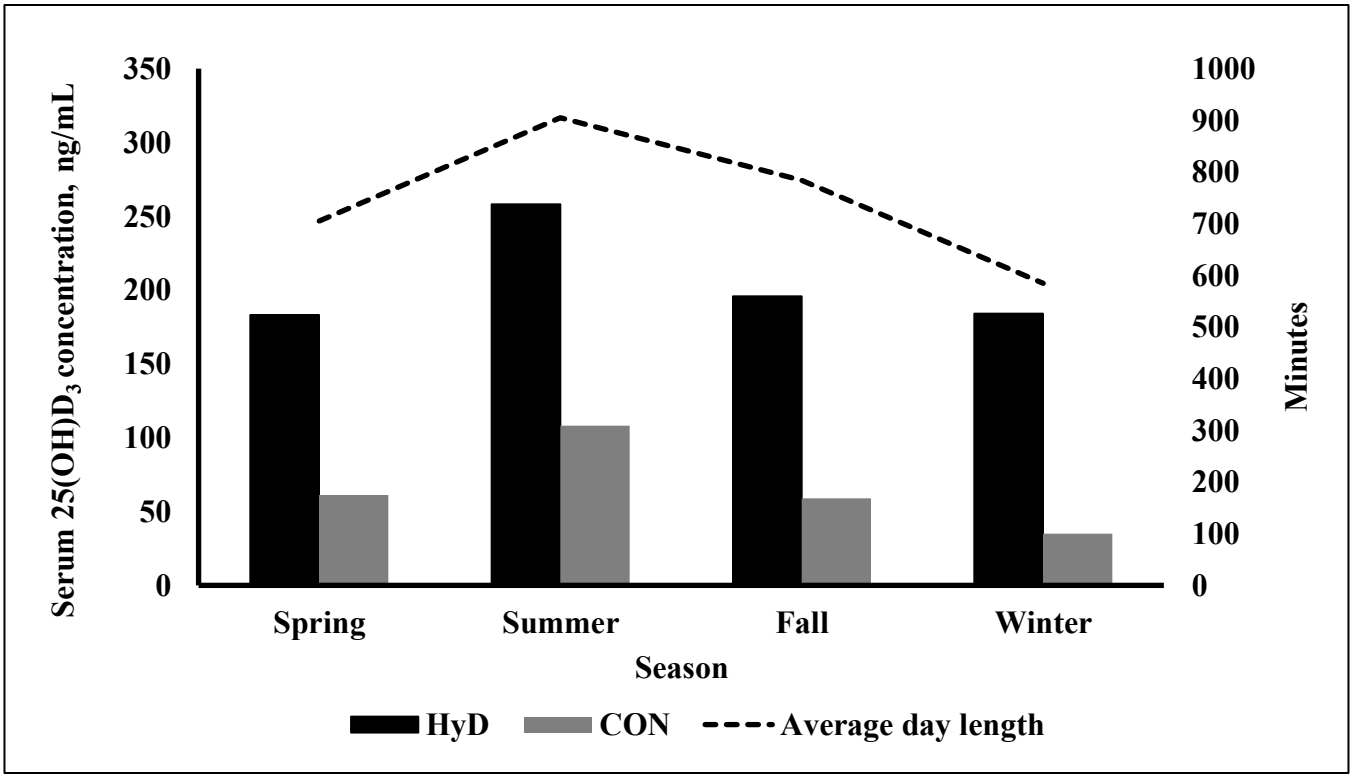

Figure 2.2. Effect of vitamin D_3 and calcidiol supplementation compared to average day length exposure across seasons in a commercial feedlot in western Kansas at the time of terminal implant. Season ($P < 0.001$); Treatment ($P < 0.001$); Season × Treatment ($P = 0.0076$); SEM = 4.74.

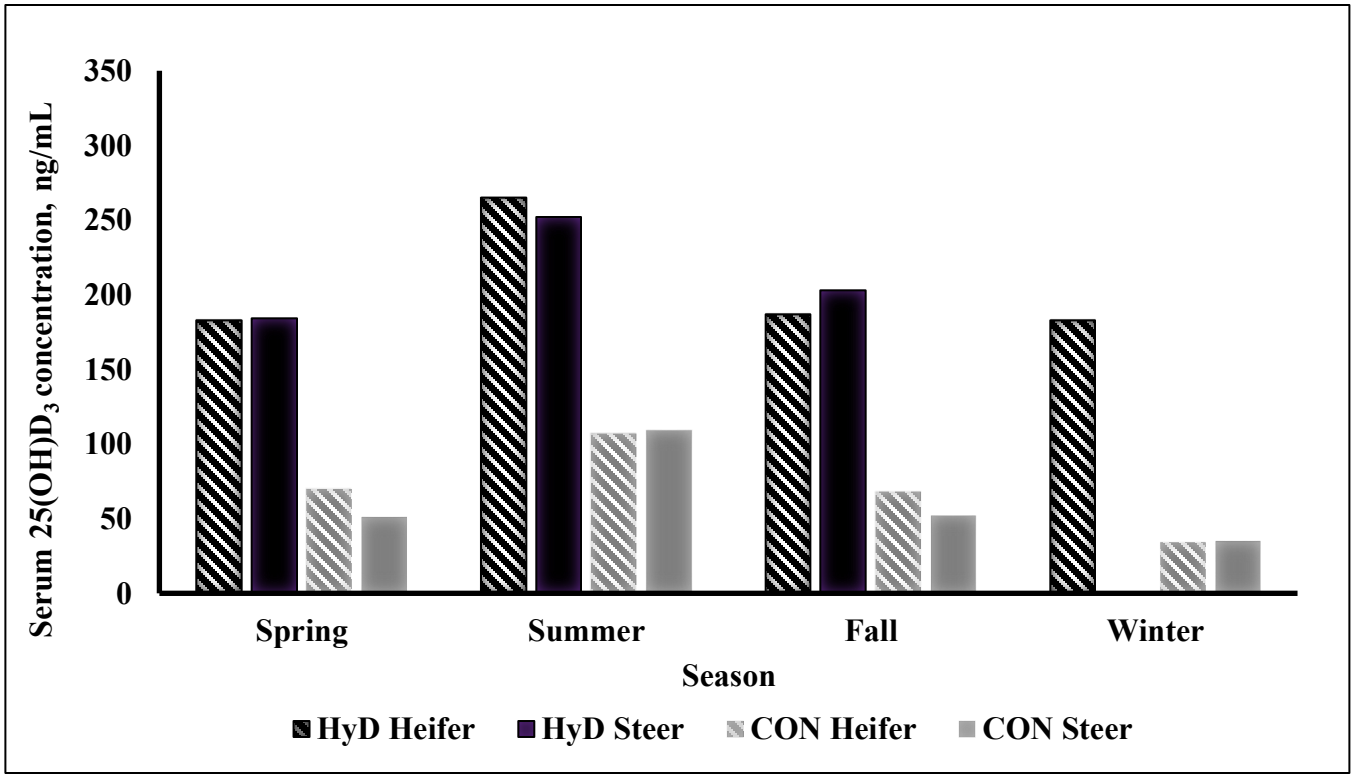

Figure 2.3. Effect of sex by treatment on 25(OH)D$_3$ serum concentration across season in a commercial feedlot in western Kansas at the time of terminal implant Sex ($P = 0.40$); Season ($P < 0.001$); Treatment ($P < 0.001$); Sex × Treatment ($P = 0.09$); SEM = 6.69. *No data was collected for winter HyD steers.

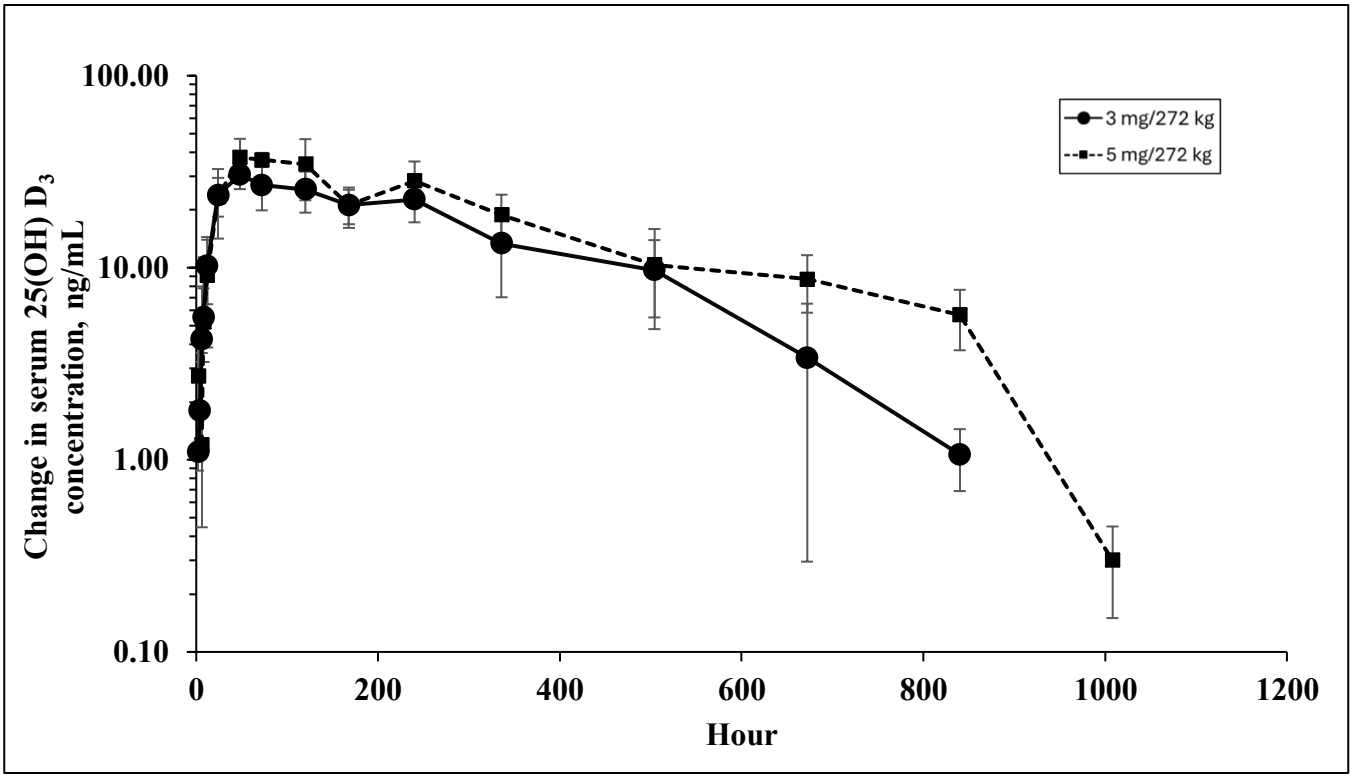

Figure 2.4. Change in serum 25(OH)D_3 concentration from baseline from d 0 to d 33 when a 3 or 5 mg pulse dose was administered on d 0 via ruminal cannula in fistulated yearling heifers.

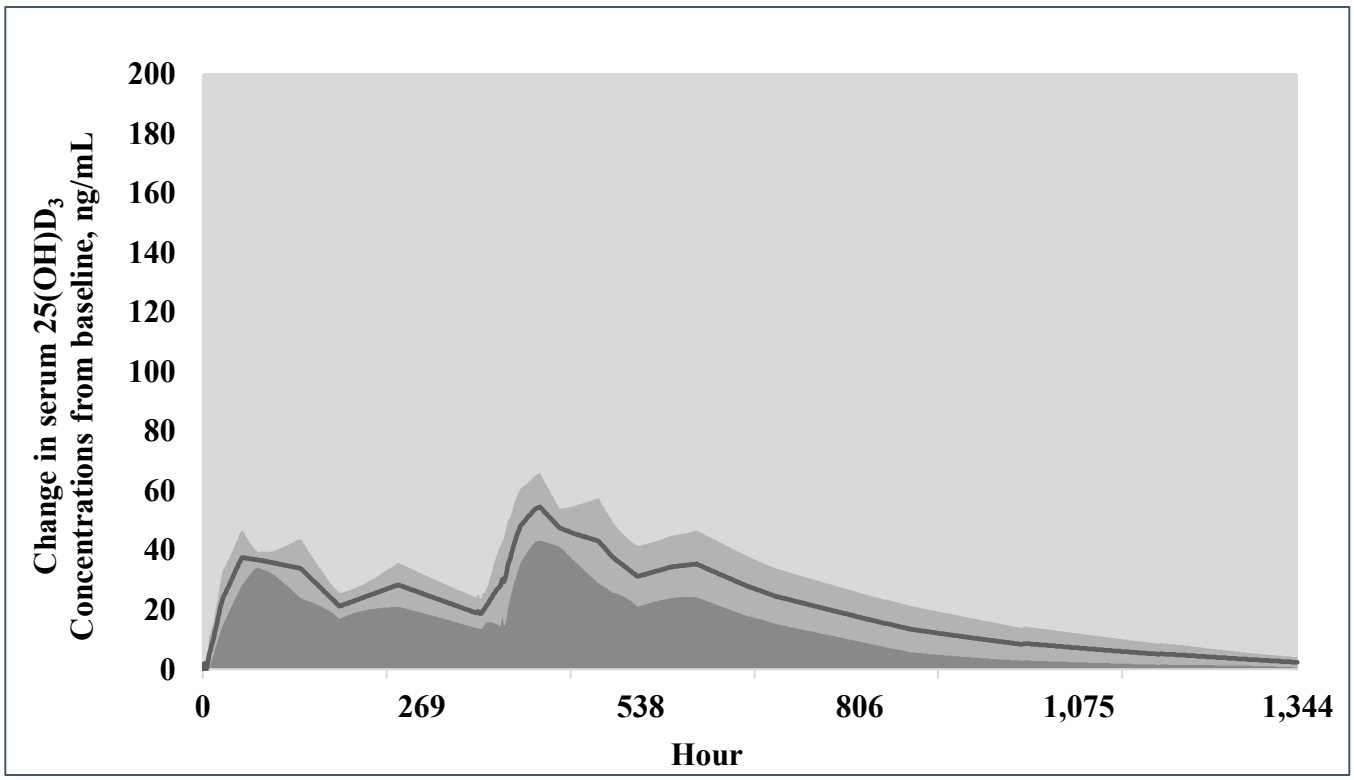

Figure 2.5. Nutrikinetic model simulation representing two separate 5 mg per 272 kg BW calcidiol pulse doses 14 days apart without any supplementation of calcidiol through the feed.

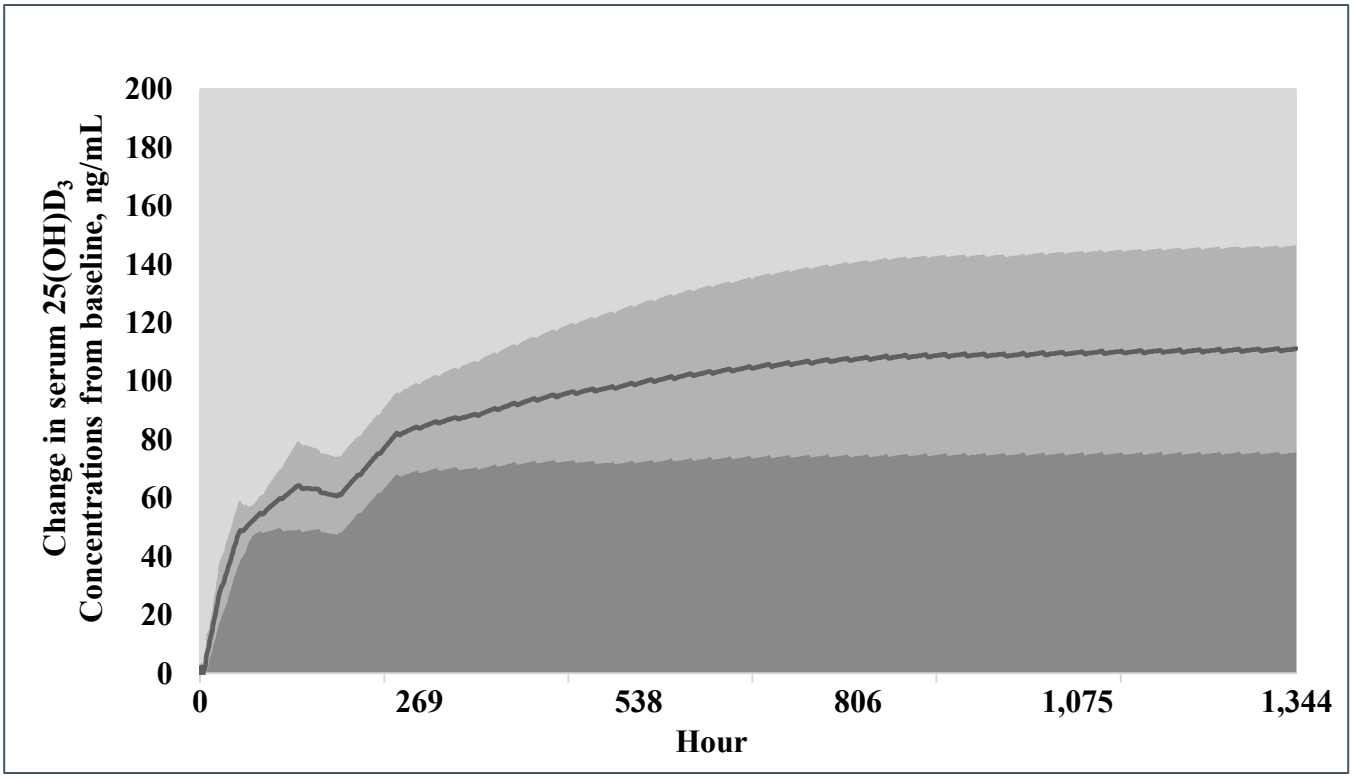

Figure 2.6. Nutrikinetic model simulation representing one 5 mg per 272 kg BW calcidiol pulse dose on day 0 with the addition of 1.0 mg per day of supplemental calcidiol through the feed.

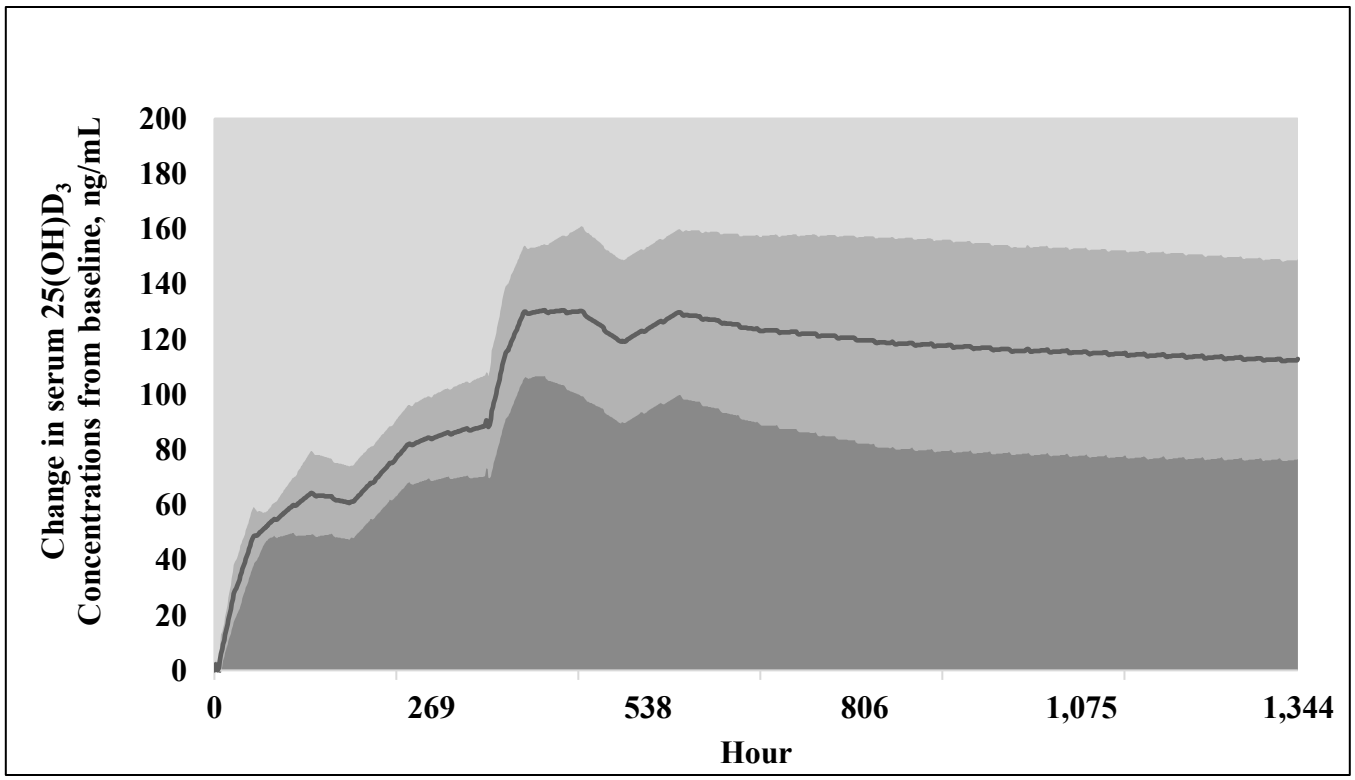

Figure 2.7. Nutrikinetic model simulation representing two separate 5 mg per 272 kg BW calcidiol pulse doses 14 days apart with the addition of 1.0 mg per day of supplemental calcidiol through the feed.

Tables

Table 2.1 Composition of dietary treatments fed to high-risk crossbred heifers used to evaluate vitamin D status.

	Treatment[1]			
Item	CON	D3	HyD Low	HyD High
Ingredient, % DM				
Dry-rolled corn	39.5	39.5	39.5	39.5
Supplement[2]	-	7.5	-	-
Supplement[3]	7.5	-	7.5	7.5
Wet corn gluten feed[4]	40.0	40.0	40.0	40.0
Warm-season grass hay	13.0	13.0	13.0	13.0

[1] No added vitamin D_3 or calcidiol (CON), 0.075 mg of vitamin D_3 (D3), 0.5 mg of calcidiol (HyD Low) or 1.0 mg calcidiol (HyD High).

[2]Supplement pellet formulated to contain (DM basis): 5,992,800 IU vitamin D_3/ton, 11.5% crude protein, 0.60% phosphorus, 4.7% salt, 0.80% potassium, 2.5% fat, and 307.2 g/ton monensin (Rumensin; Elanco, Greenfield, IN) and 307.2 grams/ton monensin (Rumensin 90; Elanco, Greenfield, IN)

[3] Supplement pellet formulated to contain (DM basis); 11.5% crude protein, 0.60% phosphorus, 4.7% salt, 0.80% potassium, 2.5% fat, and 307.2 g/ton monensin (Rumensin; Elanco, Greenfield, IN)

[4] Sweet Bran, Cargill Animal Nutrition, Blair, NE.

Table 2.2 Nutrient composition of diets fed to high-risk crossbred heifers used to evaluate vitamin D status

	Treatment[1]			
Item	CON	D3	HyD Low	HyD High
Nutrient composition[2], % of DM				
DM, % as fed	76.08	76.11	76.08	76.08
CP	14.05	14.13	14.05	14.05
NDF	23.90	24.05	23.90	23.90
ADF	10.90	10.94	10.90	10.90
Ca	0.78	0.78	0.78	0.78
P	0.53	0.53	0.53	0.53
Mcal/kg[3]				
NE_m	1.95	1.95	1.95	1.95
NE_g	1.31	1.31	1.31	1.31

[1]No added vitamin D_3 or calcidiol (CON), 0.075 mg of vitamin D_3 (D3), 0.5 mg of calcidiol (HyD Low) or 1.0 mg calcidiol (HyD High).

[2]Nutrient analysis conducted by SDK Laboratories (Hutchinson, KS)

[3] Net energy of maintenance (NEm) and net energy of gain (NEg) were calculated using NASEM (2016) values of diet ingredients.

Table 2.3 Effect of hide pigmentation on serum 25(OH)D_3 concentration across all seasons in a commercial feedlot.

	Hide Pigmentation[1]				
Item	Red	Black	White	SEM[2]	*P*-value[3]
No. of Cattle[4]	74	491	38		
Serum 25(OH)D3,[5] ng/mL	137[a]	124[b]	119[b]	7.62	<0.001

[1] Hide pigmentation category was assigned by hair color and nose pigment. Red: pink or black nose pigment and red hair,

[2] Largest standard error of the mean.

[3] Treatment main effect.

[4] Cattle were randomly sampled within pen.

[5] Represents average values across all sample time points.

[a, b] Within row, means not bearing a common superscript letter differ ($P < 0.05$).

Table 2.4 Mean (range) of nutrikinetic parameters from non-compartmental analysis for change in serum 25-hydroxyvitamin D_3 concentrations of different pulse dose concentrations.

	Pulse Dose Calcidiol Concentration	
	3 mg	5 mg
Parameters, units	Mean (range)	Mean (range)
Dose, mg	3.83 (3.37-4.39)	5.52 (3.9-6.40)
$C_{Baseline}$, ng/mL	52.4 (45.1-67.3)	56.6 (47.1-63.7)
C_{Max}, ng/mL	31.6 (28.5-36.8)	40.3 (33.8-49.6)
Dose-normalized C_{max}[b], (ng/mL)/mg	2.87 (2.59-3.35)	2.19 (1.8-2.7)
T_{Max}[a], h	60 (24-120)	48 (48-72)
λ_z, 1/h	0.004 (0.001-0.006)	0.004 (0.003-0.009)
$t_{½}$, h	177 (108-497)	164.3 (74.6-247)
$AUC_{0\text{-last}}$, h·ng/mL	10,224 (8,876-12,181)	13,028 (8,786-19,037)
Dose-normalized $AUC_{0\text{-last}}$[b] (h·ng/mL)/mg	856 (581-1,107)	709 (479-1,036)

Mean = geometric mean; Dose = oral dose of 25(OH)D_3 in mg administered to achieve target dose; $C_{baseline}$ = baseline serum concentration of 25-hydroxyvitamin D_3 prior to dosing; C_{Max} = maximum observed change in serum concentration from baseline reached; Dose-normalized C_{Max} = C_{Max} normalized to a dose of 1 mcg/kg; T_{Max}[a] = time to reach maximum observed change in serum concentration; λ_z, terminal rate constant; $t_{½}$ = terminal half-life; $AUC_{0\text{-last}}$ = area under change in concentration-time curve time 0 to the last time point that measured above baseline concentration, post-dose; Dose-normalized $AUC_{0\text{-last}}$ = $AUC_{0\text{-last}}$ normalized to a dose of 1 mcg/kg

[a] Tmax is expressed as median with minimum and maximum

[b] *P* values were > 0.05 suggesting dose proportionality (linear pharmacokinetics) between two treatments; (Dose-normalized C_{max} = 0.06) (Dose-normalized $AUC_{0\text{-last}}$ = 0.49)

Chapter 3 - Effects of Calcidiol and Beta Carotene Supplementation on Newly Received High-Risk Heifers: Growth Performance, Health, Antibody Production, Acute Phase Proteins and Cytokines of Healthy and Morbid Animals

Introduction

Nutritional strategies aimed at preventing disease in beef cattle are gaining popularity. From direct-fed microbials, prebiotics and postbiotics to vitamins and minerals, preventive nutrition seeks to provide the necessary nutrients that aid the animal's overall health and growth performance. Vitamin and mineral nutrition in beef cattle has received relatively limited research attention compared to other areas, resulting in numerous unresolved questions regarding supplementation strategies. Vitamin D_3 (cholecalciferol) plays a fundamental role in calcium and phosphorus metabolism, skeletal development, and immune function in beef cattle (Wu, 2022). Although the importance of vitamin D_3 is well recognized, research on optimal supplementation strategies in beef cattle, especially during critical periods such as receiving and backgrounding remains limited. Most dietary recommendations are extrapolated from older data or adapted from dairy cattle Nelson et al. (2016), leaving questions about the specific needs and bioavailability of different vitamin D metabolites in beef systems. Recent interest in alternative forms, such as 25-hydroxyvitamin D_3 (calcidiol), has emerged due to its higher bioavailability and faster action in raising serum vitamin D concentrations. Unlike cholecalciferol, calcidiol is a metabolically active form of vitamin D that circulates in the bloodstream and is more readily utilized by tissues, offering potential advantages in rapidly improving vitamin D status in stressed or

immunocompromised cattle. Calcidiol has been fed in the swine and poultry industry for years. The safety and efficacy of calcidiol have been extensively researched to ensure its proper use in animals and their products intended for human consumption (Yarger et al., 1995; Celi et al., 2018; Sandoval et al., 2022).

Newly received growing cattle often face multiple stressors during the early feeding period, which compromises their immune function and typically results in reduced performance and increased morbidity (Hudson et al., 2020). Nutritional interventions that support immune modulation are necessary to help combat the impacts of stress on those animals. Calcidiol supplementation has been shown to enhance immune function in dairy cattle by modulating both innate and adaptive immune responses (Nelson et al., 2012; Poindexter et al., 2020). Given its superior bioavailability and ability to rapidly elevate circulating vitamin D levels, calcidiol may be a promising nutritional strategy to support immune competence in beef cattle, particularly during periods of stress and heightened disease risk. Thus, we investigated the effects of calcidiol and beta-carotene supplementation on growth performance, health and immune status of newly received high-risk beef heifers. Calcidiol supplementation (HyD®; DSM Nutritional Products, Plainsboro, NJ) was provided to high-risk heifers through daily top-dress application.

Materials and Methods

All procedures involving the use of animals were approved by the Kansas State University Institutional Animal Care and Use Committee (IACUC # 4650).

Feed intake, growth performance, and health

Four-hundred eighty high-risk heifer calves (BW = 227 ± 16.0 kg) were purchased at auction markets and assembled at an order-buying station in Dickson, TN and then shipped 1,086 km to the Kansas State University Beef Stocker Unit in Manhattan, KS. Heifers arrived in 5 separate truckloads over an 8-d period from October 4 to October 11, 2023 and were used in a randomized complete-block design to evaluate the effects of calcidiol supplementation on growth performance, health, and immune status during a 56-d receiving period. Calves were blocked by truckload (5), stratified by individual arrival weight within each block, and assigned to pens containing 12 heifers. Pens within each block were randomly assigned to 1 of 4 treatments, resulting in10 pens per treatment for a total of 40 pens. The pens were soil-surfaced and of equal size (9.1 x 15.2 m). Concrete fence-line bunks were 9.1 m long and set on a 3.6 m apron.

Experimental diets (Table 3.1) were formulated to provide 0.075 mg (3,000 IU) per heifer per day of vitamin D_3 (D3), 0.5 mg of calcidiol per heifer per day (HyD Low), 1.0 mg calcidiol per heifer per day (HyD High), or 1.0 mg calcidiol plus 100 mg beta-carotene per heifer per day (HyD + BC). All receiving diets were corn-based and formulated to provide 1.31 Mcal of NE_g/kg of DM and consisted of 39.5% whole-shelled corn, 40.0% wet corn gluten feed (Sweet Bran; Cargill Animal Nutrition, Blair, NE), 13.0% ground, warm-season grass hay, and 7.5% pelleted supplement. Diets were limit-fed at 2.2% of BW daily (DM basis). Treatments were top-dressed immediately following feed delivery. Dried distillers' grains with solubles (DDGS) were used as

a carrier for calcidiol supplementation. Pens assigned to CON received 908 g/d of DDGS as a top dress. For HyD Low, 43.58 g/ of HyD (62.5 mg of active 25(OH)D_3 per 454 g) and 864.42 g of DDGS were mixed to make the supplement. Similarly, 87.17 g of HyD and 820.83 g of DDGS were combined to create the HyD High supplement.

At the time of arrival, heifers were individually weighed (Silencer; Moly Manufacturing Inc., Lorraine, KS), given a visual and electronic individual- identification ear tag and assessed for disease and lameness. Each animal was ear-notched and the skin samples from the ear were refrigerated at 4.4°C. After each truckload of cattle was processed, ear-notch samples were taken to the Kansas State University Veterinary Diagnostics Laboratory to identify animals persistently infected with Bovine Viral Diarrhea using a commercially available kit (VetMAX™ - Gold BVDV PI Detection Kit Applied Biosystems, Thermo Fisher Scientific, Austin, TX). Four animals tested positive (2- HyD Low and 2 - HyD High) and were removed from the experiment. Animals not displaying disease or lameness were assigned to 1 of 40 pens (12 heifers per pen). Each pen was provided ground warm-season grass hay at 1% of collective body weight on a DM basis and was given *ad libitum* access to water.

The following day (d 0), calves were weighed individually, treated for internal (Safeguard; Merck Animal Health, Madison, NJ) and external (Clean-Up II; Elanco, Greenfield, IN) parasites, vaccinated for respiratory (Titanium 5; Elanco, Des Moines, IA) and clostridial (Bovilus Vison 7 w/ somnus; Merck Animal Health, Madison, NJ) diseases, provided metaphylaxis treatment (Tulieve; Norbrook, Newry, Ireland), and tagged with a pen number. When processing was complete, heifers were allocated to their respective treatment pens. Heifers were revaccinated on d 14 for respiratory (Titanium 5; Elanco, Des Moines, IA) diseases.

Heifers were fed each day at 0700 h using a Roto-Mix feed wagon (model 414-14B; Roto-Mix, Dodge City, KS). Feed bunks were evaluated each day at 0630 h. If feed refusals were present, they were collected prior to feeding. On d 0, heifers were fed at 1.0 % of pen BW (DM basis). The following day, feed allocations were increased by 0.20% of BW daily (DM basis) if the bunk was slick(no feed remaining in the bunk); this strategy remained until the target feed call of 2.2% of BW per day (DM basis) was reached. All cattle reached 2.2% of BW daily within 7 to 10 d. Pen weights were measured biweekly (d 0, 14, 28, 42, and 56) using a pen scale (Rice Lake Weighing Systems; Rice Lake, WI) and were used biweekly to adjust feed calls. Individual feed ingredient samples were collected weekly and dried in a forced-air oven at 105°C for ~ 48 h to determine ingredient DM. The remaining sample was frozen at -20 °C. Following the completion of the experiment, feed ingredient samples were composited and sent to a commercial laboratory (SDK Laboratories, Hutchinson, KS) for nutrient analysis (Table 3.2).

All heifers were observed once daily in the pen for clinical signs of illness which manifested as general depression, nasal or ocular discharge, and reduced appetite. Any animal displaying these symptoms was removed from the pen and treated at the hospital facility where a rectal temperature was measured, and clinical illness score was recorded. Clinical illness scores (CIS) were as follows: 1 = normal, healthy animal; 2 = slightly ill with mild depression; 3 = moderately ill with severe depression, labored breathing, and nasal discharge; and 4 = severely ill and near death. Heifers with rectal temperatures ≥ 40 °C along with a CIS ≥ 2 or with a rectal temperature ≤ 40 °C and CIS ≥ 3 were treated for bovine respiratory disease following drug-label instructions. At first morbidity, animals received florfenicol (Norfenicol®; Norbrook, Newry, Ireland), at second enrofloxacin (Enroflox® 100; Norbrook), and at third oxytetracycline

(Noromycin® 300; Norbrook). Heifers requiring a third treatment were declared chronic and removed from the experiment.

Blood serum and plasma analysis

Whole blood samples were collected from heifers on arrival (day 0) and on days 14, 28, and 56 to evaluate serum 25(OH)D_3 and beta carotene concentration, antibody titers for IBR, BVD-I and BVD-II, and plasma haptoglobins. A subset of 60 animals from each dietary treatment (6 from each pen) were randomly selected after initial processing (day -1) to serve as observational measures for serum 25(OH)D_3, serum total carotenoid concentrations, and antibody titer analysis through the duration of the study. Blood samples were collected from all heifers enrolled in the study to measure haptoglobin concentrations.

All heifers were bled via jugular venipuncture using 18-gauge needles (VACUETTE® Multiple Use Drawing Needle; Greiner Bio-One, Kremsmünster, Austria). Whole blood was collected into an 8.5 mL serum-separator blood tube (BD Vacutainer SST; Becton Dickinson, Franklin Lake, NJ) to analyze serum 25(OH)D_3 and total carotenoid concentrations and antibody titers. After collection, whole blood samples destined for serum were allowed to clot for a minimum of 30 minutes before being separated by centrifugation at 1,500 × g for 12 minutes. They were then placed into a 2 mL cryovial with a transfer pipette. Whole blood samples destined for plasma analyses were places on ice until samples could be further processed by centrifugation at 1000 × g at 4 °C for 20 min; these were then placed into a 2 mL cryovial with a transfer pipette. All serum and plasma samples were placed into a freezer at -20 C° until analyses were conducted. 25-hydroxvitamin D_3 and beta carotene were performed by TMAS (dsm-firmenich, Belvidere, NJ). An HPLC coupled with tandem MS was used to analyze the serum samples for 25(OH)D_3 concentration. Beta carotene concentration was analyzed using a portable

photometer (iCheck Carotene system; BioAnalyt, Teltow, DE). Serum for antibody titer level was analyzed using a serum virus-neutralizing assay at the Kansas State University Veterinary Diagnostic Laboratory, and included for antibodies for IBR, BVD-I, and BVD-II. Whole blood used for haptoglobin analyses was collected in a 10.0 mL plastic sodium heparin tube (BD Vacutainer Sodium Heparin 158 USP Units; Becton Dickinson, Franklin Lake, NJ). Haptoglobin concentrations were analyzed using a colorimetric assay (Smith et al., 1998; Cooke et al., 2013).

Healthy and morbid animal cytokine analysis

When a heifer was first identified with a respiratory illness, it was removed from the pen and treated according to the protocol above. Morbid heifers were identified using the CIS system described above along with rectal body temperature. A healthy heifer was also pulled alongside each morbid heifer. A pre-determined random order of animals from each pen was generated on day 0 to pair healthy and sick animals randomly. Randomization was performed using Excel's random number generator. This order was used to select a healthy control animal from each pen for blood sampling and for use in pairwise comparisons. Morbid and healthy animals were bled via jugular vein with an 18-gauge needle (VACUETTE® Multiple Use Drawing Needle; Greiner Bio-One) and 10.0 mL EDTA glass blood tube (Covidien Monoject Coated EDTA Tubes with Lavender Stoppers; Cardinal Health, Dublin, OH). The morbid animal was then treated with antibiotics and returned to its pen along with the healthy pen mate. All heifers that became morbid were permanently removed from the list of healthy candidates and were never considered a healthy candidate. Plasma samples were centrifuged at 1000 × g at 4 °C for 20 min. Plasma was then harvested and stored into 2 mL cryovials at −80 °C until study completion. Following trial completion, cytokines were shipped to Eve Technologies Corporation for analysis. They performed a custom assay (Bovine Cytokine Panel 1 6-plex Custom Assay Plasma; Eve

Technologies Corporation, Calgary, AB, Canada) to analyze the following cytokines: TNFα, INfγ, Il-1β, IL-4, IL-6, and IL-10.

Statistical analysis

Performance and concentrations of haptoglobin, serum 25(OH)D_3, and beta carotene were analyzed using the MIXED procedure of SAS (version 9.4; SAS Institute, Cary, NC). Data were analyzed as a randomized complete block design with pen as the experimental unit. For the performance data analysis, the model included treatment as a fixed effect and block as a random effect. The model for haptoglobin, 25(OH)D_3, and beta carotene concentration data included treatment, day and their interactions as fixed effects, and block as a random effect. Antibody titer data were transformed as log2 (titer + 1) prior to statistical analysis to normalize the data. Transformed data were analyzed using a repeated measures analysis in randomized block design using the MIXED procedure of SAS (version 9.4; SAS Institute, Cary, NC). Pen was the experimental unit. Fixed effects in the model included treatment, day, and their interaction. Block was a random effect. Day was the repeated measure with pen as the subject, and the covariance structure was unstructured. Compound symmetry and spatial power were evaluated as potential covariance structures and, relative to unstructured, were found to have larger AIC, AICC, and BIC for titers of IBR, BVDI, and BVDII. No observations with a studentized residual > 4 were observed.

Health data were analyzed as binomial proportions of first, second, and third respiratory treatment and mortality using the GLIMMIX procedure of SAS (version 9.4; SAS Institute, Cary, NC). The model included fixed effects of treatment and random effect of block.

Treatment differences were considered significant at a *P*-value less than or equal to 0.05 and tendencies at a *P*-value less than or equal to 0.10.

Results and Discussion

Feed intake, growth performance, and health

Performance data are presented in Table 3.4. Interim and final body weights, average daily gain (ADG), gain: feed, and dry matter intake (DMI) did not differ ($P > 0.05$) among treatments. Average DMI across all treatments was 5.4 kg per day. Heifers ADG during the entirety of the study was 1.17 kg/d across all treatments. Health data shown in Table 3.5 shows overall prevalence of respiratory-related morbidity and mortality was 55.0% and 1.45%. During the trial, 7 total animals were found dead in the pen from bronchopneumonia (2 - HyD Low; 2 - HyD High; and 3 - HyD + BC). Additionally, 2 heifers were removed for lameness injury (1 - HyD High and 1 - HyD + BC), and 35 animals were removed from study for chronic morbidity related to Bovine Respiratory Disease Complex (BRD) (7 - D3; 7 - HyD Low; 9 - HyD High; and 12 - HyD + BC). Samples collected from these heifers were excluded from the data analysis. No dietary treatment differences were found to affect the prevalence of first, second, or third respiratory morbidity or overall mortality during the 56-day study. During the first 14 days of the trial, 38% of heifers were treated. A high respiratory morbidity early in the feeding period, coupled with low concentrations of circulating 25-hydroxyvitamin D_3, could explain why we observed no statistical significance in performance and health. Overall, supplementation with calcidiol or a combination of calcidiol and beta-carotene did not affect feed intake, growth performance, or health of high-risk, newly received heifers.

Blood serum and plasma analysis

In Figure 3.1, at days 14, 28 and 56, all calves supplemented with HyD had greater ($P < 0.001$) serum 25(OH)D_3 concentrations compared with calves fed D3 (Figure 3.1). Heifers fed HyD High and HyD + BC had greater ($P < 0.001$) serum 25(OH)D_3 concentrations than calves

fed HyD Low at days 14, 28, and 56. These data suggest HyD supplementation, with significantly higher levels observed in calves receiving HyD High and HyD + BC compared to HyD Low, has enhanced bioavailability and efficacy. These results align with previous research, which showed that calcidiol is more efficient in elevating serum 25(OH)D_3 than cholecalciferol (Xu et al., 2021; Blakely et al., 2023).

On arrival, total carotenoid concentrations (TCC) were 4.94 mcg/mL for D3 heifers whereas HyD + BC averaged 4.42 mcg/mL. Although heifers were stratified by body weight and randomly assigned to pen, baseline β-carotene concentration were greater ($P = 0.04$) for D3 compared to HyD + BC. On d 14, both treatments had significantly lower ($P < 0.01$) TCC with D3 and HyD + BC averaging 1.02 mcg/mL and 1.47 mcg/mL, respectively. HyD + BC tended to have greater ($P = 0.07$) TTC on d 14 than D3. By days 28 and 56, HyD + BC had greater ($P < 0.01$) TCC than D3 heifers.

Plasma haptoglobin concentrations (Figure 3.3) did not differ among treatments at any time point during the trial. However, a significant quadradic effect of time was observed, with plasma haptoglobin peaking at day 28 ($P < 0.01$). A relatively large SEM was reported from these results. Due to a large proportion of plasma samples containing some degree of hemolysis, only 624 individual animal observations (31% of total samples) of plasma haptoglobin concentration were available across the four blood sampling days. Hemolysis interferes with the haptoglobin assay; samples containing visible hemolysis were not analyzed for haptoglobin concentration.

Dietary supplementation with vitamin D_3, calcidiol, and beta-carotene on humoral immune responses in newly received high-risk heifers were evaluated by measuring antibody titers against IBR, BVD-I, and BVD-II viruses over the 56-day receiving period. A significant

day effect was observed for IBR antibody titers ($P < 0.01$; Figure 3.4), indicating an increase over time regardless of treatment. However, no significant effect of dietary treatment ($P = 0.37$) or treatment × day interaction ($P = 0.70$) was detected. Mean titers increased across all groups, with the highest numeric titers at day 28. Similarly, BVD-I titers (Figure 3.5) were significantly affected by time ($P < 0.01$), with antibody levels rising steadily throughout the study period. There were no significant effects of treatment ($P = 0.97$) or treatment × day interaction ($P = 0.95$). All treatment groups exhibited comparable increases, reaching peak titers by day 56. As with IBR and BVD-I, a significant day effect was observed for BVD-II titers ($P < 0.01$; Figure 3.6). No treatment effect ($P = 0.58$) or treatment × day interaction ($P = 0.96$) was found. Antibody titers increased over time in all dietary groups with no appreciable differences between treatments. Across all three viral pathogens (IBR, BVD-I, and BVD-II), antibody titers increased significantly over the 56-day period, indicating a successful humoral response in high-risk heifers during the receiving phase. However, no significant differences were observed among dietary treatment groups, suggesting that supplementation with vitamin D_3, calcidiol, or calcidiol combined with beta-carotene did not significantly influence antibody production against these viral antigens. The lack of treatment effects across multiple viral pathogens also supports the robustness of this conclusion and aligns with previous literature suggesting that vitamin D's influence on humoral immunity in ruminants may be limited or context-dependent (Nelson et al., 2012)

Further research is warranted to investigate the potential immune-enhancing effects of vitamin D metabolites and antioxidant co-supplementation under conditions of nutrient deficiency, pathogen challenge, or in combination with other immunomodulatory strategies. Assessing additional immune parameters such as cytokine profiles, acute-phase proteins, or

leukocyte function could offer a more comprehensive understanding of how these dietary interventions impact overall immune competency.

Healthy and morbid animal cytokine analysis

Table 3.6 displays cytokine concentrations that were measured to analyze the differences between healthy and sick animal pairs within pen across treatments. Pro-inflammatory cytokines TNFα and IL-6 as well as anti-inflammatory cytokines, INfγ, IL-1β, IL-4, , IL-10 were chosen as immune markers for vitamin D status. During cytokine analysis 94% of analyses for IL-1β were reported as out of range, therefore that data is not reported. Although treatment ($P = 0.44$) and interaction ($P = 0.45$) effects were not significantly different for IL-4, a trend for health status was detected ($P = 0.08$), with morbid animals generally exhibiting higher IL-4 concentrations, particularly in the D3 and HyD + BC heifers. Health status tended to influence IL-6 concentrations ($P = 0.0828$), with higher levels observed in morbid animals across all treatments, especially in HyD Low (292 pg/mL) and HyD + BC (145 pg/mL) heifers. Although treatment effects were not statistically significant ($P = 0.53$), the elevated IL-6 in sick calves is consistent with its role as a pro-inflammatory cytokine in acute phase responses.

No significant differences were observed for IL-10 across treatment ($P = 0.93$), health status ($P = 0.14$), or their interaction ($P = 0.57$). However, IL-10 concentrations were numerically higher in morbid calves, aligning with its anti-inflammatory function in response to infection and inflammation (Sacco et al., 2012). Although TNFα levels varied widely (range: 518 to 2600 pg/mL), no significant effects were observed for treatment ($P = 0.50$), status ($P = 0.26$), or their interaction ($P = 0.80$). These elevated TNFα concentrations in morbid calves are indicative of ongoing inflammatory processes but were not directly associated with the dietary treatments. IFNγ concentrations were low overall and showed no significant treatment ($P =$

0.63), health status ($P = 0.58$), or interaction effects ($P = 0.52$). Slightly elevated levels were found in morbid animals, particularly in the HyD Low and HyD + BC heifers.

Overall, cytokine responses were more closely associated with animal health status than with dietary treatment. Morbid animals exhibited numerically higher concentrations of IL-4, IL-6, IL-10, and TNFα, reflecting the expected immune activation during disease. This observation is consistent with previous findings indicating that vitamin D metabolites, particularly calcidiol, can modulate cytokine expression during disease conditions (Nelson et al., 2012).

Implications

Supplementation with calcidiol (HyD) increased serum 25(OH)D_3 concentrations compared with vitamin D_3, confirming greater bioavailability. However, no differences were observed in feed intake, growth performance, morbidity, or humoral immune response among treatments. Morbid calves exhibited numerically higher cytokine concentrations, suggesting immune activation during disease, but dietary treatments did not significantly alter immune markers. These findings indicate that while calcidiol enhances vitamin D status, its impact on health and immunity under high-risk conditions may be limited.

Literature Cited

Blakely, L. P., T. L. Wells, M. F. Kweh, S. Buoniconti, M. Reese, P. Celi. C. Cortinhas and C.D. Nelson. 2023. Effect of vitamin D source and amount on vitamin D status and response to endotoxin challenge. J. Dairy Sci. 106:912-926. doi: 10.3168/jds.2022-22354

Celi, P., S. Williams, M. Engstrom, J. McGrath, and J. La Marta. 2018. Safety evaluation of dietary levels of 25-hydroxyvitamin D3 in growing calves. Food Chem. Toxicol. 111:641–649. doi:10.1016/j.fct.2017.11.053

Cooke, R. F., and J. D. Arthington. 2013. Concentrations of haptoglobin in bovine plasma determined by ELISA or a colorimetric method based on peroxidase activity. J. Anim. Physiol. Anim. Nutr. 97:531–536. doi:10.1111/j.1439-0396.2012.01298.x.

Hudson, R.E., D. J. Tomczak, E. L. Kaufman, A. M. Adams, J. A. Carroll, P. R. Broadway, M. A. Ballou, and J. T. Richeson. 2020. Immune responses and performance are influenced by respiratory vaccine antigen type and stress in beef calves. Animals (Basel). 30:1119. doi: 10.3390/ani10071119

Nelson, C. D., T. A. Reinhardt, J. D. Lippolis, R. E. Sacco, and B. J. Nonnecke. 2012. Vitamin D signaling in the bovine immune system: A model for understanding human vitamin D requirements. Nutrients 4:181–196. doi:10.3390/nu4030181

Nelson, C. D., J. L. Powell, D. M. Price, M. J. Hersom, J. V. Yelich, M. E. Drewnoski, S. L. Bird, and G. A. Bridges. 2016. Assessment of serum 25-hydroxyvitamin D concentrations of beef cows and calves across seasons and geographical locations. J. Anim. Sci. 94:3958–3965. doi:10.2527/jas.2016-0611

Poindexter, M. B., M. F. Kweh, R. Zimpel, J. Zuniga, C. Lopera, M. G. Zenobi, Y. Jiang, M. Engstrom, P. Celi, J. E. P. Santos, and C. D. Nelson. 2020. Feeding supplemental 25-

hydroxyvitamin D_3 increases serum mineral concentrations and alters mammary immunity of lactating dairy cows. J. Dairy Sci. 103:805–822. doi:10.3168/jds.2019-16999

Sacco, R. E., B. J. Nonnecke, M. V. Palmer, W. R. Waters, J. D. Lippolis, and T. A. Reinhardt. 2012. Differential expression of cytokine in response to respiratory syncytial virus infection of calves with high and low circulating 25-hydroxyvitamin D_3. PLoS ONE. 7(3):e33074. doi:10.1371/journal.pone.0033074

Sandoval, J.L., D. E Ventura, o. B. Fiallos, B. L. Anderson, J. C. Sparks, J. D. Starkey, C. W. Starkey. 2022. Efficacy and safety of a novel source of dietary 25-hydroxycholecalciferol in growing pigs. J Anim Sci. 1;100(9):skac260. doi: 10.1093/jas/skac260.

Smith, J. E., P. S. Chavey, and G. A. Andrews. 1998. Semiautomatic and robotic methods for determining serum haptoglobin levels. Vet Clin Pathol. 27:11–14. doi:10.1111/j.1939-165X.1998.tb01073.x.

Xu, H. J., L. H. Wang. Q. Y. Zhang, X. Jiang, C. R. Zhang and Y. G. Zhang. 2021. Effects of 25-hydroxyvitamin D3 on growth performance, fecal scores, vitamin d3 metabolites, antioxidant status, and inflammatory and stress-related parameters in weaning calves. Anim. Feed Sci. Technol. 281:114946. doi:10.1016/j.anifeedsci.2021.114946

Wu, G. 2022. Nutrition and metabolism: Foundations for animal growth, development, reproduction, and health. Adv. Exp. Med. Biol. 1354:1–24. doi:10.1007/978-3-030-85686-1_

Yarger, J.G., C. L. Quarles, BW Hollis and RW gray. 1995. Safety of 25-hydroxycholecalciferol as a source of cholecalciferol in poultry rations. Poultry science. 74:1437-1446

Figures

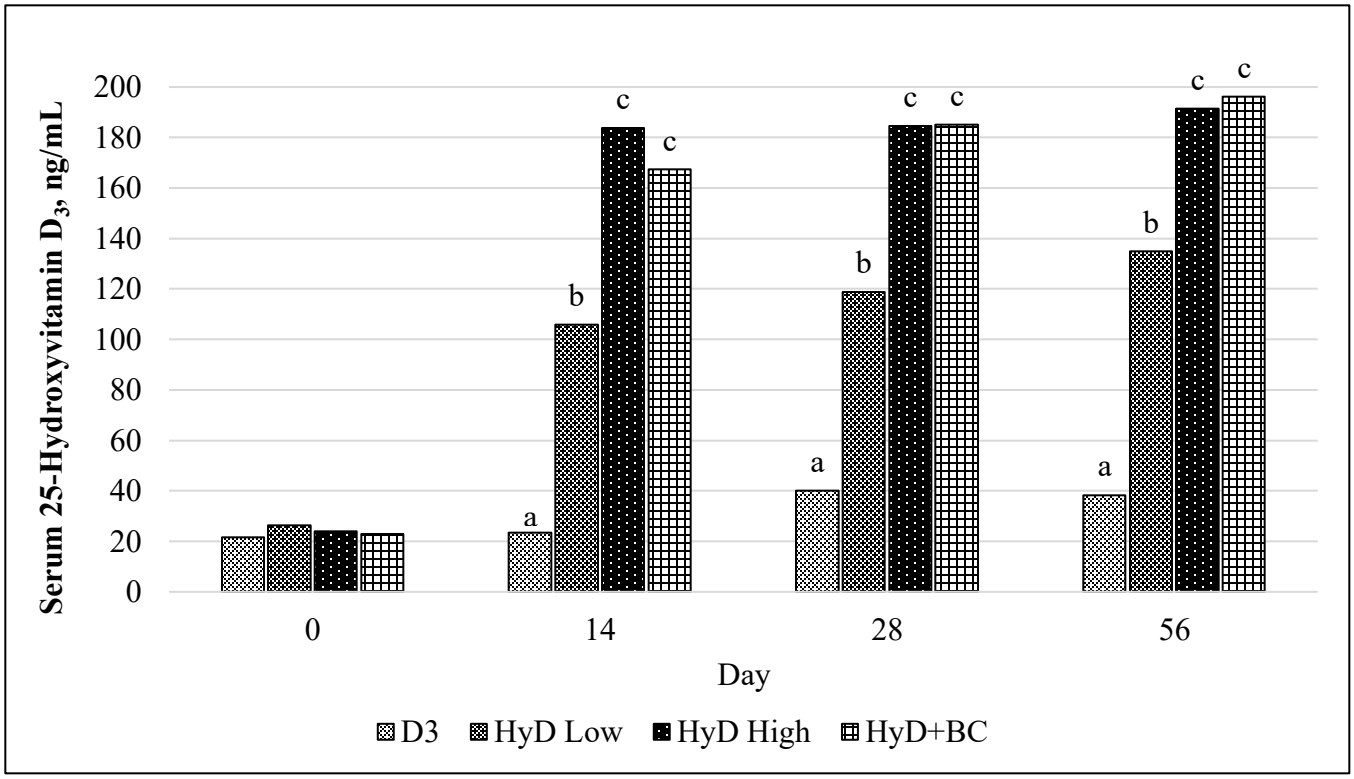

Figure 3.1 Effect of dietary treatment on serum 25-hydrovitamin D_3 concentrations in newly received high-risk heifers. Effect of day ($P < 0.01$), treatment ($P < 0.01$), and day × treatment ($P < 0.01$); SEM = 7.41.

[a, b] Unlike superscripts above bars in chart differ $P \leq 0.05$

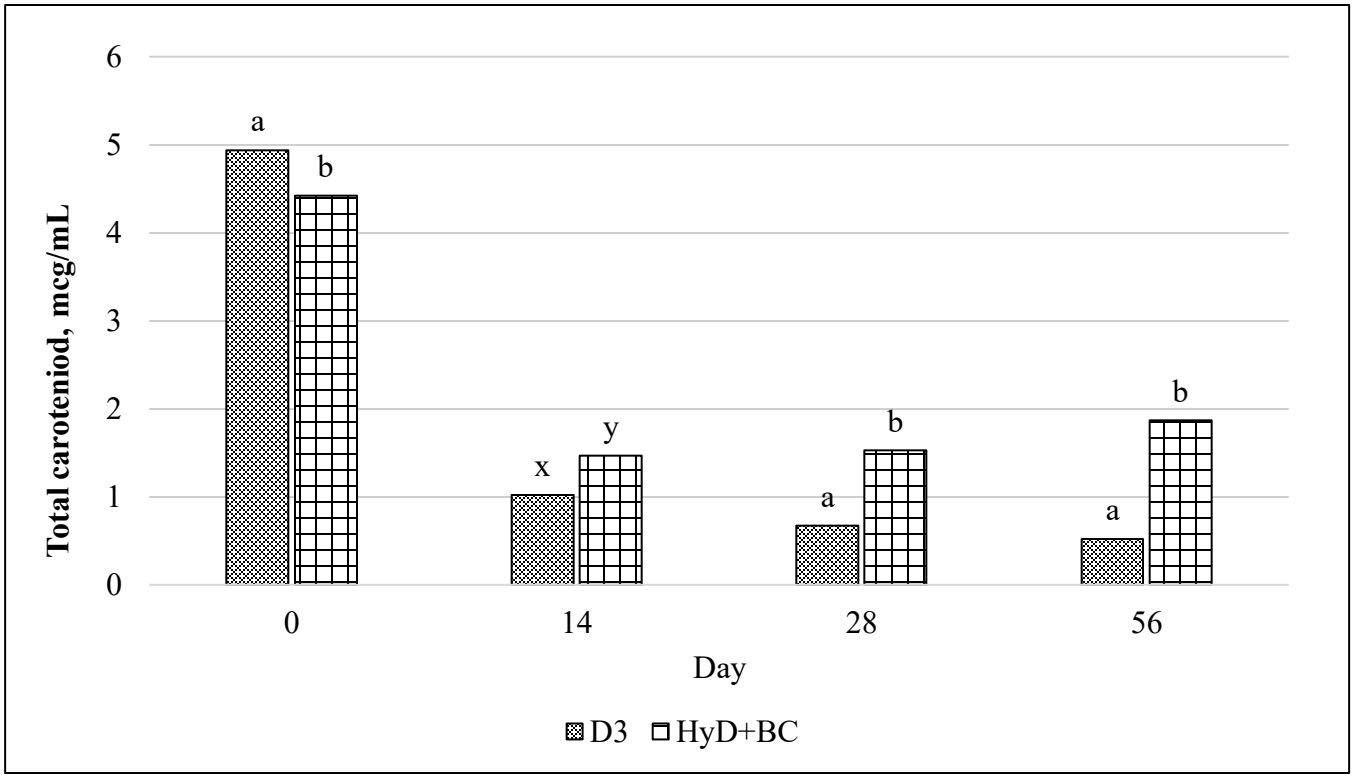

Figure 3.2 Effect of beta carotene supplementation on serum carotenoid concentrations in newly received high-risk heifers. Effect of day ($P < 0.01$), treatment ($P < 0.01$), and day × treatment ($P < 0.01$); SEM=0.190

[a, b] Unlike superscripts above bars in chart differ $P \leq 0.05$

[x, y] Unlike superscripts above bars in chart differ $P \leq 0.1$

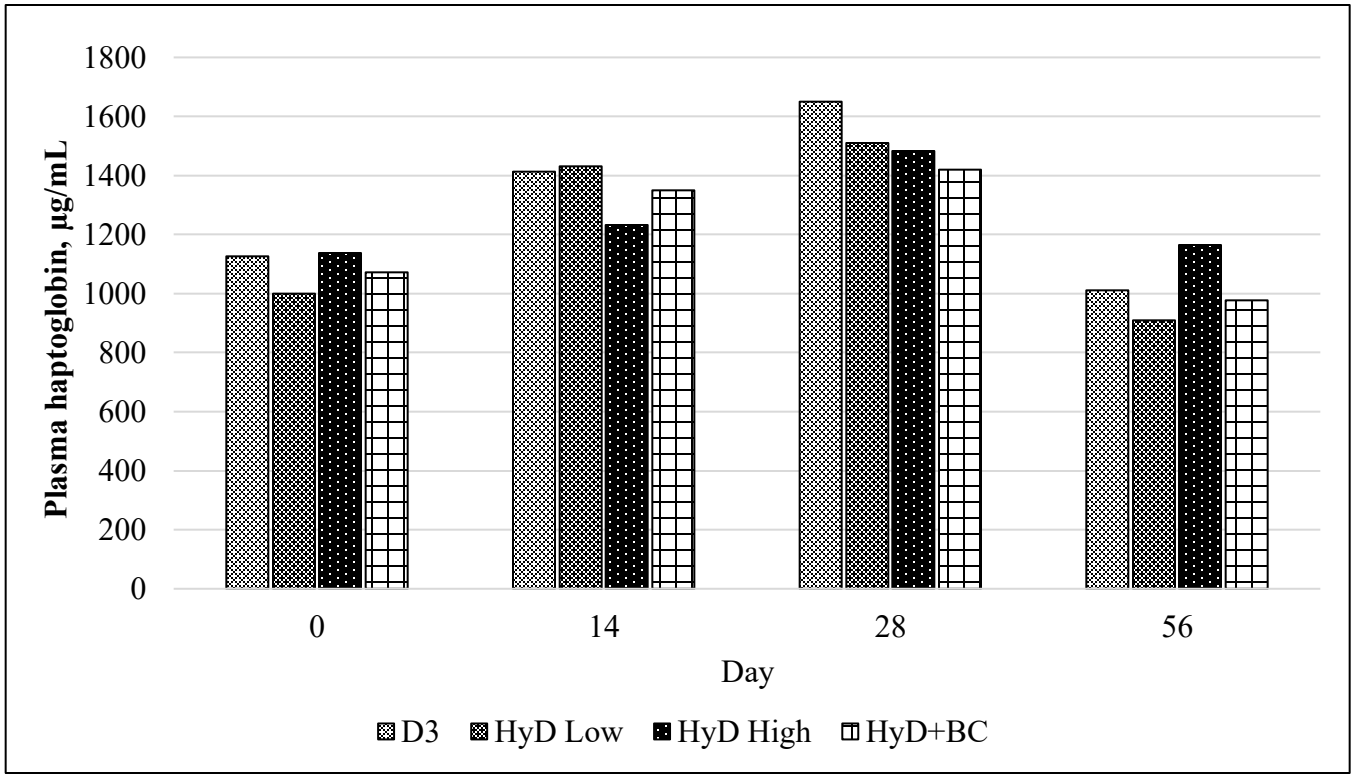

Figure 3.3 Effect of dietary treatment on plasma haptoglobin concentration in high-risk newly received heifers. Effect of treatment ($P = 0.33$), day ($P < 0.01$), treatment × day ($P = 0.60$) SEM = 113.62

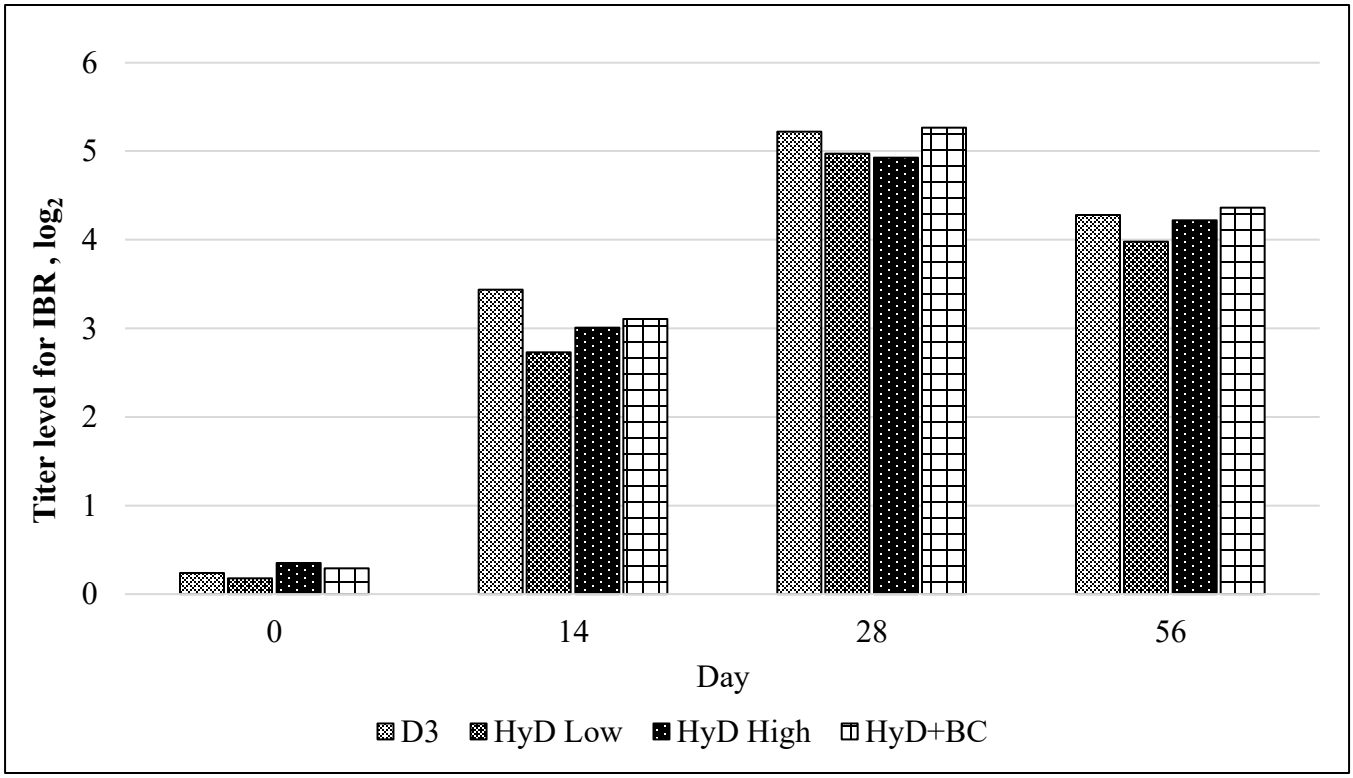

Figure 3.4 Effect of dietary treatment of antibody titers of IBR in high-risk newly received heifers. Effect of treatment ($P = 0.37$), day ($P < 0.01$), treatment × day ($P = 0.70$); SEM = 0.299

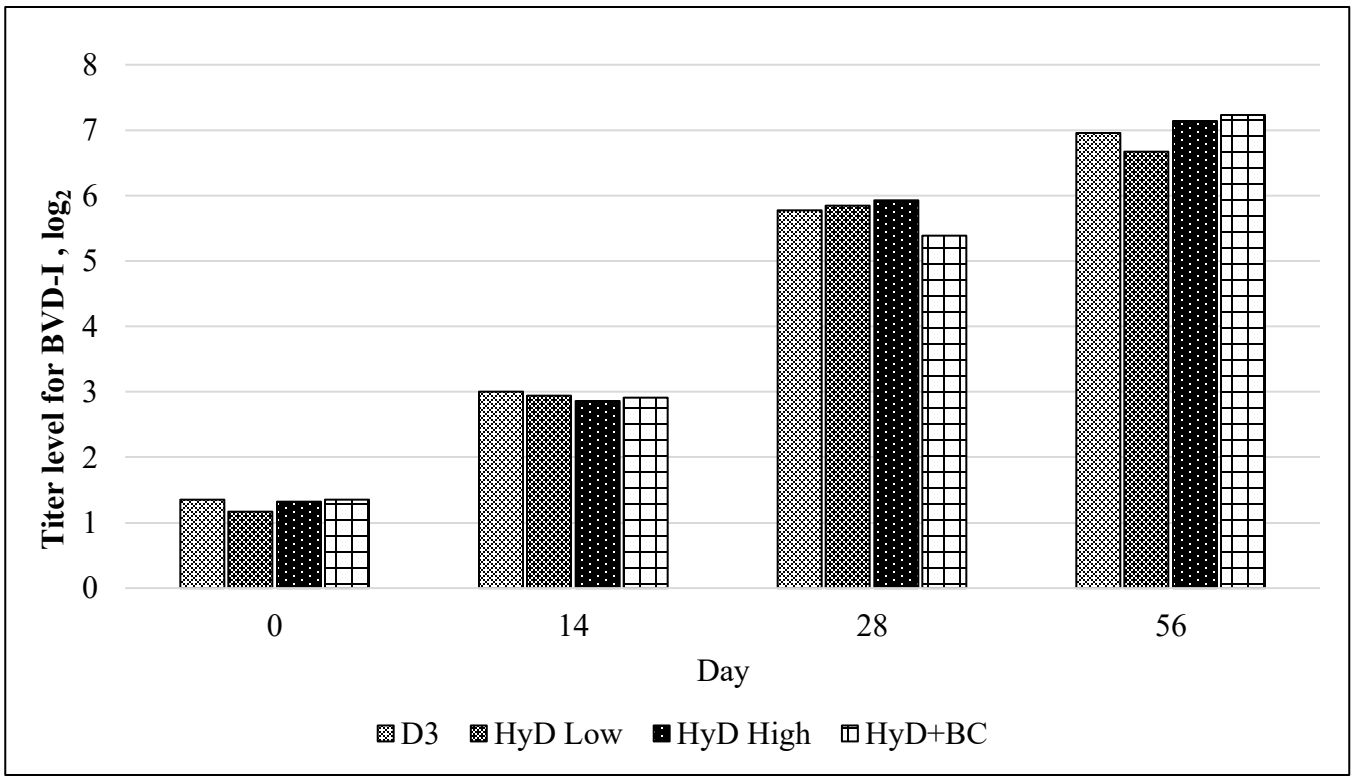

Figure 3.5 Effect of dietary treatment of antibody titers of BVD-I in high-risk newly received heifers. Effect of treatment ($P = 0.97$), day ($P < 0.01$), treatment × day ($P = 0.95$); SEM = 0.504

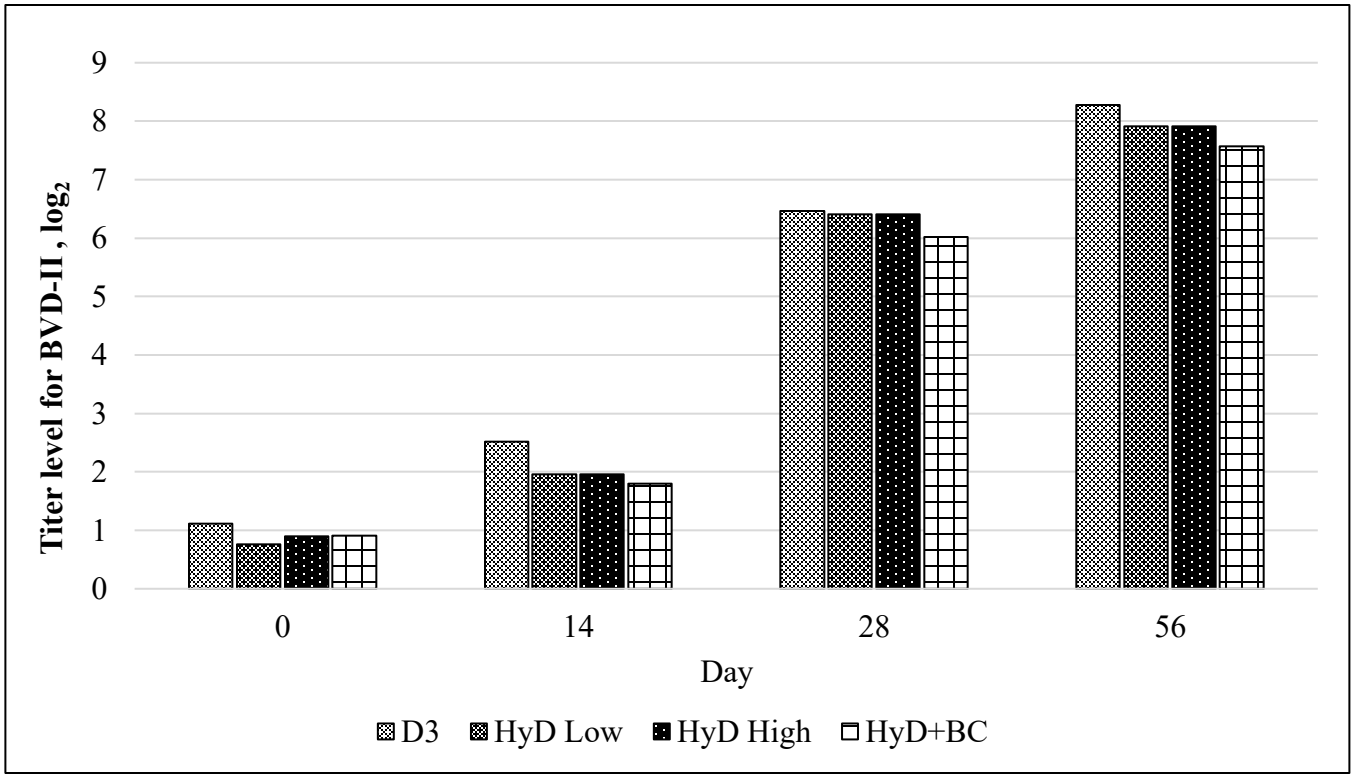

Figure 3.6 Effect of dietary treatment of antibody titers of BVD-II in high-risk newly received heifers. Effect of treatment ($P = 0.58$), day ($P < 0.01$), treatment × day ($P = 0.96$); SEM = 0.537

Tables

Table 3.1 Composition of experimental diets.

	Dietary Treatment[1]			
Item	D3	HyD Low	HyD High	HyD + BC
Ingredient, % DM				
Whole-shelled corn	39.5	39.5	39.5	39.5
Supplement[2, 3]	7.5	-	-	-
Supplement[4]	-	7.5	7.5	7.5
Wet corn gluten feed[5]	40.0	40.0	40.0	40.0
Warm-season grass hay	13.0	13.0	13.0	13.0

[1] 0.075 mg/head/day added vitamin D_3 (D3), 0.5 mg/head/day calcidiol; [HyD; DSM Nutritional Products, Plainsboro, NJ] (HyD Low); 1.0 mg/head/day calcidiol (HyD High); 1.0 mg/head/day calcidiol and 100 mg beta-carotene; [Victus Transition; DSM Nutritional Products, Plainsboro, NJ] (HyD+BC).

[2] Two different supplements were used in this trial to supply vitamin D_3

[3] Supplement pellet formulated to contain (DM basis): 5,992,800 IU vitamin D_3/ton, 11.5% crude protein, 0.60% phosphorus, 4.7% salt, 0.80% potassium, 2.5% fat, and 307.2 g/ton monensin (Rumensin; Elanco, Greenfield, IN) and 307.2 grams/ton monensin (Rumensin 90; Elanco, Greenfield, IN)

[4] Supplement pellet formulated to contain (DM basis); 11.5% crude protein, 0.60% phosphorus, 4.7% salt, 0.80% potassium, 2.5% fat, and 307.2 g/ton monensin (Rumensin; Elanco, Greenfield, IN)

[5] Sweet Bran, Cargill Animal Nutrition, Blair, NE.

Table 3.2 Nutrient composition of dietary treatments.

	Dietary Treatment[1]			
Item	D3	HyD Low	HyD High	HyD + BC
Nutrient composition[2], % of DM				
DM, % as fed	77.9	77.9	77.9	77.9
CP	13.6	13.6	13.6	13.6
NDF	25.2	25.3	25.3	25.3
OM	94.5	94.6	94.6	94.6
ADF	14.0	13.7	13.7	13.7
Ca	0.81	0.80	0.80	0.80
P	0.58	0.57	0.57	0.57
Mcal/kg[3]				
NE_m	1.95	1.95	1.95	1.95
NE_g	1.31	1.31	1.31	1.31

[1] 0.075 mg/head/day added vitamin D_3 (D3), 0.5 mg/head/day calcidiol; [HyD; DSM Nutritional Products, Plainsboro, NJ] (HyD Low); 1.0 mg/head/day calcidiol (HyD High); 1.0 mg/head/day calcidiol and 100 mg beta-carotene; [Victus® Transition; DSM Nutritional Products, Plainsboro, NJ] (HyD + BC).

[2] Nutrient analysis conducted by SDK Laboratories (Hutchinson, KS)

[3] Net energy of maintenance (NEm) and net energy of gain (NEg) were calculated using NASEM (2016) values of diet ingredients.

Table 3.3 Effects of calcidiol 25(OH)D_3 supplementation on growth performance of limit-fed high-risk newly received heifers.

	Dietary Treatment[1]					
Item	D3	HyD Low	HyD High	HyD + BC	SEM[2]	*P*-value[3]
No. of pens	10	10	10	10	--	--
No. of animals[4]	113	109	106	104	--	--
Body weight, kg						
d 0	226	226	227	226	1.24	0.88
d 14	248	246	244	244	2.52	0.29
d 28	259	258	255	255	2.95	0.56
d 56	295	291	289	290	3.31	0.54
Average daily gain, kg/d						
0 to 14	1.54	1.44	1.25	1.24	0.131	0.13
0 to 28	1.17	1.13	1.01	1.04	0.068	0.34
14 to 28	0.80	0.81	0.77	0.85	0.111	0.96
14 to 56	1.13	1.07	1.08	1.11	0.066	0.85
28 to 56	1.29	1.21	1.23	1.24	0.105	0.80
0 to 56	1.23	1.17	1.12	1.14	0.059	0.40
Dry matter intake, kg/d						
0 to 14	4.82	4.76	4.80	4.74	0.198	0.90
0 to 28	5.15	5.12	5.12	5.01	0.153	0.46
14 to 28	5.84	5.84	5.81	5.63	0.149	0.27
14 to 56	5.82	5.69	5.70	5.69	0.108	0.35
28 to 56	6.00	5.81	5.83	5.91	0.096	0.22
0 to 56	5.48	5.37	5.38	5.36	0.113	0.36
G:F, kg/kg						
0 to 14	0.320	0.295	0.262	0.256	0.0108	0.16
0 to 28	0.227	0.214	0.198	0.207	0.0058	0.43
14 to 28	0.137	0.135	0.132	0.154	0.0088	0.82
14 to 56	0.194	0.185	0.190	0.194	0.0048	0.81
28 to 56	0.216	0.203	0.212	0.209	0.0076	0.87
0 to 56	0.225	0.212	0.209	0.212	0.0043	0.53

[1]0.075 mg/head/day added vitamin D_3 (D3), 0.5 mg/head/day calcidiol; [HyD; DSM Nutritional Products, Plainsboro, NJ] (HyD low); 1.0 mg/head/day calcidiol (HyD high); 1.0 mg/head/day calcidiol and 100 mg beta-carotene; [Victus® Transition; DSM Nutritional Products, Plainsboro, NJ] (HyD+BC).

[2] Standard error of the mean.

[3] Treatment main effect.

Table 3.4 Effects of calcidiol 25(OH)D_3 supplementation morbidity and mortality of limit-fed high-risk newly received heifers.

	Dietary Treatment[1]					
Item	D3	HyD Low	HyD High	HyD + BC	SEM[2]	*P*-value[3]
Morbidity, %						
Treated once[4]	50.00	58.33	53.33	63.33	0.276	0.17
Treated twice[5]	13.33	16.67	15.83	25.83	0.408	0.34
Treated thrice[6]	5.83	5.83	7.50	9.17	0.625	0.83
Mortality[7], %	0.00	1.67	1.67	2.50	0.739	1.00

[1]0.075 mg/head/day added vitamin D_3 (D3), 0.5 mg/head/day calcidiol; [HyD; DSM Nutritional Products, Plainsboro, NJ] (HyD low); 1.0 mg/head/day calcidiol (HyD high); 1.0 mg/head/day calcidiol and 100 mg beta-carotene; [Victus® Transition; DSM Nutritional Products, Plainsboro, NJ] (HyD+BC).

[2] Standard error of the mean.

[3] Treatment main effect.

[4] Percentage within treatment of heifers treated once for bovine respiratory disease (BRD).

[5] Percentage within treatment of heifers treated a second time for BRD out of total population (heifers treated twice ÷ total population).

[6] Percentage within treatment of heifers treated a third time for BRD out of total population (heifers treated thrice ÷ total population).

[7] Percentage within treatment of heifers within treatment that died from BRD-related illness.

Table 3.5 Cytokine concentration measured to analysis the differences in healthy and sick animal pairs within pen across dietary treatments in high-risk newly received heifer calves.

	Dietary Treatment[1]					*P*-value		
Item	D3	HyD Low	HyD High	HyD + BC	SEM[2]	Trt	Status	Trt x Status
No. of animals	60	70	64	76				
Cytokine, OC[3]								
IL-4, pg/mL					10.7	0.44	0.08	0.45
H[4]	12	23	12	18				
M1[5]	36	24	14	43				
IL-6, pg/mL					84.2	0.53	0.08	0.64
H	72	70	62	42				
M1	134	292	131	145				
IL-10, pg/mL					15.7	0.93	0.14	0.57
H	40	56	48	58				
M1	70	70	78	58				
TNFα, pg/mL					756	0.50	0.26	0.80
H	519	1327	1267	1272				
M1	1153	1407	2600	1657				
IFNγ, pg/mL					2.04	0.63	0.58	0.52
H	3.62	4.10	4.23	3.13				
M1	3.57	6.70	2.93	6.00				

[1] 0.075 mg/head/day added vitamin D_3 (D3), 0.5 mg/head/day calcidiol; [HyD; DSM Nutritional Products, Plainsboro, NJ] (HyD low); 1.0 mg/head/day calcidiol (HyD high); 1.0 mg/head/day calcidiol and 100 mg beta-carotene; [Victus® Transition; DSM Nutritional Products, Plainsboro, NJ] (HyD+BC).

[2] Standard error of the mean

[3] Observed concentrations

[4] Healthy animals

[5] First morbidity treatment animals