AN ABSTRACT OF THE DISSERTATION OF

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Title: Management Strategies to Improve Immune Function and Performance of Feeder Cattle.

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Three experiments evaluated the effects of different management strategies on immune function and performance of feeder cattle. The objective of experiment 1 was to compare the effects of anticipating, delaying, or vaccinating against Bovine Respiratory Disease (BRD) at the time of weaning and feedlot entry on growth, DMI, and plasma antibody parameters of feeder cattle. The objective of experiment 2 was to evaluate the effects of Cu, Mn, Zn, and Co supplementation, either as inorganic or organic complexed sources, during a 45-day preconditioning program on productive, immunity, and physiological parameters of cattle through preconditioning followed by a 58-day feedlot receiving period. The objective of experiment 3 was to evaluate the effects of supplementing Omnigen-AF or Immune Primer formula products on performance, health, and physiological responses of receiving cattle.

In experiment 1, 90 Angus × Hereford calves were ranked by sex, BW, and age, and assigned to 1 of 3 vaccination schemes against the BRD complex: 1) vaccination at weaning (d 0) and booster at feedlot entry (d 30; CON, n = 30), 2) vaccination 15 d before weaning (d -15) and booster 15 d before feedlot entry (d 15; EARLY, n = 30),

and 3) vaccination 15 d after weaning (d 15) and booster 15 d after feedlot entry (d 45; DELAYED, n = 30). From d -15 to 7, calves were maintained as a single group on pasture. On d 8, calves were placed into 1 of 18 drylot pens (6 pens/treatment; 5 calves/pen) and fed alfalfa-triticale hay. On d 29, calves were transported 1,440 km in a livestock trailer and unloaded on d 30 at the same feedyard with the same pen arrangement used prior to transport. From d 30 to 75, calves were fed a receiving diet based on alfalfa-triticale hay + corn-based concentrate. Calf BW was recorded on 2 consecutive days (d -15, -14, 0, 1, 28, 29, 75, and 76). Blood samples were collected on d -15, 0, 15, 30, 45, 60, and 75. The EARLY calves had less ($P \leq 0.09$) ADG pre-weaning (d -15 to -1), however had greater ($P \leq 0.01$) ADG during feedlot receiving (d 30 to 75) compared to the other treatments. During preconditioning (d 0 to 29), CON had greater ($P \leq 0.04$) DMI compared with EARLY and DELAYED. During feedlot receiving, no treatment differences were detected ($P \geq 0.17$) for hay or concentrate DMI, G:F, morbidity, and mortality rates. There were no treatment effects on calf BW at weaning, and at the end of preconditioning or receiving periods ($P \geq 0.65$). Plasma concentrations of antibodies against *Mannheimia haemolytica* were greater ($P \leq 0.05$) in EARLY vs. CON and DELAYED on d 0, greater ($P \leq 0.04$) for CON vs. EARLY and DELAYED on d 15, greater ($P \leq 0.02$) in DELAYED and EARLY vs. CON on d 30, and greater ($P = 0.03$) in EARLY vs. CON on d 75. Plasma concentrations of antibodies against bovine viral diarrhea viruses were greater ($P \leq 0.04$) in EARLY vs. CON and DELAYED on d 15, and greater for EARLY and CON vs. DELAYED on d 30 and 45. Collectively, EARLY calves had greater plasma concentrations of

antibodies against the evaluated pathogens at feedlot entry, and increased ADG during receiving compared with CON and DELAYED cohorts.

In experiment 2, 90 Angus x Hereford calves were weaned at 7 mo (d − 1), sorted by sex, weaning BW and age (261 ± 2 kg; 224 ± 2 days), and allocated to 18 drylot pens (one heifer and four steers per pen) on day 0; thus, all pens had equivalent initial BW and age. Pens were randomly assigned to receive a corn-based preconditioning concentrate containing: (1) Cu, Co, Mn and Zn sulfate sources (INR), (2) Cu, Mn, Co and Zn complexed organic source (AAC) or (3) no Cu, Co, Mn and Zn supplementation (CON). From day 0 to 45, cattle received concentrate treatments (2.7 kg/animal daily, as-fed basis) and had free-choice access to orchardgrass (*Dactylis glomerata L.*) long-stem hay and water. The INR and AAC treatments were formulated to provide the same daily amount of Co, Cu, Mn and Zn at a 50-, 16-, 8- and ninefold increase, respectively, compared with the CON treatment. On day 46, cattle were transported to a commercial feedlot, maintained as a single pen, and offered a free-choice receiving diet until day 103. Calf full BW was recorded on days − 1 and 0, 45 and 46, and 102 and 103 for average daily gain (ADG) calculation. Liver biopsy was performed on days 0 (used as covariate), 22 and 45. Cattle were vaccinated against respiratory pathogens on days 15, 29 and 46. Blood samples were collected on days 15, 29, 45, 47, 49, 53 and 60. During preconditioning, mean liver concentrations of Co, Zn and Cu were greater ($P \leq 0.03$) in AAC and INR compared with CON. No treatment effects were detected ($P \geq 0.17$) for preconditioning feed intake, ADG or feed efficiency. No treatment effects were detected ($P \geq 0.48$) for plasma concentrations of antibodies against *M. haemolytica*, bovine viral diarrhea types 1 and 2 viruses. Plasma

haptoglobin concentrations were similar among treatments ($P = 0.98$). Mean plasma cortisol concentration was greater ($P \leq 0.04$) in CON compared with INR and AAC. No treatment effects were detected ($P \geq 0.37$) for cattle ADG during feedlot receiving.

In experiment 3, 108 Angus × Hereford steers, originating from 7 cow–calf were obtained from an auction yard on d −2 and transported by road (800 km; 12 h) to an experimental feedlot facility. Upon arrival on d −1, shrunk BW was recorded and steers were grouped with free-choice access to grass hay, mineral supplement, and water. On d 0, steers were ranked by source and shrunk BW and assigned to 1 of 18 pens (6 steers/pen). Pens were allocated to 1) no immunomodulatory ingredient supplementation during feedlot receiving (CON), 2) supplementation with OmniGen-AF (OMN; 22 g/steer daily, as-fed basis; Phibro Animal Health Corp., Teaneck, NJ) from d 0 to 30, or 3) 2 oral capsules of Stocker Immune Primer on d 0 + 15 g/ steer daily (as-fed basis) of Stocker Preconditioned Premix (Ramaekers Nutrition, Santa Cruz, CA) from d 7 to 30 (IPF). From d 0 to 80, steers had free-choice access to grass hay and water and received a corn-based concentrate. Feed DMI was recorded from each pen, and steers were assessed for BRD signs daily. Steers were vaccinated against BRD pathogens on d 0 and 21. Final shrunk BW was recorded on d 81, and blood samples were collected on d 0, 3, 7, 10, 14, 21, 31, 42, 56, and 73. Steer ADG and final BW were greater ($P \leq 0.05$) in CON steers than in OMN and IPF steers [1.23, 0.76, and 1.06 kg/d (SEM = 0.06), respectively, and 320, 282, and 307 kg (SEM = 4), respectively] and ($P < 0.01$) in IPF steers than in OMN steers. No treatment effects were detected ($P \geq 0.76$) for BRD incidence (66 ± 4%) and DMI, whereas G:F was greater ($P < 0.01$) in OMN steers than in CON steers. Mean plasma cortisol

concentration was greater ($P = 0.01$) in CON steers than in OMN and IPF steers. Plasma haptoglobin concentrations tended ($P = 0.10$) to be greater in CON steers than in IPF steers on d 3, were greater ($P = 0.04$) in IPF steers than in CON steers on d 7, and tended ($P = 0.10$) to be less in OMN steers than in IPF and CON steers on d 21. Blood mRNA expression of interleukin 8 was greater ($P \leq 0.05$) in OMN and IPF steers than in CON steers on d 3 and in OMN steers than in CON and IPF steers on d 14. Blood mRNA expression of tumor necrosis-α was greater ($P \leq 0.05$) in OMN and IPF steers than in CON steers on d 10. Plasma IGF-I concentrations, serum antibody titers to BRD pathogens, and blood mRNA expression of chemokine ligand 5, cyclooxygenase 2, interleukin 8 receptor, and L-selectin did not differ ($P \geq 0.21$) among treatments.

Collectively, anticipating initial and booster vaccination against respiratory pathogens to provide both doses prior to feedlot entry appears to be a valid strategy to enhance cattle health and performance during feedlot receiving. Regarding mineral supplementation, INR and AAC increased liver concentrations of Co, Zn and Cu through preconditioning, but did not impact cattle performance and immunity responses during preconditioning and feedlot receiving. Lastly, the immunomodulatory feed ingredients evaluated herein impacted adrenocortical and innate immune responses but failed to mitigate BRD incidence and improve performance of receiving cattle.

Management Strategies to Improve Immune Function and Performance of Feeder Cattle.

by
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I understand that my dissertation will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my dissertation to any reader upon request.

Katana D. Lippolis, Author

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CHAPTER I

INTRODUCTION

The beef cattle industry is highly segmented, with the major sectors being cow-calf and feedlot operations. The focus of cow-calf operations is to raise cow-calf pairs, predominantly on rangeland and pasture, until the calf is weaned. As of January 1, 2018, the number of beef cows in the United States was 31.7 million head (USDA, 2018). While cow/calf operations can be found in all 50 states, the majority of operations are in the western U.S. Following weaning, calves are often transferred to feedlots as "feeder cattle". Here, they begin to consume high-energy, concentrate-based diets until reaching desired finishing weight and condition. With the exception of Texas, the top states for total cattle on feed are located near the "corn-belt" region of the U.S., including Nebraska, Kansas, Colorado, and Iowa (USDA, 2018). At the start of 2018, the total number of calves and cattle on feed was 14.4 million head (USDA, 2018). In the first few weeks following feedlot entry, cattle are considered "receiving cattle" who require specific management to recover from the variety of stressors associated with the transition.

One of the most stressful periods in a beef animal's life is the transition from the cow-calf operation to the feedlot. During this time, cattle are subjected to numerous stressful events during a short period of time, such as weaning, transportation, nutrient depravation, fatigue, commingling with cattle from various locations, and exposure to a new environment and feedstuffs (Araujo et al., 2010; Carroll and Forsberg, 2007). Additionally, cattle are often processed on arrival which may include tagging, receiving

growth-promoting implants, castration of bulls, dehorning of horned cattle, deworming, and vaccination (USDA-NAHMS, 2011a).

Loerch and Fluharty (1999) describe that the cumulative result of the various stressors experienced by receiving cattle lead to: "1) transient endocrine responses, 2) altered products of energy and protein metabolism, 3) changes in appetite and growth rate, 4) possible limited compromise of digestive and rumen function, and 5) a challenged immune system." Subsequently, feed intake is often low during the receiving period. Weaning, transportation, and feedlot entry are associated with decreased dry matter intake (**DMI**) and body weight (**BW**; Araujo et al., 2010; Arthington et al., 2013). Historically, feed intake during the first week of the receiving period has been reported as low as 1.55% of BW in healthy calves (Hutcheson and Cole, 1986), and may take cattle as long as 21 d to reach normal feed intake (Lofgreen, 1988). Further, newly received feeder cattle may be subjected to stress-induced immunosuppression and increased risk of disease incidence (Duff and Galyean, 2007).

Bovine respiratory disease (**BRD**), or "shipping fever", is the most detrimental disease impacting the beef cattle industry today (Hilton, 2014) affecting both economic and performance efficiency. Prevalence of this disease can be attributed to numerous factors, including environmental challenges, nutrition, and management decisions prior to and during feedlot entry. Bovine respiratory disease incidence most often occurs during the several weeks after weaning and feedlot entry, with morbidity rates as high as 34.7 – 75.0% (Roeber et al., 2001; Richeson et al., 2008). According to the National Animal Health Monitoring System (USDA-NAHMS; 2011b), treatment for BRD averages $23.60 per treatment. Further, there is a negative relationship between

incidence of disease with animal performance and carcass quality, as ADG, final BW, and percent of steers grading Choice or better decreases with treatment for disease (Reinhardt et al., 2012; Schneider et al., 2009). Subsequently, BRD costs the beef industry in the US about $500 million every year (Miles, 2009) and demonstrates an area to improve feedlot production efficiency by improving receiving cattle health and performance. As prevalence of BRD is associated with the cumulative effect of the variety of stressors surrounding feedlot entry, implementing management strategies that better prepare feeder cattle for the receiving period are warranted.

CHAPTER II

LITERATURE REVIEW

The immune system

The immune system is generally categorized into two components: the innate and adaptive immune responses (Parham, 2005). The innate immune response is composed of macrophages, dendritic cells, neutrophils, eosinophils, basophils, and mast cells, and is non-specific to pathogens. This response serves as the body's first line of defense against pathogen and is aided by the acute phase response (**APR**). The adaptive immune response is composed of B- and T-lymphocytes and is antigen-specific, leading to immunological memory of pathogens and lifelong protective immunity. While the immune system is a complex and intrinsic combination of cellular processes, the two systems communicate and work together to clear the body of pathogens and return to homeostasis (Murphy et al., 2011).

Acute inflammation is a biological response to infection or trauma that aims to resolve the damage and heal the affected area. In order to do this, pro-inflammatory cytokines such as interleukin-1β (**IL-1β**), interleukin-6 (**IL-6**), and tumor necrosis factor α (**TNFα**) are released in order to induce inflammation and activate and proliferate cells of the immune response (Parham, 2005). Inflammation causes the release or decrease of acute phase proteins from the liver, which function to sequester nutrients from bacteria, resolve infection, and promote healing. (Murphy et al., 2011). Chemokines help to draw immune cells to the infected area, so they can work to resolve the infection or damaged tissue. Generally, inflammation due to trauma or external injury can be visualized as redness, heat, swelling, and pain. This is primarily because

the proinflammatory cytokines and histamine allow for the dilation of blood vessels and separation of endothelial cells, causing swelling. Immune cells, such as neutrophils, follow the chemokines to the site of the infection or injury and attach to the lining of endothelial cells via ligand binding of L-selectin on the cell surface (Murphy et al., 2011). Swelling of the infected area allows the immune cells easier access between the endothelial cells, where they begin to phagocytose pathogens, foreign debris, and dead or damaged host cells.

Following recognition and destruction of bacteria, antigen presenting cells (**APC**) such as dendritic cells and macrophages present antigen to lymphocytes. Naïve T-lymphocytes respond to the interleukin 12 (**IL-12**) from APC and interferon γ (**IFN-γ**) from natural killer (**NK**) cells and differentiate into Type 1 T helper (**Th1**) cells of the cell-mediated immune response (Elenkov and Chrousos, 1999). These Th1 cells also release proinflammatory cytokines to further activate naïve T-lymphocytes and macrophages, as well as provide help to B-lymphocytes to begin producing antibodies specific to the pathogen (Murphy et al., 2011). Once the B-lymphocytes are activated, they begin dividing into short-lived plasma cells that secrete antibodies and long-lasting memory cells that provide immunological memory to the specific pathogen.

Alternatively, naïve T-lymphocytes may also be activated by APC to differentiate into Type 2 T helper (**Th2**) cells of the humoral immune response (Elenkov and Chrousos, 1999). This response is often triggered by extracellular pathogens such as helminths and allergens and drives the production of antibodies from B-lymphocytes (Murphy et al., 2011). The activated Th2 cells secrete anti-inflammatory cytokines such as interleukin 4 (**IL-4**), interleukin 10 (**IL-10**), and

interleukin 13 (**IL-13**). The cytokines responsible for initiating either Th1 or Th2 responses act as a negative feedback regulator of the other, therefore the activation of the Th1 response inhibits the Th2 response and vice versa (Elenkov and Chrousos, 1999). While this may serve as a mechanism by which the immune system self-regulates, the induction of the hypothalamic-pituitary-adrenal (**HPA**) axis as a result of perceived stress may also have immune modulating properties.

The HPA axis

Stress of the body includes the cumulative biological responses to physical, emotion, and/or mental stimuli that alter homeostasis; thus, initiating a cascade of physiological processes in the body's attempt to reestablish it. The stress response in humans and animals is a complex and multifaceted response that includes the central nervous system (**CNS**), the sympathetic nervous system (**SNS**), and the HPA axis. When the brain senses physical or psychological stress, the hypothalamus releases Corticotropin Releasing Hormone (**CRH**), also known as Corticotropin Releasing Factor (**CRF**), which acts on the pituitary gland to produce adrenocorticotropic hormone (**ACTH**). This ACTH then travels to the adrenal gland to induce the production of glucocorticoids (Tsigos and Chrousos, 2002), which can then translocate into the cell to bind to glucocorticoid receptors and regulate gene transcription. This allows glucocorticoids, such as cortisol, to act on both the physiology and the behavior of the animal in order to ensure survival.

The production of glucocorticoids stimulated by the activation of the HPA axis can be due to inflammatory cytokines following an inflammatory response to pathogen, as discussed previously. This mechanism uses cortisol as a lipolytic agent to break

down body stores, subsequently increasing levels of glucose and fatty acids in the blood (Nelson and Cox, 2005). This provides the body with increased energy to combat the pathogen or stressor. Alternatively, cortisol also functions as a negative feedback loop in order to stop the production of inflammatory cytokines to bring the body back to homeostasis. However, the body activates the HPA axis in other perceived stressors. As glucocorticoids exhibit immunosuppressive properties, this can be detrimental to the animal (Tsigos and Chrousos, 2002). Scheinman et al. (1995) discovered that glucocorticoid binding to glucocorticoid receptors inhibit cytokine transcription via the inactivation of nuclear factor kappa-light-chain-enhancer of activated B cells (**NF-kB**), a key regulator in the production of cytokines. Key immune cells such as macrophages and lymphocytes possess glucocorticoid receptors (Carroll and Forsberg, 2007), thus are susceptible to high circulating glucocorticoids. Further, it has been postulated that the action of glucocorticoids on cytokine production drives the immune system towards a Th2 response instead of the Th1 response needed to combat pathogens (Elenkov and Chrousos, 1999). Therefore, the activation of the HPA-axis independent of pathogen may lead to increased susceptibility to disease.

The role of acute-phase proteins (**APP**) of the early innate immune response in beef animals has been a focus of recent research to evaluate the impact of stress on the immune system. These hepatic proteins, such as haptoglobin and ceruloplasmin, can serve as a marker for inflammation and either increase or decrease depending on their mode of action in response to events such as stress and inflammation. Stressful events such as weaning and subsequent transportation have been associated with the acute phase protein response (Arthington et al., 2003; Arthington et al., 2005), and are also

associated with decreased DMI and BW (Cooke et al., 2009; Araujo et al., 2010; Arthington et al., 2013). Additionally, research investigating 24-h transportation or 24-h nutrient deprivation has observed elevated APP and subsequent decreased ADG in the 28-d feedlot receiving period (Marques et al., 2012). However, the mechanism behind the acute phase protein reaction in response to stress is not completely understood in beef cattle. To further understand the initiation of this response, Cooke and Bohnert (2011) hypothesized that they can initiate a neuroendocrine response and the acute phase reaction through intravenous injection with CRF. The infusion successfully initiated an increase in plasma cortisol, as well as increased body temperature and concentrations of IL-6, ceruloplasmin, and haptoglobin. These data indicate that CRF and cortisol play a role in inducing the inflammatory response in beef cattle independently of pathogen, represented by increased acute phase proteins. However, the mechanism depicting the relationship between elevated stress hormones in cattle and decreased forage intake has yet to be elucidated.

Cattle management-induced stress and immune function

The first stressor experienced by feeder cattle transitioning from the cow-calf operation to the feedlot is when they are weaned from their dams. Sudden separation from the dam leads to a multifaceted perceived stress to the calf, including loss of their dam, elimination of milk from their diet, and altered social structure (Weary et al., 2007). Due to this separation, abruptly weaned calves exhibit numerous signs of distress including increased vocalization and walking behavior (Haley et al., 2005). The stress associated with abrupt weaning of calves leads to increased cortisol 3 d post weaning and increased haptoglobin 3 and 5 d post weaning (Kim et al., 2011).

Following weaning stress, calves have been found to exhibit decreased plasma lymphocytes 2 and 5 d post weaning (Lynch et al., 2010; Kim et al., 2011). Furthermore, neutrophils from weaned calves exhibit decreased phagocytic function and increased concentration in the blood, which the authors hypothesize to be due to suppressed L-selectin on the surface of the immune cells (Lynch et al., 2010). Further, abrupt weaning decreases *in vitro* IFN-γ responses of lymphocytes when exposed to keyhole limpet hemocyanin (**KLH**) antigen and Concanavalin A mitogen (Hickey et al., 2003). Collectively, these data suggest that the stress of weaning alone can induce physiologic responses that alter immune function.

Following weaning, calves are often subjected to transportation directly to the feedlot. During this time, cattle may experience increased handling during loading and unloading, crowding, harsh weather conditions, bruising, and fatigue. Transportation is known to be a major risk factor for BRD incidence, hence the classical term of "shipping fever." Cortisol concentrations are markedly increased following transportation (Crookshank et al., 1979), which activates the APP response as indicated by elevated serum amyloid-A and ceruloplasmin on d 2 and 3 following transportation (Arthington et al., 2003). The activation of the APP response due to transportation is not limited to the transportation itself, but also due to the other stressors experienced by transported cattle. Marques et al. (2012) sought to investigate the effects of 24-h transportation compared to 24-h nutrient deprivation and control cohorts that were neither transported or had feed and water withheld. Both transportation and nutrient deprivation increased cortisol and NEFA concentrations on d 1 after treatment. Interestingly, cattle subjected to nutrient deprivation exhibited greater NEFA

concentrations on d 1 and greater cortisol concentrations in the 28-d following treatment. Both transportation and nutrient deprivation activated the APP response, indicated by increased haptoglobin and ceruloplasmin concentrations. However, while ceruloplasmin and haptoglobin concentrations were greatest for transported cattle on d 1, there was no difference from controls throughout the 28-d experimental period. Alternatively, cattle subjected to nutrient deprivation exhibited greater ceruloplasmin than control on d 4, greater ceruloplasmin than transported and controls on d 14, and greater haptoglobin on d 7 than transported or control cohorts. The mechanism behind why the nutrient deprived cattle elicited a longer duration APP response is unclear, however demonstrates the effect of transportation on cortisol secretion, stress induced tissue mobilization, and APP response.

Transportation-induced immunosuppression has long been recognized; early reports from Blecha et al. (1984) who observed decreased lymphocyte blastogenic responses. Similar results were reported by Van Engen et al. (2014), who observed decreased blood lymphocytes for up to 5 d after 16-h transportation, as well as neutrophilia immediately following transportation. A study investigating immune function following transportation of *Bos indicus* steers for 72-h did not observe decreased lymphocyte concentrations; however, leukocyte concentrations and lymphocyte phagocytic ability were decreased immediately following transportation (Stanger et al., 2005). Further, decreased respiratory burst capability of phagocytes in the bronchoalveolar fluid of the lungs have been observed immediately after 4 h transportation and in the 3-d following transportation (Ishizaki et al., 2004), indicating decreased ability to combat pathogens due to the stress of hauling.

When unloaded at the feedlot, cattle from a variety of sources are joined into pens and commingled with each other. This presents a multitude of stressors including the introduction of a new herd hierarchy and environment, but also presents the physical challenge of being exposed to cattle who may be sick and shedding BRD pathogens. A study by Step et al. (2008) compared morbidity rates during a 42-d feedlot receiving period of calves from a single source, calves weaned and sold at auction, and calves from a single source commingled with market calves. Calves from a single source had the lowest morbidity of 11.1%, whereas calves from a single source commingled with market calves experienced 22.6% morbidity. Calves purchased from auction market had the greatest overall morbidity of 41.9%. The exact reasoning is unclear, as the differences in morbidity may have been due to the stress of increased interactions with new animals. However, the authors explain it may have also been due to potential novel exposure to pathogens. Burciaga-Robles et al. (2010) sought to investigate the effects of a 3-d exposure to a persistently infected (**PI**) bovine viral diarrhea virus (**BVDV**) infected calf and simultaneous challenge with *M. haemolytica*. Steers exposed to the PI calf had greater concentrations of the anti-inflammatory cytokine IL-4 compared to steers not exposed. By d 4, calves exposed to the PI calf began showing marked increases in serum antibodies against BVD and remained elevated throughout the 28-d sampling period. Additionally, calves challenged with both BVDV and *M. haemolytica* exhibited suppressed white blood cells, decreased antibodies against *M. haemolytica*. Therefore, it is likely that exposure infected animals and novel pathogens during feedlot entry and commingling may decrease the body's immune defenses and increase susceptibility to secondary infection. Cumulatively with

the other stressors discussed previously, receiving cattle are often quite stressed, fatigued, and immunocompromised, leading to increased BRD incidence.

Alleviation of stress induced by cattle management

Various strategies have been investigated to reduce the impact of stress associated with management procedures on cattle morbidity and performance, including the use of pharmaceuticals. The use of meloxicam, a non-steroidal anti-inflammatory drug (**NSAID**), in bulls castrated at feedlot entry significantly decreased morbidity rate from 35.0% to 21.1% and first pull rate for disease treatment but did not mitigate suppressed ADG or G:F due to castration (Coetzee et al., 2012). Meloxicam use has also been investigated to alleviate stress-induced inflammation following transportation. Meloxicam administered to beef steers immediately prior to 16 h transportation exhibited decreased neutrophilia immediately after transportation, decreased blood lymphocyte concentration for 5 d following transportation, and tended to decrease cortisol concentrations following transportation (Van Engen et al., 2014). Another trial by Guarnieri Filho et al. (2014) investigated meloxicam treatment immediately prior to 24 h transportation, immediately afterward, and on d 2 to 7 of a 21-d receiving period. In this study, meloxicam mitigated feed intake losses during the first 7 d of receiving, and mitigated ADG losses throughout the receiving period. Meloxicam treatment also mitigated the increases in the APP ceruloplasmin and haptoglobin. Therefore, the use of NSAIDs may be beneficial in mitigating intake depression associated with feedlot receiving.

Other pharmaceutical strategies to mitigate transportation stress include flunixin meglumine, a non-steroidal analgesic. When treated immediately prior to 1,280 km transportation and upon feedlot entry, flunixin tended to decrease cortisol, decreased haptoglobin on d 1 and 4 following transportation, and decreased ceruloplasmin on d 1, 4, and 7 compared to untreated transported cattle (Cooke et al., 2013). While flunixin was able to mitigate the APP response, there were no differences observed for cattle performance.

Metaphylaxis or prophylactic treatment with antibiotics at feedlot entry has been the industry standard in reducing BRD incidence in "high stress" feeder cattle. According to USDA-NAHMS (2011b), 59.3% of feedlots utilize prophylactic treatment, with tilmicosin being the most common. When tilmicosin was provided to incoming feeder cattle following a transport of 1,930 miles, calves exhibited 20.8% greater ADG and greater serum total antioxidant capacity following a 28-d receiving period compared to untreated cohorts (Chirase et al., 2004). Additionally, the inclusion of antibiotics in feed and water have historically been common in managing health of feeder cattle. In 2011, 73.4% of feedlots used antibiotics in feed and 4.7% used antibiotics in water for health or production purposes (USDA-NAHMS, 2011b). However, beginning December of 2016, the Veterinary Feed Directive (**VFD**) increased the regulation of antibiotics in feed and water of food-producing animals in an effort to support the judicious use of antibiotics. (US Food and Drug Administration, 2015). According to the new regulations, medically important antibiotics will no longer be allowed for production purposes and require all VFD medications to be prescribed by a veterinarian. Therefore, the development of management practices for cattle prior

to and after feedlot entry that decrease the incidence of disease and necessity of antibiotic treatment is of increased importance.

Management strategies to separate the stressors surrounding weaning and feedlot entry, such as via a preconditioning program, can have a positive effect on cattle health and performance as the stress effects are not compounded. Many states have developed preconditioning programs that incorporate vaccination protocols while introducing calves to dry feed and feed bunks to help combat the prevalence of BRD. Most of these programs include weaning calves for 30 to 45 d and providing 2 rounds of vaccinations against BRD pathogens prior to sale or feedlot entry. The goal of these programs is to provide increased adaptive protection against BRD pathogens and prepare cattle for the feedlot (Duff and Galyean, 2007). Schwartz-Genswein et al. (2007) observed that calves preconditioned for 30 d and receiving both vaccinations prior to transport exhibited decreased cortisol concentration following transportation and feedlot entry than non-preconditioned cohorts when transported for either 2.7 or 15 h. Work by Step et al. (2008) observed decreased morbidity in calves weaned for 45-d prior to transportation (5.9%) and calves weaned and vaccinated prior to transportation (9.5%) compared to calves weaned at shipping or sold via auction (35.1% and 41.9%, respectively). Further, calves weaned prior to feedlot entry have been observed to have greater DMI (% of BW) during feedlot receiving than calves weaned at transportation (2.84% vs. 2.50%, respectively). Subsequently, preconditioning for 42-d prior to feedlot entry has been observed to increase final BW, hot carcass weight (**HCW**), and carcass fat thickness (Anderson et al., 2016). Thus, preconditioning programs offer a unique opportunity to implement various

management strategies to weaned calves in order to prepare them for the feedlot, including via vaccination and nutritional strategies, and may mitigate disease incidence.

Vaccination against pathogens of the BRD Complex

One of the most important components of a preconditioning program is the administration of vaccinations prior to cattle entry into the feedlot. Vaccinated cattle are 0.68 times as likely to be treated for disease than market calves (Macartney et al., 2003). Traditionally, this includes an initial vaccine against BRD pathogens prior to weaning, followed by a booster 3 to 4 weeks later at weaning. Unfortunately, the latest published NAHMS survey of cow-calf producers reported that 60.6% of cow-calf operations, representing 30.9% of calves, did not vaccinate calves against bovine respiratory disease pathogens prior to sale (USDA-NAHMS, 2008). Further, calves are often vaccinated at weaning and feedlot entry when highly stressed. As glucocorticoids have immunosuppressive properties, vaccinating stressed animals may decrease the effectiveness of the vaccine (Blecha et al., 1984), thus decreasing protection against BRD pathogens and increasing susceptibility to disease. Therefore, research has investigated the impact of alternative vaccination strategies to improve health of feeder cattle.

Preweaning vaccination against BRD pathogens with booster at weaning have been found to be successful in improving vaccination response and decreasing costs associated with BRD. Kirkpatrick et al. (2008) investigated vaccinating calves at 67 and 190 d of age or 167 and 190 d of age compared to unvaccinated, control cohorts,

weaned at 190 d of age. Both groups of vaccinated calves had greater seroconversion to infectious bovine rhinotracheitis (**IBR**) and bovine viral diarrhea virus (**BVDV**) than the unvaccinated group and had less treatment costs. Additionally, mortality was greater in unvaccinated calves than calves vaccinated at 67 and 190 d of age. However, as vaccinating at weaning may suppress antibody response, providing vaccines after weaning and prior to feedlot entry may be more beneficial.

Step et al. (2008) investigated the effects of different weaning and vaccination strategies on cattle performance and BRD incidence. Treatments were steer calves weaned at transport to feedyard, calves weaned for 45 d prior to transport, calves weaned for 45 d prior to transport and vaccinated, or calves purchased from multiple sources at a commercial livestock market (control). Calves weaned and vaccinated prior to feedlot entry had 9.5% incidence of morbidity compared to 35.1% of calves not vaccinated and weaned at transport, and 41.9% of calves purchased from auction. Subsequently, calves weaned and vaccinated prior to feedlot entry had decreased health costs compared to unvaccinated calves weaned at transport and market calves. However, many calves entering the feedlot are considered high-risk for BRD. Therefore, vaccination strategies at the feedlot are of increased interest.

Unfortunately, vaccination can also have a detrimental impact on animal performance and may therefore compound performance losses when administered at feedlot entry. Vaccination induces the inflammatory response and the subsequent APP response. Arthington et al. (2013) observed increased haptoglobin, ceruloplasmin, and fibrinogen following vaccination against *M. haemolytica* and *Clostridium*, and subsequently decreased ADG and G:F. Another trial was conducted by Rodrigues et

al. (2015), in which vaccination against BRD pathogens induced increased levels of TNFα, haptoglobin, insulin, and leptin. This physiological response was associated with subsequent decreases in forage DMI and total DMI during the 2-d following vaccination. The authors suggested that the decreased DMI induced by vaccination could be partially due to elevated concentration of leptin, which is produced by adipocytes during the inflammatory response and is known to impact feed intake via satiety (Houseknecht et al., 1998). Therefore, delaying vaccination until after the stress of feedlot entry may be beneficial to improve immune response to vaccination and prevent production losses.

Richeson et al. (2008) investigated the effect of delaying vaccination of high risk calves against BRD pathogens on infectious bovine rhinotracheitis (IBR) titers and performance. Calves were purchased from a commercial livestock auction and vaccinated upon arrival or 14 d after arrival. Morbidity rates did not differ and were 71.5% and 63.5% for on arrival vaccination and delayed vaccination, respectively, with the majority of BRD incidence occurring within the first 14-d receiving. However, calves receiving the delayed vaccination had greater BW gain during the first 14-d receiving and had greater seroconversion to IBR at the end of the 42-d receiving period. These results demonstrate the impact of BRD incidence of high risk calves, and vaccination upon feedlot entry did not mitigate incidence. However, delaying vaccination did mitigate the negative effects of vaccination on BW, and provided greater protection against BRD following the receiving period.

A similar trial by Richeson et al. (2009) investigated the effects of delaying vaccination against clostridial and BRD pathogens on high risk calves purchased from

several commercial auction markets. In this trial, calves that received vaccination on arrival had greater seroconversion to BVDV than delayed cohorts, and decreased days to initial BRD treatment. Further, there was no difference BW gain, however there was also no difference found in morbidity rates. Similar results were found by Poe et al. (2013), with greater BVDV antibody response in calves vaccinated on arrival compared to delayed vaccination. These results may indicate a difference in immune response to vaccination against particular pathogens, however vaccination after feedlot entry is still unable to mitigate BRD incidence.

Due to continued prevalence of BRD, 92.7% of feedlots vaccinate for IBR and 96.6% vaccinate for BVDV (USDA-NAHMS, 2011a) upon cattle arrival. As discussed previously, vaccination impairs cattle performance and intake (Rodrigues et al., 2015; Arthington et al., 2013), thus may contribute to poor performance during feedlot receiving. However, as the majority of BRD cases occur within the first few weeks following feedlot entry, waiting to provide vaccination until 2 weeks after feedlot entry may leave cattle vulnerable to disease. Therefore, the development of alternative vaccination strategies to improve immune function of calves prior to feedlot entry are warranted.

Mineral supplementation and performance

The utilization of preconditioning programs provides beef cattle producers an opportunity to implement nutritional interventions that may aid in immune function prior to feedlot entry. Due to their importance in a variety of physiological functions, supplementation of minerals may serve an important role in preparing the immune

system of feeder cattle prior to feedlot entry. In particular, the trace minerals Zinc (Zn), Copper (Cu), Cobalt (Co), and Manganese (Mn) play crucial roles in the physiological mechanisms needed for growth, health, and the stress response (Spears, 2000), and may benefit stressed and/or immune challenged calves. Additionally, the source of trace mineral supplementation via organic or inorganic sources may determine the extent of the effects observed following supplementation (Spears, 1996). As trace mineral deficiencies will alter antibody response and the inflammatory response (Galyean et al., 1999; Engle et al., 1997), their supplementation is crucial to feeder cattle health.

Copper: Copper has a variety of roles in the body, including as a component of 2 copper-dependent enzymes: ceruloplasmin, an APP, and superoxide dismutase (**SOD**). Both of these enzymes play antioxidant roles in reducing inflammation, particularly in mitigating oxidative damage due to pathogenic infection or inflammation (Suttle and Jones, 1986). Subsequently, serum copper concentrations are elevated following cumulative transportation, co-mingling, castration, and vaccination, as well as following an IBR challenge (Orr et al., 1990). Copper also plays a role in immune cell function, as neutrophils from Cu-deficient cattle exhibited decreased activity and killing ability of neutrophils (Boyne and Arthur, 1986). Further, Cu-deficient heifers immunized against *Brucella abortus* displayed impaired ceruloplasmin, immunoglobulin G production, and peripheral blood mononuclear cells (Cerone et al., 1995). Subsequently, calves supplemented with Cu prior to and after weaning exhibited a tendency for greater neutrophil SOD and bactericidal activity, and higher plasma TNFα concentrations following intranasal inoculation with IBR (Gengelbach et al., 1997). When cattle are supplemented with Cu in the feedlot, they

have been found to exhibit increased DMI during receiving and growing phases, and increased ADG during the growing phase (Ward and Spears, 1997).

Cobalt: Cobalt serves as an essential component of cobalamins, most importantly of the coenzyme vitamin B12. Vitamin B12 functions as a coenzyme for 2 important enzymes: methylcobalamin for DNA synthesis, and deoxyadenosylcobalamin for bacteria-derived propionic acid utilization. Deficiency in cobalt can lead to decreased neutrophil function (Paterson and MacPherson, 1990), lower lymphoblastic responses (Vallema et al., 1996), reduced parasite resistance (Spears et al., 2000), and a shift from T-lymphocytes from TH1 (inflammatory) to TH2 (anti-inflammatory; Funada et al., 2001). Feeding a diet with no Co supplementation has been shown to decrease ADG compared to supplemented cattle (Schwarz et al., 2000; Stangle et al., 1999), and cattle fed Co-deficient diets have exhibited decreased DMI and vitamin B12 concentration in the serum and liver (Stangl et al., 1999). However, supplementation to weaned calves can improve antibody response to vaccination for *Brucella abortus* (Sager, 2013), indicating its importance in immune function.

Manganese: Manganese is a trace mineral widely distributed in the body that is important for a variety of physiological processes, such as a component of the antioxidant Mn superoxide dismutase (MnSOD), Mn-activated metabolic enzymes, and bone development (Underwood and Suttle, 1999). One of the most important Mn-activated enzymes is glycosyltransferase, which plays a large role in mucopolysaccharide production for growth, cartilage, and bone development (Underwood and Suttle, 1999). Further, Mn deficiency during pregnancy has been

associated with altered fetal development, indicating its importance in growth (Hansen et al., 2006). Additionally, broiler chickens challenged with Salmonella and supplemented with high Mn experienced an improved Th1 immune response, indicated by greater T-lymphocytes in the blood and greater gene expression of proinflammatory genes IL-1β, IL-6, and IFN-γ (Pan et al., 2017).

Zinc: Zinc is another mineral that has a variety of functions in the body, including antioxidant, catalytic, regulatory, and structural roles. Specific regulatory roles include gene transcription, hormone release, and cell signaling (Underwood and Suttle, 1999), which are all important in the immune response. Further, the antioxidant capacity of Zn is important, as cell damage due to the inflammatory response can produce reactive oxygen species (Prasad et al., 2008). Supplementation to stressed calves during the feedlot receiving period with Zn from organic (Zn-methionine) and inorganic (Zn-oxide) sources has been shown to improve DMI during the 28-d following feedlot entry, and supplementation with Zn-methionine tended to improve antibody response to BHV-1 by d 14 following vaccination (Spears et al., 1991). Similar improvement of immune function has been seen by Mandal et al. (2007), who observed greater antibody responses to *Brucella abortus* vaccination in Zn-propionate supplemented bulls compared to bulls receiving Zn-sulfate supplementation or bulls receiving no Zn supplementation. Therefore, Cu, Co, Mn, and Zn are important components of immune function and should be considered in a receiving diet where stress and morbidity rates are elevated.

Organic vs. inorganic sourced minerals: Trace mineral source can have an effect on the bioavailability, rate of absorption, and utilization by the animal. Inorganic

source minerals are commonly found as a sulfate, oxide, chloride, and carbonate form. Organic source minerals are minerals chelated to amino acids, hydrolyzed proteins, or polysaccharides. Research has been growing in the area of organic source mineral supplementation, due to reported enhanced absorption, retention, and biological activity compared to inorganic source minerals (Spears, 1996; George et al., 1997; Marques et al., 2016). While the exact mechanism is unclear, there are several theories, including: protection against breakdown in the GI tract, absorption via amino acid transporters instead of metal transporters, decreased competition with other minerals, increased solubility through membranes, and increased stability at low pH (Miles and Henry, 2000). As the trace minerals discussed above are involved in immune function, more efficient mineral absorption and utilization may improve cattle health during times of stress.

Nockels et al. (1993) observed the effects of inorganic or organic source Zn and Cu mineral supplementation for 28 d prior to an 18-d mineral balance trial, including a 3-d nutrient deprivation and ACTH injection challenge. Following the challenge, calves were repleted with their mineral treatment. During repletion, calves that received Cu-lysine exhibited 53% greater apparent Cu absorption and increased retention compared to those receiving Cu-sulfate. Additionally, Cu retention throughout the 18-d mineral balance trial was greater in Cu-lysine supplemented calves, and greater for Zn methionine supplemented calves compared to Zn-sulfate supplemented calves. These results are directly applicable to stress observed during feedlot entry, as Zn methionine supplementation to newly received feeder steers receiving vaccination had a tendency for higher antibody titers against BHV-1 compared to steers receiving Zn-

oxide supplementation or no Zn supplementation (Spears et al., 1991). Similarly, bulls supplemented with Zn-propionate exhibited greater Zn retention and higher antibody response to *Brucella abortus* compared to Zn-sulfate supplementation or no Zn supplementation (Mandal et al., 2006). Therefore, organic trace mineral supplementation of these minerals appears to have an added benefit of mineral retention and immune function compared to inorganic source.

As intake is often limited in receiving cattle due to physiological and psychological stressors associated with weaning and transport, offering calves a trace mineral supplementation prior to weaning and feedlot entry may serve as a means to support the immune system prior to the activation of the stress response. Moriel and Arthington (2013) fed a creep feed fortified or not with inorganic trace minerals (Co sulfate, Cu sulfate, Mn oxide, sodium selenite, Cu sulfate, iron sulfate, Mn oxide, sodium selenite, and Zn sulfate) compared to no mineral supplementation to observe the effects on calves from the preweaning phase throughout the first 30 d of the feedlot receiving phase. Further, a subset of calves was injected with porcine red blood cells (**PRBC**) in order to analyze humoral immune response to an unknown antigen. While calves receiving preweaning trace mineral supplementation had greater liver concentrations of Co, Cu, and Se compared to non-supplemented calves, there was no difference in weaning BW or preweaning ADG in supplemented calves even though they had decreased DMI of the creep feed. However, although trace mineral supplementation improved liver trace mineral status, there was no difference in plasma ceruloplasmin concentrations following feedlot entry and no difference in antibody response to PRBC. In this experiment, incidence of BRD was not recorded. However,

inorganic trace mineral supplementation was able to improve liver status of trace minerals at feedlot receiving. While performance effects were not observed in the aforementioned study, it is possible that trace mineral status was adequate in the non-supplemented calves to withstand the stress of weaning and feedlot entry. Additionally, the incorporation of organic source trace minerals may have provided different results. Thus, mineral supplementation may be more beneficial with organic trace minerals or to calves who are deficient in the key trace minerals necessary for immune function to prevent performance losses during feedlot receiving.

Another nutritional strategy to support immune function prior to feedlot entry is the incorporation of trace mineral supplementation during a preconditioning program. Following traditional weaning of 7-mo old calves, Dorton et al. (2006) sought to observe the effects of Cu, Zn, Mn, and Co trace mineral supplementation and source during a period including a 30-d preconditioning program and the subsequent 28 d following feedlot receiving. Treatments were either no supplementation (control), inorganic Cu, Zn, Mn, and Co supplementation, or organic Cu, Zn, Mn, and Co supplementation. No differences in performance were observed during the preconditioning phase, and there were no differences observed in morbidity in the feedlot receiving phase. However, steers supplemented with organic source trace minerals had greater ADG during the feedlot receiving phase than those supplemented with inorganic source trace minerals. The difference observed in ADG but not morbidity may be due to the fact that morbidity rates were relatively low (7.3%-13.5%) compared to that seen in industry in traditionally weaned and high-risk calves as a result of the 30-d preconditioning protocol, or perhaps to the duration of the supplemental

period. Nonetheless, this trial, as well as those discussed previously, details the potential benefits of organic sourced trace minerals compared to inorganic sourced trace minerals and no supplementation.

The variance of effects observed in mineral supplementation may be due to several factors. Current mineral status, supplementation duration, management decisions, and immune challenge may all have an effect as to the success of mineral supplementation in calves and impact on performance and immunity. Even so, the benefits observed by organic source trace minerals on immune function and performance are encouraging. Faber et al. (1999) encourages the incorporation of a 45-d preconditioning program, compared to 30-d, in order to allow for proper vaccine administration. As previous 45-d preconditioning programs have been successful at reducing feedlot receiving morbidity rates compared to traditional weaning (Step et al., 2008), incorporating organic source trace mineral supplementation during a 45-d preconditioning period may allow for greater immune function and decreased morbidity following feedlot entry.

Immunomodulatory products on cattle health

Although preconditioning programs are available to producers, many feedlots continue to experience “high-risk” cattle that are at elevated risk of BRD. These are often cattle with no vaccination history, and/or purchased from commercial livestock auctions, who may have increased susceptibility to stress-induced immunosuppression. As 30.9% of weaned calves did not receive vaccination against bovine respiratory disease pathogens prior to sale (USDA-NAHMS, 2008), and only 38% of feedlots >

1,000 head capacity always obtain pre-arrival information of receiving cattle, the quantity of feedlots frequently purchasing high risk cattle is likely high. The first few weeks of the feedlot receiving period are crucial to long-term cattle health, therefore the development of management strategies that support immune function during this critical time are necessary.

Due to the high rate of morbidity during the feedlot receiving period, fed antimicrobials are often used to mitigate the incidence of disease (Wilson et al., 2017). However, their use is under increased regulations (U.S. Food and Drug Administration, 2015), thus driving the need for alternative feed ingredients to improve immune function and performance. Several ingredients that stimulate the immune system have been investigated in beef cattle to determine their effects on performance and immune function. These ingredients offer the potential to activate the immune system to prepare calves for the stress of feedlot receiving and to possibly combat immunosuppression.

One such type of ingredient includes the use of live yeast and yeast cell wall (**YCW**) supplements derived from *Saccharomyces cerevisiae*. These molecules interact with the immune system via beta-glucans that stimulate the release of pro-inflammatory cytokines such as TNFα from macrophages and increased T lymphocyte INF-γ (Broadway et al., 2015). Therefore, these ingredients could be used to prime the immune system prior to a stress event. Burdick Sanchez et al. (2012) sought to investigate the use of yeast cell wall supplementation on the acute phase response of heifers following a lipopolysaccharide (**LPS**) challenge. Treatments included no YCW supplementation for 52 d, or YCW supplementation with either A or C *Saccharomyces cerevisiae* strains. Heifers were challenged with LPS on d 37 of the supplemental

period. Although no differences were observed in sickness behavior or TNF-a concentrations, YCW supplementation decreased cortisol concentrations and serum IL-6 following LPS challenge compared to control heifers, and the C strain of *Saccharomyces cerevisiae* decreased vaginal temperatures compared to other cohorts. These data suggest an ability of YCW supplements to lessen the effect of the inflammatory response, which may allow the animal to recover more quickly after the challenge.

The same research group followed their previous inquiry to further investigate the effects of YCW during the feedlot receiving period (Young et al., 2017). Heifers were sourced from two different operations, and received either no supplementation or supplementation with 1 of 3 *Saccharomyces cerevisiae* strains for 56 d. An LPS challenge was conducted at d 63 and performance variables were monitored. Results were dependent on heifer source, where the C strain of *Saccharomyces cerevisiae* was more effective to one source of heifers, and the A strain was more effective for the other source of heifers. In one group of heifers, those supplemented with the C strain of *Saccharomyces cerevisiae* had greater BW and ADG by d 42 of the feedlot receiving period, as well as greater BW and ADG after the LPS challenge. In the other group of heifers, those supplemented with the A strain of *Saccharomyces cerevisiae*, a positive linear relationship was found between supplementation, BW, ADG, and G:F. These results support the ability of YCW products to influence the inflammatory response to allow for improved performance during a stress event. The ability of one strain of YCW to influence performance within the first 2 weeks of supplementation indicates the potential for these products to activate the immune system quickly. Further, the ability

of another strain of YCW to improve weight gain following an immune challenge indicates the potential to use these products during the feedlot receiving period to lessen the impact of the physiological challenge on cattle performance.

A commercial product that uses YCW as a component is Omnigen-AF and has been well researched in dairy cattle to mitigate mastitis. The use of this supplement when fed for a month prior to calving has been found to increase the gene expression of IL-1b converting enzyme and IL-4R (Wang et al., 2009). Following 30 d of supplementation in dairy heifer calves, neutrophils from supplemented heifers had greater capacity to bind and destroy E. Coli (Ryman et al., 2013). Further, L-selectin mRNA expression was improved, indicating an increased adhesion capability by leukocytes of Omnigen supplemented heifers. Similar results were observed when Omnigen was fed 60-d prepartum until calving (Nace et al., 2014). In addition to a tendency of greater L-selectin on their surface, leukocytes exhibited a greater potential to internalize and kill pathogens. Further, heifers supplemented with Omnigen had fewer incidences of udder edema and mastitis than non-supplemented heifers. These results demonstrate the capability of Omnigen to enhance immune function and improve the immune system's ability to recognize and destroy pathogenic bacteria that can lead to disease.

Research by Brandão et al. (2016) investigated the effects of Omnigen on performance and physiological responses of transition dairy cows. Treatments were either no supplementation, or supplementation of 56 g/cow of Omnigen from 35 d prior to the expected calving date through 46 d post calving. A subset of cows was then subsequently challenged with LPS at 48.7 $\pm$ 1.6 d in milk. Cows receiving Omnigen

supplementation had increased milk yield compared to non-supplemented cows. Further, Omnigen supplemented cows had enhanced response to LPS exhibited by increased haptoglobin and increased TNFα during the 3 hours post-LPS challenge. Collectively, these results indicate the capacity of Omnigen to not only enhance immune function and prevent disease in dairy cows, but also improve milk yield. This indicates an immunomodulatory ability of Omnigen to alleviate the impact of disease via immune stimulation, thus leading to improved animal performance. Therefore, research investigating the use of this immunomodulatory product during the feedlot receiving period is warranted.

Another commercial product advertised to enhance the immune system of cattle is Stocker Preconditioned Premix (Ramaekers Nutrition). This product is composed of primarily transfer factor proteins and lactate-producing antibiotics. While research is limited in the use of this product, research investigating the effects of its ingredients show promise to improve cattle health.

Transfer factor proteins are extracted from bovine colostrum and egg yolks, which are high in immunoglobulins and essential components for developing the immune system of young calves and chicks. A component of T-lymphocyte derived dialyzable leukocyte extracts, transfer factor has the ability to transfer immunity when extracted from one animal and administered to another (Fudenberg and Fudenberg, 1989). For example, Louie et al. (1987) investigated the effect of cryptosporidiosis upon treatment with oral bovine transfer factor. Calves that tested positive for delayed-type hypersensitivity to *Eimeria bovis* were used to provide transfer factor, which were subsequently used to orally inoculate immune naïve calves and mice. Treatment was

successful, with a dramatic decrease in bowel movement prevalence, increased firmness of stools, and eradication of *Cryptosporidium* in half of the treated animals.

Observation of the use of transfer factor in cattle was continued by Montgomery et al. (2008), who evaluated the effects of oral transfer factor on newly received feedlot heifers, as well as the impact of ruminal degradation of transfer factor. Treatments were either 10mg/kg of tilmicosin phosphate (a macrolide antibiotic used to treat BRD), or an oral drench containing 700 mg of transfer factor extracted from bovine colostrum followed by 700 mg of transfer factor in the feed from d 2-5. Incidence of disease was greater for heifers given transfer factor, however no differences were observed for DMI, ADG, or G:F. Transfer factor was found to be highly degraded in the rumen, which likely negated the beneficial effects observed in the prior study. Nonetheless, research to date has not observed the impact of supplementation with transfer factor during feedlot receiving compared to a negative control of no treatment, therefore further research may be necessary.

The other active ingredient in Stocker Preconditioned Premix are lactate-producing bacteria. A survey inquiring on the impact of these direct-fed microbials (**DFM**) found that cattle in feedyards claiming to use DFMs reported 1.9% higher ADG in feeder steers and 1.4% higher ADG in feeder heifers (McDonald et al., 2005). Little research has investigated the use of DFMs in receiving cattle; however, a study by Crawford et al. (1980) observed a reduction in morbidity rates when newly received feeder cattle were fed DFMs during the first 14-d of the receiving, indicating DFM supplementation may improve immune function and subsequent performance. Neuhold et al. (2012) supplemented Charolais steers with DFMs during the finishing period. No

differences in performance or carcass quality were observed, however this supplementation strategy may have been provided too late in the feeding period to have an effect. Therefore, while research is limited in the relationship between immunomodulatory feed ingredients and BRD incidence, research is needed to further elucidate the possible mechanisms of these ingredients on morbidity, immune function, and performance during feedlot receiving.

Development of management strategies to improve immune function and performance of feeder cattle

Bovine respiratory disease (**BRD**) is the most detrimental disease impacting the U.S. beef cattle industry today (NASS, 2006), costing about $500 million every year (Miles, 2009). As the majority of BRD cases in the feedlot occur in the first few weeks of the receiving period due to a combination of stressors, the development of management strategies to increase feeder cattle health and performance during this fundamental time period are warranted.

Preconditioning programs separate the stress of weaning and feedlot entry and offer producers a unique opportunity to implement vaccination and nutritional strategies to better prepare calves for feedlot entry. Therefore, the development of these strategies that are effective in mitigating BRD incidence and improving performance of feeder cattle is necessary. However, although these strategies exist, many receiving cattle are considered “high risk”. With increased regulation of fed-antimicrobials, the incorporation of immunomodulatory ingredients in receiving cattle diets may hold promise to improve immune function and performance. To address the challenges

indicated herein, three experiments were conducted to evaluate the effects of different management strategies surrounding weaning, preconditioning, and feedlot entry on immune function, health, and performance of feeder cattle. The methods and results from these experiments are reported and discussed in the following chapters.

CHAPTER III

ALTERING THE TIME OF VACCINATION AGAINST RESPIRATORY PATHOGENS TO ENHANCE ANTIBODY RESPONSE AND PERFORMANCE OF FEEDER CATTLE[1]

Introduction

The bovine respiratory disease (BRD) complex is the most common and costly disease in U.S. feedlots (NASS, 2006), and strategies that mitigate incidence of BRD are warranted for optimal welfare and productivity of feedlot cattle (Duff and Galyean, 2007). An example is the adoption of preconditioning programs that include vaccination against pathogens that cause BRD (Martin et al., 1999; Duff and Galyean, 2007). It is common for preconditioned calves to receive a vaccination against BRD pathogens at weaning and a booster 30 d later at feedlot entry (England et al., 2009). However, weaning and feedlot entry are 2 of the most stressful situations encountered by feeder cattle, and vaccine efficacy can be reduced if administered to highly stressed animals (Blecha et al., 1984). Therefore, altering the time of vaccination against BRD has been investigated to enhance health and performance of feeder cattle.

Richeson et al. (2008) compared vaccination against BRD pathogens on arrival at the feedlot or 14 d later and reported that delaying vaccination increased seroconversion[1] to infectious bovine rhinotracheitis during feedlot receiving. Nevertheless, the majority of BRD cases often occur within the first 14 d on feedlot arrival (Kirkpatrick et al., 2008), and delaying the booster by 2 wk may not provide

[1] This is a copy-edited, author-produced version of an article accepted for publication in the Journal of Animal Science. The version of record K. D. Lippolis et al. Altering the time of vaccination against respiratory pathogens to enhance antibody response and performance of feeder cattle. *Journal of Animal Science* (2016) 94 (9): 3987-3995 is available at: https://doi.org/10.2527/jas.2016-0673.

proper immunological protection against BRD pathogens to newly received feeder calves. In addition, vaccination against BRD pathogens has been shown to reduce cattle DMI, ADG, and G:F (Arthington et al., 2013; Rodrigues et al., 2015). Based on this rationale, we hypothesized that anticipating vaccination and a booster against BRD pathogens by 15 d to provide both doses prior to feedlot entry further enhances cattle health and performance during feedlot receiving. Hence, this experiment compared the effects of anticipating, delaying, or vaccinating against BRD at the time of weaning and feedlot entry on growth, DMI, and plasma antibody parameters of feeder cattle.

Materials and Methods

This experiment (d −15 to 75) was conducted at the Oregon State University – Eastern Oregon Agricultural Research Center (Burns station; Burns, OR) and divided into preweaning (d −15 to −1), preconditioning (d 0 to 29), and feedlot receiving (d 30 to 75) phases. All animals were cared for in accordance with acceptable practices and experimental protocols reviewed and approved by the Oregon State University, Institutional Animal Care and Use Committee (number 4738).

Animals and Treatments

Ninety Angus × Hereford calves (n = 69; n = 21 heifers) were used in this experiment. All calves were vaccinated against clostridial diseases (Clostrishield 7; Novartis Animal Health, Bucyrus, KS) and the BRD complex (Virashield 6 + Somnus; Novartis Animal Health) at approximately 45 d of age. On d −18 of the experiment, calves were ranked by sex, BW, and age (215 ± 4 kg initial BW and 184 ± 18 d initial

age) and assigned to 1 of 3 treatments: 1) vaccination at weaning (d 0) and a booster at feedlot entry (d 30; **CON**; n = 30), 2) vaccination 15 d before weaning (d −15) and a booster 15 d before feedlot entry (d 15; **EARLY**; n = 30), and 3) vaccination 15 d after weaning (d 15) and a booster 15 d after feedlot entry (d 45; **DELAYED**; n = 30). Treatment groups contained 23 steers and 7 heifers each and were balanced for initial calf BW and age. Vaccines administered during the experiment were against Clostridium (2 mL, subcutaneously; One Shot Ultra 7; Zoetis, Florham Park, NJ); parainfluenza virus (TSV-2; Zoetis); and infectious bovine rhinotracheitis virus, bovine viral diarrhea virus (**BVDV**) Types 1 and 2, and *M. haemolytica* (**MH**; 2 mL, s.c.; Bovi-Shield Gold One Shot; Zoetis). Calves not receiving a vaccination were administered 4 mL (s.c.) of sterile saline.

From d −15 to −1, calves were managed as a single group with their respective dams in a semiarid rangeland pasture (Ganskopp and Bohnert, 2009). All dams were multiparous, and dam age during the experiment was 6.2 ± 0.7 yr for CON, 5.8 ± 0.6 yr for DELAYED, and 5.9 ± 0.6 yr for EARLY. Calves were weaned and administered an anthelmintic (subcutaneous injection at 1 mL/50 kg of BW of Dectomax; Zoetis) on d 0. From d 0 to 7, calves were managed as a single group in a meadow foxtail pasture (Alopecurus pratensis L.) with ad libitum access to long-stem alfalfa–triticale hay and no concentrate supplementation. On d 8, calves within each treatment were ranked by sex and BW, allocated to 1 of 18 drylot pens (5 calves/pen, 4 steers and 1 heifer; 6 pens/treatment), and fed longstem alfalfa–triticale hay ad libitum until d 29. Calves were not assigned to the drylot pens immediately after weaning so they could acclimate to the absence of their dams as a single group. On d 29, all calves were loaded into a

single double-deck commercial livestock trailer (Legend 50' cattle liner; Barrett LLC, Purcell, OK) and transported 1,440 km. During transport, the driver stopped every 6 h to rest for 60 min but cattle remained in the truck at all times, and total transport time was 24 h. Transport length and duration were selected to elicit the stress challenges of a long haul (Arthington et al., 2008; Cooke et al., 2013c). Minimum, maximum, and average environmental temperatures during transport were −9, 10, and 1°C, respectively, whereas average humidity was 64% and no precipitation was observed. Upon arrival (d 30), calves were unloaded at the same feed yard and with the same pen distribution used prior to transport but allocated to different drylot pens (Cooke et al., 2013c). From d 30 to 75, calves were fed long-stem alfalfa–triticale hay ad libitum and offered corn-based concentrate (Table 3.1) twice daily (0800 and 1600 h), which was offered separately from hay. Water and a commercial mineral mix (Cattleman's Choice; Performix Nutrition Systems, Nampa, ID; contained 14% Ca, 10% P, 16% NaCl, 1.5% Mg, 3,200 mg/kg of Cu, 65 mg/kg of I, 900 mg/kg of Mn, 140 mg/kg of Se, 6,000 mg/kg of Zn, 136,000 IU/kg of vitamin A, 13,000 IU/kg of vitamin D3, and 50 IU/kg of vitamin E) were offered for ad libitum consumption throughout the experimental period (d −15 to 75).

Sampling

Samples of hay and concentrate ingredients were collected weekly, pooled across all weeks, and analyzed for nutrient content by a commercial laboratory (Dairy One Forage Laboratory, Ithaca, NY). All samples were analyzed by wet chemistry procedures for concentrations of CP (method 984.13; AOAC, 2006), ADF (method

973.18 modified for use in an Ankom 200 fiber analyzer [Ankom Technology Corp., Fairport, NY]; AOAC, 2006), and NDF (Van Soest et al., 1991; modified for use in an Ankom 200 fiber analyzer [Ankom Technology Corp.]). Calculations for TDN used the equation proposed by Weiss et al. (1992), whereas NEm and NEg were calculated with the equations proposed by the NRC (2000). Hay nutritional profile was (DM basis) 59% TDN, 59% NDF, 41% ADF, 1.20 Mcal/kg of NEm, 0.62 Mcal/kg of NEg, and 11.3% CP. Concentrate nutritional profile is described in Table 1.

Calf BW was recorded on 2 consecutive days (d −15 and −14, d 0 and 1, d 28 and 29, and d 75 and 76), and values from both days were averaged for ADG calculation. Calves were observed daily (0800 to 1000 h and 1600 to 1800 h) for BRD symptoms according to the subjective criteria described by Berry et al. (2004) and received 0.1 mL/kg of BW of Hexasol LA Solution (Norbrook Inc. USA, Overland Park, KS) when symptoms were observed. Concentrate, hay, and total DMI were evaluated daily from d 8 to 75 from each pen by collecting and weighing refusals daily. Samples of the offered and nonconsumed feed were collected daily from each pen and dried for 96 h at 50°C in forced-air ovens for DM calculation. Hay, concentrate, and total daily DMI of each pen were divided by the number of calves within each pen and ex-pressed as kilograms per calf per day. Total BW gain and DMI of each pen from d 30 to 75 were used for receiving G:F calculation.

Blood samples were collected on d −15, 0, 15, 30, 45, 60, and 75 via jugular venipuncture into commercial blood collection tubes (Vacutainer, 10 mL; Becton, Dickinson and Company, Franklin Lakes, NJ) with 158 US pharmacopeia units of freeze-dried sodium heparin for plasma collection. Blood samples were collected prior

to treatment administration (d −15 to 45) and prior to concentrate and hay feeding of the day (d 15 to 75).

Blood Analysis

All blood samples were placed immediately on ice, centrifuged (2,500 × *g* for 30 min at 4°C) for plasma harvest, and stored at −80°C on the same day of collection. Plasma was analyzed for concentrations of MH leukotoxin antibodies (Confer et al., 1996; Burciaga-Robles et al., 2010) and BVDV type I and II strains (BVDV Ab ELISA number 99-44000; IDEXX Switzerland AG, Liebefeld-Bern, Switzerland; Gonda et al., 2012), which are 2 of the most common pathogens associated with BRD in cattle (Edwards, 2010). Plasma concentrations of antibodies against these pathogens were evaluated based on day of the experiment (d −15 to 75) or based on equivalent days relative to the vaccination and booster.

Statistical Analysis

Calf was considered the experimental unit. Quantitative data were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC), and bi-nary data were analyzed using the GLIMMIX procedure of SAS and Satterthwaite approximation to determine the denominator degrees of freedom for tests of fixed effects. The model statement for BW, ADG, G:F, and morbidity and mortality rates contained the effects of treatment, sex, and the resultant interaction. The model statement for DMI and plasma variables contained the effects of treatment, sex, day, and all resultant interactions. Data were analyzed using calf (pen × treatment × sex). Nevertheless, DMI

and G:F data used pen(treatment) as random variable and did not include sex in the fixed model because DMI was recorded from each pen. G:F that used pen(treatment) as random variable and did not include sex in the fixed model because DMI was recorded from each pen. The specified term for the repeated statements was day, and the subject for DMI and plasma variables were, respectively, pen(treatment) or calf(pen × treatment × sex). The covariance structure used was first-order autoregressive, which provided the smallest Akaike information criterion and, hence, the best fit for all variables analyzed. Although calves were not managed in pens from d −15 to 7, pen was included in random and repeated statements to ensure equal statistical structure across the experimental period. Results are reported as least squares means and were separated using PDIFF. Significance was set at $P \leq 0.05$ and tendencies were determined if $P > 0.05$ and ≤ 0.10. Results are reported according to main effects if no interactions were significant or according to the highest-order interaction detected.

Results and Discussion

Performance Traits

During the preweaning phase, a treatment effect was detected ($P = 0.04$) for ADG (Table 3.2). Calves assigned to EARLY had decreased ($P \leq 0.05$) ADG compared with calves assigned to CON and tended ($P = 0.09$) to have decreased ADG compared with DELAYED calves, whereas ADG was similar ($P = 0.35$) between CON and DELAYED calves. This outcome is supported by Arthington et al. (2013), who observed reduced ADG in heifers vaccinated for MH and *Clostridium* compared with

unvaccinated heifers. However, treatment differences in ADG were not sufficient to impact calf weaning BW, which were similar ($P = 0.76$) across treatments (Table 3.2).

During the preconditioning phase, a treatment × day interaction was detected ($P = 0.03$) for hay DMI, which was less for EARLY and DELAYED calves compared with CON calves from d 15 to 18 (Fig. 3.1). Supporting our findings, Rodrigues et al. (2015) also reported that DMI was reduced due to vaccination against BRD pathogens for 72 h and returned to levels similar to those of nonvaccinated cohorts 4 d after vaccination. Moreover, overall preconditioning hay DMI was greater ($P \leq 0.04$) for CON calves compared with EARLY and DELAYED calves and similar ($P = 0.86$) between EARLY and DELAYED calves (Table 3.3; treatment effect, $P = 0.05$). It is important to note that hay DMI was not evaluated during the initial 7 d of preconditioning and that CON calves received a vaccination at weaning on d 0. Hence, hay DMI was evaluated after the expected decrease in DMI caused by vaccination to CON calves, which helps explaining the treatment effect detected for overall preconditioning hay DMI. Nevertheless, such difference was not sufficient to impact ($P \geq 0.65$) preconditioning ADG or final preconditioning BW (Table 3.2).

During the feedlot receiving phase, no treatment effects were detected ($P \geq 0.22$) for hay, concentrate, and total DMI (Table 3.3). A treatment effect was detected ($P = 0.01$) for feedlot receiving ADG, which was greater ($P \leq 0.01$) for EARLY calves compared with DELAYED and CON calves and similar ($P = 0.87$) between DELAYED and CON calves (Table 2). Yet no treatment differences were detected ($P \geq 0.16$) for receiving G:F (198.5, 193.4, and 175.0 g/kg of G:F for EARLY, CON, and DELAYED calves, respectively; SEM = 8.6) and final receiving BW (Table 3.2).

Rodrigues et al. (2015) also reported that vaccination against BRD pathogens did not impact concentrate intake, despite differences detected in hay DMI. Supporting our findings, Arthington et al. (2013) did not detect differences in total DMI in feeder heifers vaccinated or not against BRD pathogens but did observe an ADG decrease in vaccinated heifers.

These results support our hypothesis that anticipating vaccination against BRD pathogens, in a manner such that both injections are administered prior to feedlot entry, improve receiving performance of feeder cattle. The vaccines administered herein contained a freeze-dried preparation of modified-live virus strains, a product from whole cultures of inactivated MH, and a proprietary (Zoetis) adjuvant formulation. The viral fraction and adjuvant contained in the vaccines used herein assist in recruiting leukocytes to the site of vaccine delivery, which, in turn, synthesize proinflammatory cytokines and elicit a systemic acute-phase response (Carroll and Forsberg, 2007). These responses are known to impair animal performance via several metabolic mechanisms, including fever, reduced appetite, and impaired tissue anabolism (Johnson, 1997; Rodrigues et al., 2015). Nevertheless, the negative impact of vaccination on ADG of EARLY calves during the preweaning phase cannot be fully elucidated as calf DMI was not assessed. Moreover, the greater ADG in EARLY calves during feedlot receiving did not result from increased DMI, suggesting that vaccination against BRD pathogens impacts cattle performance beyond feed intake modulation (Arthington et al., 2013).

Health Variables

A treatment × day interaction was detected ($P < 0.01$) for plasma concentrations of antibodies against MH when data were analyzed based on days relative to the initial vaccination and the booster (Fig. 3.2a). Plasma MH antibody concentrations were less ($P \leq 0.03$) in EARLY calves compared with CON and DELAYED calves 15 and 30 d after the initial vaccination and also greater ($P < 0.01$) for DELAYED vs. CON calves 15 d after the initial vaccination. Conversely, EARLY calves had greater ($P \leq 0.02$) MH antibody concentrations compared with CON and DELAYED calves 15 and 30 d after the booster, which was also greater ($P = 0.05$) for CON vs. DELAYED calves 15 d after the booster. No further treatment differences were detected ($P \geq 0.33$). Circulating concentrations of neutralizing anti-bodies provide an indication of immune protection, dis-ease prevention, and vaccine efficacy in cattle (Howard et al., 1989; Bolin and Ridpath, 1990; Callan, 2001). Supporting our hypothesis, delaying vaccination against BRD pathogens to avoid the stress of weaning enhanced their initial MH antibody response, whereas anticipating vaccination by 15 d impaired such response. This latter outcome was unexpected but can be associated with a potential decrease in nutrient intake of EARLY calves, as suggested by treatment differences in preweaning ADG (Table 3.2; Arthington et al., 2013; Rodrigues et al., 2015), to levels that impaired antibody production by their adaptive immune system (Galyean et al., 1999; Downey et al., 2013; Moriel et al., 2015).

Antibody response to the MH booster among treatments, however, was the opposite of the response to the initial vaccination. A booster vaccination against MH is not required, although this is a common practice in commercial feedlots due to the frequent lack of health history in high-risk receiving cattle (Edwards, 2010). Yet one

of the immunological purposes of a booster vaccination is to provide repeated antigen exposure in calves that lacked an adequate immune response to the initial vaccination (Edwards, 2010). Hence, it seems plausible that EARLY calves benefited from and likely a required booster against MH due to their inadequate MH antibody response to the initial vaccination. Conversely, the same outcome was not observed in DELAYED calves due to their elevated MH antibody response to the initial vaccination, given that existing circulating antibodies can bind to antigen provided by the booster vaccine and prevent its recognition and subsequent antibody production by the adaptive immune system (Zimmerman et al., 2006; Downey et al., 2013).

A treatment × day interaction was also detected ($P < 0.01$) for plasma concentrations of antibodies against MH when data were analyzed based on day of the experiment (Fig. 3.2b). Calves assigned to EARLY had greater ($P \leq 0.05$) MH antibody concentrations compared with calves assigned to CON and DELAYED on d 0 of the experiment, given that only EARLY calves were vaccinated prior to weaning. On d 15 of the experiment, CON calves had the greatest ($P \leq 0.04$) antibody concentrations due to their improved MH antibody response to the initial vaccination compared with EARLY calves and the fact that DELAYED calves were yet to be vaccinated. On d 30 of the experiment, MH antibody concentrations were greater ($P \leq 0.02$) in DELAYED and EARLY calves compared with CON calves. On d 75 of the experiment, MH antibody concentrations were greater ($P = 0.03$) in EARLY calves compared with CON calves. No further treatment differences were detected ($P \geq 0.20$). These results indicate that both EARLY and DELAYED calves had greater plasma concentrations of antibodies against MH at feedlot entry (d 30 of the experiment) compared with CON

calves, suggesting improved immune protection against this pathogen (Callan, 2001) despite differences detected for MH antibody response to the initial and booster vaccinations (Fig. 3.2a).

No treatment differences were detected ($P = 0.33$) when plasma concentrations of antibodies against BVDV were analyzed based on equivalent days relative to the vaccination and booster (Fig. 3.3a). This outcome suggests that, contrarily to MH, treatments did not impact BVDV antibody response to the initial or booster vaccination. It is important to note that increased plasma concentration of antibodies against BVDV were only detected 30 d after the initial vaccination (day effect, $P < 0.01$; Fig. 3.3a). Richeson et al. (2009) also reported a similar interval between vaccination against BVDV and substantial increases in serum BVDV titers, whereas Rodrigues et al. (2015) did not detect such increase within 14 d after vaccination. Hence, the interval between vaccination and synthesis of BVDV antibodies (>15 d) likely prevented benefits of DELAYED calves over CON calves because the immunological consequences of stress endure for up to 15 d (Purdy et al., 2000). Similarly, the antibody response to the initial BVDV vaccination in EARLY calves was not impaired by the decrease in preweaning ADG, given that this outcome was observed at least 15 d before the increase in plasma BVDV antibodies in these calves.

A treatment × day interaction was detected ($P< 0.01$) for plasma concentrations of antibodies against BVDV when data were analyzed based on day of the experiment (Fig. 3.3b). On d 15, EARLY calves had greater ($P\leq 0.04$) antibody concentrations compared with CON and DELAYED calves. On d 30 and 45, BVDV antibody concentrations were greater ($P\leq 0.01$) in EARLY and CON calves compared with

DELAYED calves. No further treatment differences were detected ($P \geq 0.28$). These outcomes can be directly attributed to lack of differences in BVDV antibody response (Fig. 3.3a), treatment design, and interval between vaccination and increased plasma concentrations of BVDV antibodies. Therefore, EARLY and CON calves had greater plasma concentrations of antibodies against BVDV, suggestive of improved immune protection against this pathogen (Callan, 2001), at feedlot entry compared with DELAYED calves.

No treatment effects were detected ($P > 0.05$) for morbidity or mortality data during the preconditioning or feedlot receiving phases (Table 3.4), despite treatment differences being detected for plasma concentrations of MH and BVDV antibodies. Morbidity during the receiving period was not as prevalent compared with values from research conducted at commercial receiving yards (Snowder et al., 2006; Marques et al., 2016), which may have hindered proper assessment of this variable. This outcome can be associated with the fact that although calves were subjected to the stress of weaning and long transportation (Arthington et al., 2008; Cooke et al., 2013c), they returned to the same facility with the same pen members and were not exposed to calves from other sources in a novel environment (Step et al., 2008). Yet it is important to note that morbidity was less ($P = 0.05$) in EARLY calves compared with CON cohorts during the feedlot receiving phase (Table 3.4), despite the lack of main treatment effect ($P = 0.17$) for this variable. According to the results observed herein and the G*power 3 software (Faul et al., 2007), at least 50 calves/treatment were needed to yield a significant ($P \leq 0.05$) main treatment effect for receiving morbidity. Hence, additional research with greater treatment replication and inclusion of commingling stress is

warranted to further investigate how the treatments evaluated herein impact morbidity and mortality rates in high-stress feedlot receiving scenarios.

Overall Conclusions

Collectively, the EARLY treatment resulted in increased plasma concentrations of antibodies against MH and BVDV at feedlot entry and increased ADG during feedlot receiving compared with the CON and DELAYED treatments. Moreover, treatment effects on plasma BVDV and MH antibodies at feedlot entry should not be associated with increased antibody response in EARLY calves but with a greater interval between vaccinations and feedlot entry. Further research is warranted to validate these outcomes in high-stress feedlot receiving scenarios where morbidity and mortality are traditionally greater, as observed herein, including evaluation of antibodies against other BRD pathogens and calf performance until slaughter. Nevertheless, anticipating the vaccination and booster against BRD pathogens to provide both doses prior to feedlot entry appears to be a valid strategy to enhance cattle health and performance during feedlot receiving.

Table 3.1. Ingredient composition and nutrient profile of concentrate offered to cattle during the receiving phase.[1]

Concentrate type	**A**	**B**	**C**	**D**
DM intake per calf, kg/d	1.20	2.40	4.20	5.40
Ingredient, % DM				
Whole corn	68.7	84.6	87.3	87.7
Soybean meal	27.5	13.5	11.6	11.5
Mineral mix[2]	3.8	1.9	1.1	0.8
Nutrient profile[3] (DM basis)				
TDN,[4] %	82	85	86	86
NE_m,[5] Mcal/kg	2.03	2.12	2.14	2.14
NE_g,[5] Mcal/kg	1.39	1.45	1.47	1.48
CP, %	21.5	15.6	14.9	14.8

[1] A = offered for 5 d upon receiving; B = offered for 10 d after concentrate A; C = offered for 10 d after concentrate B; D = offered for 20 d after concentrate C.

[2] Cattleman's Choice (Performix Nutrition Systems, Nampa, ID) containing 14% Ca, 10% P, 16% NaCl, 1.5% Mg, 3,200 mg/kg of Cu, 65 mg/kg of I, 900 mg/kg of Mn, 140 mg/kg of Se, 6,000 mg/kg of Zn, 136,000 IU/kg of vitamin A, 13,000 IU/kg of vitamin D3, and 50 IU/kg of vitamin E.

[3] Values obtained via wet chemistry analysis (Dairy One Forage Laboratory, Ithaca, NY).

[4] Calculated according to the equations described by Weiss et al. (1992).

[5] Calculated according to the equations described by NRC (2000).

Table 3.2. Performance parameters of calves vaccinated against respiratory pathogens at: 1) weaning (d 0) and revaccination at feedlot entry (d 30; **CON**, n = 30), 2) 15 d before weaning (d -15) and revaccination 15 d before feedlot entry (d 15; **EARLY**, n = 30), and 3) 15 d after weaning (d 15) and revaccination 15 d after feedlot entry (d 45; **DELAYED**, n = 30).[1]

Item	EARLY	CON	DELAYED	SEM	*P-value*
BW, kg					
Pre-weaning (d -15)	215	215	215	4	0.99
Weaning (d 0)	220	225	223	4	0.76
Final preconditioning (d 29)	228	234	231	4	0.65
Final feedlot receiving (d 75)	275	273	271	4	0.78
ADG, kg/d					
Pre-weaning (d -15 to -1)	0.38[a]	0.65[b]	0.55[b]	0.08	0.04
Preconditioning (d 0 to 29)	0.26	0.30	0.26	0.07	0.88
Feedlot receiving (d 30 to 75)	1.04[a]	0.87[b]	0.88[b]	0.04	0.01

[1]Vaccines administered were against *Clostridium* (2 mL s.c; One Shot Ultra 7; Zoetis, Florham Park, NJ), parainfluenza virus (TSV-2; Zoetis), infectious bovine rhinotracheitis virus, bovine viral diarrhea Types 1 and 2 viruses, and *Mannheimia haemolytica* (2 mL s.c.; Bovi-Shield Gold One Shot; Zoetis). Calves not receiving vaccination were administered 4 mL (s.c.) of sterile saline. Means with different superscripts differ ($P \leq 0.05$).

Table 3.3. Feed intake parameters (kg/d; DM basis) of calves vaccinated against respiratory pathogens at: 1) weaning (d 0) and revaccination at feedlot entry (d 30; **CON**, n = 30), 2) 15 d before weaning (d -15) and revaccination 15 d before feedlot entry (d 15; **EARLY**, n = 30), and 3) 15 d after weaning (d 15) and revaccination 15 d after feedlot entry (d 45; **DELAYED**, n = 30).[1]

Item	EARLY	CON	DELAYED	SEM	*P-value*
Preconditioning (d 8 to 29)					
Hay	5.08[a]	5.60[b]	5.03[a]	0.16	0.05
Feedlot receiving (d 31 to 75)					
Hay	4.10	3.67	3.98	0.16	0.22
Concentrate	3.72	3.71	3.71	0.07	0.99
Total	7.81	7.39	7.69	0.18	0.28

[1]Vaccines administered were against *Clostridium* (2 mL s.c; One Shot Ultra 7; Zoetis, Florham Park, NJ), parainfluenza virus (TSV-2; Zoetis), infectious bovine rhinotracheitis virus, bovine viral diarrhea Types 1 and 2 viruses, and *Mannheimia haemolytica* (2 mL s.c.; Bovi-Shield Gold One Shot; Zoetis). Calves not receiving vaccination were administered 4 mL (s.c.) of sterile saline. Means with different superscripts differ ($P \leq 0.05$).

Table 3.4. Health responses of calves vaccinated against respiratory pathogens at: 1) weaning (d 0) and revaccination at feedlot entry (d 30; **CON**, n = 30), 2) 15 d before weaning (d -15) and revaccination 15 d before feedlot entry (d 15; **EARLY**, n = 30), and 3) 15 d after weaning (d 15) and revaccination 15 d after feedlot entry (d 45; **DELAYED**, n = 30).[1,2]

Item	EARLY	CON	DELAYED	SEM	*P-value*
Morbidity, %					
Preconditioning, % (d 0 to 30)	27.9	34.5	32.3	9.5	0.88
Feedlot receiving, % (d 31 to 75)	2.1[a]	16.5[b]	9.3[ab]	5.3	0.17
Mortality, %	0.0	4.4	4.4	4.5	0.73
Preconditioning, % (d 0 to 30)	-	-	-	-	-
Feedlot receiving, % (d 31 to 75)	0.0	4.4	4.4	4.5	0.73

[1]Vaccines administered were against *Clostridium* (2 mL s.c; One Shot Ultra 7; Zoetis, Florham Park, NJ), parainfluenza virus (TSV-2; Zoetis), infectious bovine rhinotracheitis virus, bovine viral diarrhea Types 1 and 2 viruses, and *Mannheimia haemolytica* (2 mL s.c.; Bovi-Shield Gold One Shot; Zoetis). Calves not receiving vaccination were administered 4 mL (s.c.) of sterile saline. Means with different superscripts differ ($P \leq 0.05$).

[2] Calves were observed daily for morbidity according to the subjective criteria described by Berry et al. (2004), and received 0.1 mL/kg of BW of Hexasol LA Solution (Norbrook® Inc. USA; Overland Park, KS) when symptoms were observed.

Figure 3.1. Hay DMI during preconditioning in calves vaccinated against respiratory pathogens at: 1) weaning (d 0) and revaccination at feedlot entry (d 30; **CON**, n = 30), 2) 15 d before weaning (d -15) and revaccination 15 d before feedlot entry (d 15; **EARLY**, n = 30), and 3) 15 d after weaning (d 15) and revaccination 15 d after feedlot entry (d 45; **DELAYED**, n = 30). Vaccines administered were against *Clostridium* (2 mL s.c; One Shot Ultra 7; Zoetis, Florham Park, NJ), parainfluenza virus (TSV-2; Zoetis), infectious bovine rhinotracheitis virus, bovine viral diarrhea Types 1 and 2 viruses, and *Mannheimia haemolytica* (2 mL s.c.; Bovi-Shield Gold One Shot; Zoetis). Calves not receiving vaccination were administered 4 mL (s.c.) of sterile saline. A treatment × day interaction was detected ($P < 0.01$). Within days, letters indicate ($P \leq 0.05$); a = CON vs. DELAYED, b = CON vs. EARLY.

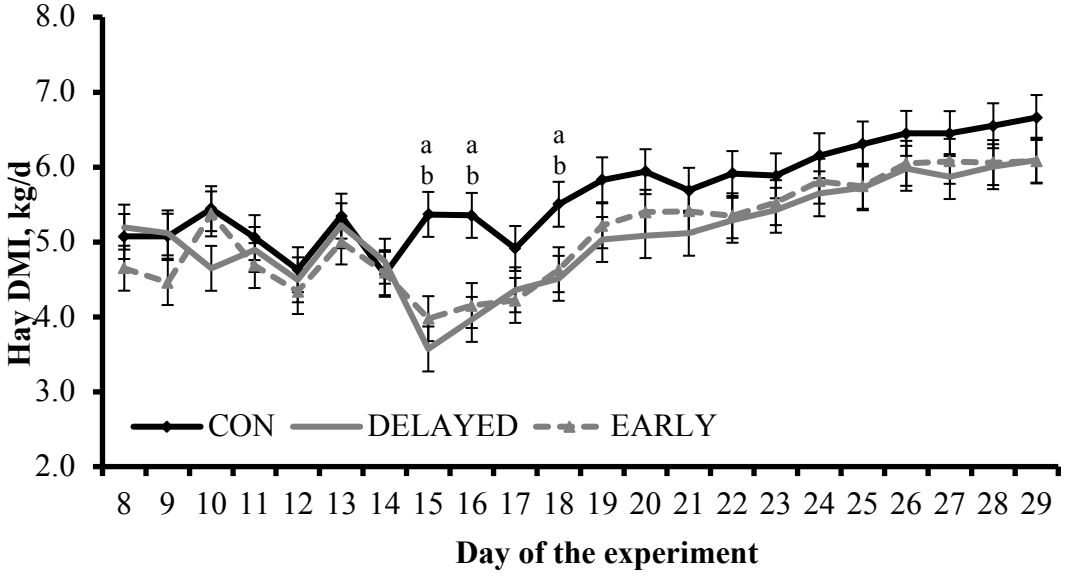


Figure 3.2. Plasma concentrations of antibodies against *Mannheimia haemolytica* (ng/antibody bound) in calves vaccinated against respiratory pathogens at: 1) weaning (d 0) and revaccination at feedlot entry (d 30; **CON**, n = 30), 2) 15 d before weaning (d -15) and revaccination 15 d before feedlot entry (d 15; **EARLY**, n = 30), and 3) 15 d after weaning (d 15) and revaccination 15 d after feedlot entry (d 45; **DELAYED**, n = 30). Vaccines administered were against *Clostridium* (2 mL s.c; One Shot Ultra 7; Zoetis, Florham Park, NJ), parainfluenza virus (TSV-2; Zoetis), infectious bovine rhinotracheitis virus, bovine viral diarrhea Types 1 and 2 viruses, and *M. haemolytica* (2 mL s.c.; Bovi-Shield Gold One Shot; Zoetis). Calves not receiving vaccination were administered 4 mL (s.c.) of sterile saline. Panel A reports values relative to the day of the first (d 0) and revaccination (d 30) within each treatment, and Panel B reports values during the experimental period (d -15 to 75). Treatment × day interactions were detected ($P < 0.01$). Within days, letters indicate ($P \leq 0.05$); a = EARLY vs. DELAYED, b = EARLY vs. CON, c = DELAYED vs. CON.

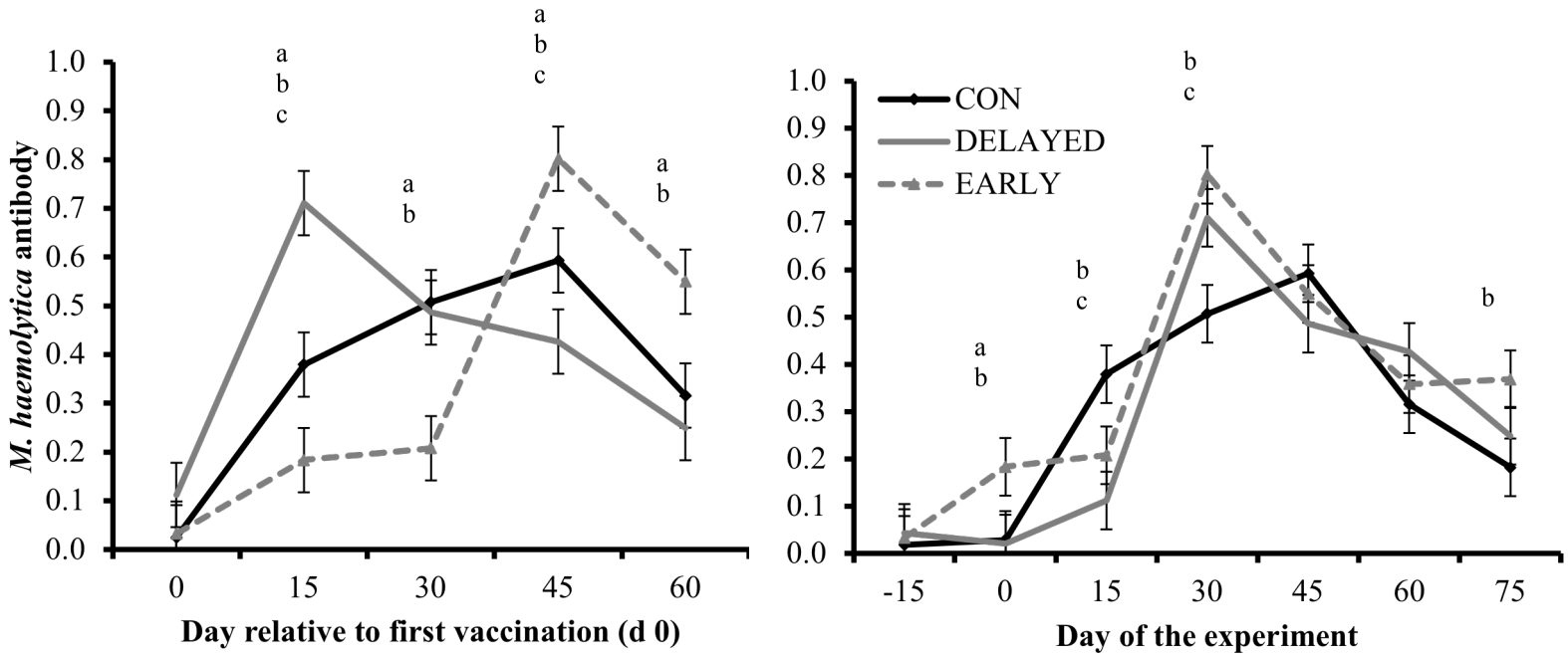
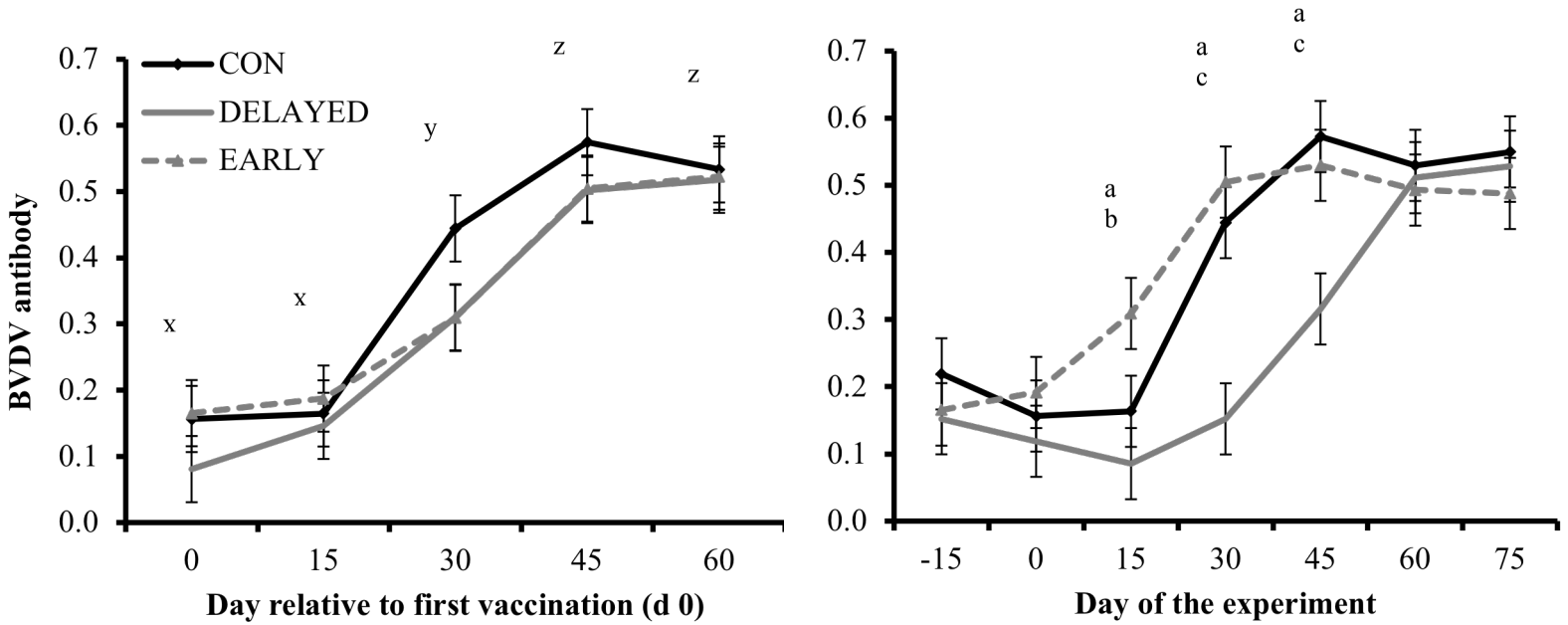


Figure 3.3. Plasma concentrations of antibodies against *bovine viral diarrhea virus* (**BVDV**; sample:positive control ratio as in Gonda et al., 2012) in calves vaccinated against respiratory pathogens at: 1) weaning (d 0) and revaccination at feedlot entry (d 30; **CON**, n = 30), 2) 15 d before weaning (d -15) and revaccination 15 d before feedlot entry (d 15; **EARLY**, n = 30), and 3) 15 d after weaning (d 15) and revaccination 15 d after feedlot entry (d 45; **DELAYED**, n = 30). Vaccines administered were against *Clostridium* (2 mL s.c; One Shot Ultra 7; Zoetis, Florham Park, NJ), parainfluenza virus (TSV-2; Zoetis), infectious bovine rhinotracheitis virus, bovine viral diarrhea Types 1 and 2 viruses, and *M. haemolytica* (2 mL s.c.; Bovi-Shield Gold One Shot; Zoetis). Calves not receiving vaccination were administered 4 mL (s.c.) of sterile saline. Panel A reports values relative to the day of the first (d 0) and revaccination (d 30) within each treatment, and Panel B reports values during the experimental period (d -15 to 75). A day effect was detected in Panel A ($P < 0.01$), and means with different letters (x,y,z) differ ($P < 0.01$). A treatment × day interaction was detected ($P < 0.01$) for Panel B only, and within days letters indicate ($P \leq 0.05$); a = EARLY vs. DELAYED, b = EARLY vs. CON, c = DELAYED vs. CON.



CHAPTER IV

EFFECTS OF ORGANIC COMPLEXED OR INORGANIC CO, CU, MN, AND ZN SUPPLEMENTATION DURING A 45-DAY PRECONDITIONING PERIOD ON PRODUCTIVE AND HEALTH RESPONSES OF FEEDER CATTLE [1]

Introduction

Preconditioning programs prepare weaned beef calves to face stress and immune challenges associated with feedlot entry, mainly through a complete vaccination program and introduction of cattle to dry feeds (Duff and Galyean, 2007). Accordingly, preconditioning programs allow for specific nutritional approaches targeted to optimize cattle health and productive traits post-weaning and during feedlot receiving (Roeber et al., 2001; Arthington et al., 2008). An example of such approach is Zn, Cu, Mn and Co supplementation due to their role on stress, health and growth responses in cattle (Spears, 2000). Moreover, supplementing organic complexed Zn, Cu, Mn and Co during preconditioning may be of further benefit based on their enhanced absorption, retention and biological activity compared with inorganic sulfate sources (Spears, 1996; George et al., 1997; Marques et al., 2016).

Tradition[2]al preconditioning recommendations include a 30-day period between weaning and feedlot entry (Pritchard and Mendez, 1990). Research results indicated that supplementing organic complexed Zn, Cu, Mn and Co to beef cattle during a 30-day preconditioning increased feedlot receiving growth, but not health, compared with cattle non-supplemented or receiving inorganic Zn, Cu, Mn and Co

[1] This is a copy-edited, author-produced version of an article accepted for publication in Animal. The version of record K. Lippolis et al. Effects of organic complexed or inorganic Co, Cu, Mn and Zn supplementation during a 45-day preconditioning period on productive and health responses of feeder cattle. *Animal,* (2017) *11*(11), 1949-1956 is available at: https://doi.org/10.1017/S1751731117001033.

(Dorton et al., 2006). Currently, a 45-day preconditioning program is recommended to allow proper vaccine administration and enhance cattle health during feedlot receiving (Faber et al., 1999). However, the impacts of Zn, Cu, Mn and Co supplementation during a 45-day preconditioning are still unknown and should be investigated, given that extending the supplementation period may further increase the benefits of these trace minerals. Therefore, this experiment evaluated the effects of Cu, Mn, Zn and Co supplementation, either as inorganic or organic complexed sources, during a 45-day preconditioning program on productive, immunity and physiologic parameters of cattle through preconditioning followed by a 58-day feedlot receiving period.

Material and Methods

This experiment was conducted at the Oregon State University – Eastern Oregon Agricultural Research Center (Union Station). Animals utilized were cared for in accordance with acceptable practices and experimental protocols reviewed and approved by the Oregon State University, Institutional Animal Care and Use Committee (no. 4739). The experiment was divided into a preconditioning (day 0 to 45) and feedlot receiving phase (day 46 to 103).

Animals and treatments

In total, 90 Angus × Hereford calves (72 steers and 18 heifers) were weaned at 7 mo (d −1), sorted by sex, weaning BW and age (initial BW = 261 ± 2 kg; initial age = 224 ± 2 d), and allocated to 1 of 18 drylot pens (5 calves/pen, 1 heifer and 4 steers/pen) on d 0, in a manner that pens had equivalent initial BW and age. Pens (14

× 35 m, dirt surfaced with covered feed bunks) were balanced for initial calf BW and age, and randomly assigned to receive a preconditioning concentrate containing one of three treatments: (1) Cu, Co, Mn and Zn sulfate sources (INR; custom blend manufactured by Performix Nutrition Systems, Nampa, ID, USA), (2) Cu, Mn, Co and Zn complexed organic source (AAC; Availa®4; Zinpro Corporation, Eden Prairie, MN, USA) or (3) no Cu, Co, Mn and Zn supple-mentation (CON). The AAC trace mineral source was based on a metal : amino acid complex ratio of 1 : 1 for Zn, Cu and Mn, in addition to Co glucoheptonate (Zinpro Corporation). Steers and heifers were used due to availability of cattle at the research station. Nevertheless, all pens had the same proportion of steers and heifers to ensure that calf sex would not bias the experimental objectives.

During the preconditioning phase (day 0 to 45), cattle received concentrate treatments (Table 4.1) while having free-choice access to orchardgrass (*Dactylis glomerata* L.), long-stem hay and water. The INR and AAC sources were formulated to provide the same daily amount of Cu, Co, Mn and Zn based on 7 g/calf daily of the AAC source as recommended by the manufacturer and previous research with this ingredient (Marques et al., 2016). Concentrate and hay were offered twice daily (0700 and 1500 h) in different sections of the same feed bunk. On days 15, cattle were vaccinated against *Clostridium* (One Shot Ultra 7; Zoetis, Florham Park, NJ, USA), parainfluenza virus, infectious bovine rhinotracheitis virus, bovine respiratory syncytial virus, bovine viral diarrhea virus (BVDV) types 1 and 2, and *M. haemolytica* (MH; Bovi-Shield Gold One Shot; Zoetis). On day 29, cattle were re-vaccinated against all the aforementioned pathogens but for MH (Bovi-Shield Gold 5; Zoetis).

On day 46, cattle were loaded into a single double-deck commercial livestock trailer (Legend 50′ cattle liner; Barrett LLC, Purcell, OK, USA) and transported for 192 km to a commercial feedlot (Lighting Feeders, Nyssa, OR, USA) for a 58-day receiving phase (day 46 to 103). Upon arrival on day 46, cattle were vaccinated against *Clostridium* (Ultrabac 8; Zoetis), parainfluenza virus, infectious bovine rhinotracheitis virus and MH (Pyramid 5 + Presponse; Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, USA), and received anti-helminthic (Vetrimec Plus; VetOne, Boise, ID, USA) and hormonal implant (Component TE 200; Elanco Animal Health, Greensfield, IN, USA). During the feedlot receiving phase, cattle were maintained in a single pen and offered the same free-choice diets (Table 4.2).

Sampling

Feedstuffs.

Samples of hay and concentrate ingredients offered during preconditioning were collected weekly, pooled across all weeks, and analyzed for nutrient content by a commercial laboratory (Dairy One Forage Laboratory, Ithaca, NY, USA). Each sample was analyzed in triplicate by wet chemistry procedures for concentrations of CP (method 984.13; Association of Official Analytical Chemists (AOAC), 2006), ADF (method 973.18 modified for use in an Ankom 200 fiber analyzer, Ankom Technology Corp., Fairport, NY, USA; AOAC, 2006), NDF (Van Soest et al., 1991; modified for Ankom 200 fiber analyzer, Ankom Technology Corp.), macro and trace minerals using inductively coupled plasma emission spectroscopy (Sirois et al., 1991), as well as Se according to method 996.16 of the AOAC (2006). Calculations for net energy for

maintenance and growth were calculated with the equations proposed by the National Research Council (NRC) (2000).

Performance traits.

Calf full BW were recorded on days −1 and 0, days 45 and 46 before truck loading, and on days 102 and 103. Calf BW recorded on consecutive days were used for ADG calculation. Calf ADG during preconditioning was calculated based on initial preconditioning BW (average BW on days -1 and 0) and final preconditioning BW (average BW on days 45 and 46). Calf feedlot receiving BW (average BW on days 102 and 103).

During the preconditioning phase, concentrate, hay and total dry matter (DM) intake were evaluated from each pen by collecting and weighing refusals daily. Samples of the offered and non-consumed feed were collected daily from each pen and dried for 96 h at 50°C in forced-air ovens for DM calculation. Hay, concentrate and total daily DM intake of each pen were divided by the number of cattle within each pen, and expressed as kg per calf/day. Daily intake of Co, Cu, Mn and Zn were estimated by hay and concentrate intake of each pen, in addition to trace mineral content of hay and concentrate (Table 4.1). Total BW gain and DM intake of each pen from day 0 to 46 were used for preconditioning feed efficiency calculation.

Health and physiologic variables.

During preconditioning, cattle were observed daily for sickness, including the BRD symptoms according to the subjective criteria described by Berry et al. (2004),

and received 0.1 ml/kg of BW of Hexasol LA Solution (Norbrook® Inc. USA, Overland Park, KS, USA) when symptoms were observed. During the receiving period, cattle were observed daily for sickness and BRD symptoms (DART system; Zoetis), and received medication according to Wilson et al. (2015).

Liver samples were collected from all animals via needle biopsy on day 0, 22 and 45 of the preconditioning phase via needle biopsy (Tru-Cut biopsy needle; Becton Dickinson, Franklin Lakes, NJ, USA) according to the procedures described by Marques et al. (2016). Liver samples were analyzed via inductively coupled plasma mass spectrometry for concentrations of Co, Cu, Mn and Zn by the Michigan State University – Diagnostic Center for Population and Animal Health (Lansing, MI, USA). Blood samples were collected via jugular venipuncture into commercial heparinized blood collection tubes (Vacutainer, 10 ml; Becton Dickinson) on days 15, 29, 45, 47, 49, 53 and 60 of the experiment. Blood samples were placed immediately on ice, centrifuged (2500 × g for 30 min; 4°C) for plasma harvest, and stored at −80°C on the same day of collection. Plasma samples collected on days 15, 29, 45 and 60 were analyzed for concentrations of MH leukotoxin antibodies (Confer et al., 1996) and BVDV type I and II strains (BVDV Ab ELISA no. 99-44000; IDEXX Switzerland AG, Westbrook, ME, USA; Gonda et al., 2012). Plasma samples collected on days 45 to 60 were analyzed for plasma concentrations of haptoglobin (Cooke and Arthington, 2013) and cortisol (Immulite 1000; Siemens Medical Solutions Diagnostics, Los Angeles, CA, USA). The intra- and inter-assay CV for haptoglobin were 2.2% and 6.1%, respectively. Plasma cortisol was analyzed within a single assay, and the intra-assay CV was 6.5%.

Statistical analysis

Data were analyzed using pen as the experimental unit, with Satterthwaite approximation to determine the denominator degrees of freedom for tests of fixed effects. Quantitative data were analyzed using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC, USA) and binary data were analyzed using the GLIMMIX procedure of SAS (SAS Institute Inc.). Model statements for BW, ADG, feed efficiency, and morbidity and mortality rates within each phase contained the effects of treatment and calf sex as an independent covariate. Model statement for DM intake and plasma variables contained the effects of treatment, day, the resultant interaction and calf sex as an independent covariate. Model statement for liver Co, Cu, Zn and Mn concentrations contained the effects of treatment, day, the resultant interaction, in addition to calf sex and values from day 0 as independent covariates. All data were analyzed using pen(treatment) and calf(pen) as random variables, but for DM intake and feed efficiency that used pen (treatment) as random variable and did not include sex in the fixed model because DM intake was recorded from each pen. The specified term for the repeated statements was day, with pen(treatment) or calf(pen) as subject for DM intake or plasma and liver variables, respectively. The covariance structure used was first-order autoregressive, which provided the smallest Akaike information criterion and hence the best fit for all variables. Results are reported as least square means and were separated using least square differences. Significance was set at $P \leq 0.05$ and tendencies were determined if $P > 0.05$ and ≤ 0.10. Results are reported according to main effects if no interactions were significant, or according to the highest-order interaction detected.

Results

During the 45-day preconditioning phase, no treatment differences were detected ($P \geq 0.47$) for hay, concentrate and total DM intake (Table 4.3). As designed, estimated daily intake of Co, Cu, Mn and Zn were greater ($P \leq 0.01$) in AAC and INR compared with CON cattle (Table 4.3), whereas INR cattle consumed more ($P < 0.01$) Cu and Zn, but less ($P < 0.01$) Co compared with AAC cohorts (Table 4.3). However, no treatment differences were detected ($P \geq 0.17$) during preconditioning for BW, ADG, feed efficiency, as well as morbidity and mortality rates (Table 4.3).

Treatment effects were detected for liver Co and Zn (P 0.03), whereas a treatment × day interaction was detected ($P < 0.01$) for liver Cu concentrations (Table 4.4). No treatment effects were detected ($P = 0.23$) for liver Mn concentrations (Table 4.4). It is important to note that liver Co, Cu, Mn and Zn concentrations were similar ($P \geq 0.15$) among CON, INR and AAC cattle on day 0 of the experiment (0.177, 0.181 and 0.184 ppm of Co, respectively, SEM = 0.006; 44.0, 30.0 and 41.1 ppm of Cu, respectively, SEM = 5.7; 10.8, 10.8 and 10.7 ppm of Mn, respectively, SEM = 0.4; and 271, 286 and 304 ppm of Zn, respectively, SEM = 13), whereas liver Cu, Mn and Zn values were significant ($P \leq 0.04$) covariates. During the preconditioning period, mean liver Co and Zn concentrations were greater ($P \leq 0.02$) in AAC and INR compared with CON, and similar ($P \geq 0.14$) between AAC and INR cattle (Table 4.4). On days 22 and 45, liver Cu concentrations were also greater ($P < 0.01$) in AAC and INR compared with CON, and similar ($P \geq 0.16$) between AAC and INR cattle (Table 4.4). However, liver Cu concentrations increased (day effect, $P < 0.01$) from day 22 to 45 in AAC and INR, but not in CON cattle (day effect, $P < 0.62$).

No treatment effects were detected ($P \geq 0.48$) for plasma concentrations of antibodies against MH and BVDV (Table 4.5), although day effects were detected ($P < 0.01$) for both variables as they increased from day 15 to 60 of the experiment (Table 4.6). No treatment effect was detected ($P = 0.98$) for plasma haptoglobin concentrations (Table 4.5), which peaked (day effect, $P = 0.05$) on day 53 of the experiment (Table 4.6). A treatment effect was detected for plasma cortisol concentrations, which were greater ($P \leq 0.04$) for CON compared with INR and AAC during the experiment, and similar ($P = 0.97$) between INR and AAC cattle (Table 4.5). A day effect was also detected ($P < 0.01$) for plasma cortisol concentrations, which peaked on day 47 followed by a steady decrease until day 60 of the experiment (Table 4.6). During the receiving period, no treatment effects were detected ($P \geq 0.37$) for cattle ADG, final receiving BW, as well as health parameters (Table 4.7).

Discussion

As previously mentioned, steers and heifers were used herein due to cattle availability at the research station, while all pens had the same proportion of steers and heifers. Moreover, all calf performance and immune responses were analyzed using calf sex as an independent covariate, and the treatment × sex interaction was not tested because experimental units were not replicated by calf sex (Marques et al., 2016). Hence, calf sex was properly balanced among experimental units and used to adjust calf-related responses to ensure that calf sex did not bias the experimental outcomes.

As expected based on the experimental design, both INR and AAC treatments increased estimated daily Co, Cu, Mn and Zn intake during the preconditioning period

compared with the CON treatment (Table 4.3). Although daily Co, Cu and Zn intake also differed between AAC and INR cattle, such differences seem biologically irrelevant given that intake (Table 4.3) of these trace minerals was beyond NRC (2000) requirements for growing cattle (0.74 mg/day of Co, 74 mg/ day of Cu, 148 mg/day of Mn and 222 mg/day of Zn). Moreover, daily Cu and Zn intake in CON cattle were below NRC (2000) requirements during the preconditioning phase (Table 4.3).

The similar liver Co, Cu, Mn and Zn concentrations on day 0 indicates that cattle in all treatments had similar, as well as adequate (Kincaid, 2000; McDowell, 2003) Co, Cu, Mn and Zn liver status before the beginning of the experiment. During the preconditioning period, treatment effects detected for liver Co, Cu and Zn corroborate with increased daily intake of these trace minerals in AAC and INR compared with CON cattle (Table 4.4) and are supported by previous research (Stanton et al., 2000; Akins et al., 2013; Marques et al., 2016). Although organic mineral forms are expected to have enhanced absorption, retention and biological activity com-pared with sulfate minerals (Spears, 1996; George et al., 1997), similar liver concentrations of Cu, Co and Zn between AAC and INR cattle validates that differences in estimated daily Co, Cu and Zn intake between AAC and INR were biologically irrelevant. Accordingly, the effects of supplementing organic or inorganic Zn, Cu and Co on liver mineral status of beef cattle have been variable, with research reporting similar effects or advantage in cattle supplemented with organic forms (Stanton et al., 2000; Arthington and Swenson, 2004; Marques et al., 2016). The lack of treatment differences in liver Mn concentrations during preconditioning (Table 4.4) has also been reported by others (Ahola et al., 2004; Marques et al., 2016), suggesting that hepatic

Mn concentrations in ruminants are not influenced by increased dietary Mn intake (Underwood and Suttle, 1999).

It is important to note, however, that liver Co, Cu, Mn and Zn status during preconditioning (Table 4.4) were either marginal or adequate across all treatments (Kincaid, 2000; McDowell, 2003), indicating that inadequate Cu and Zn intake by CON cattle (NRC, 2000) was not sufficient to result in hepatic deficiency for these trace elements. It is well known that Cu, Zn, Mn and Co (as component of vitamin B_{12}; NRC, 2000) play essential roles on growth and immune responses in cattle (Spears, 2000). Therefore, marginal and adequate liver status of these trace minerals among all treatments likely contributed to the similar preconditioning DM intake, ADG, feed efficiency and morbidity (Table 4.3). The same rationale can be applied to the lack of treatment differences for plasma concentrations of antibody against MH and BVDV (Table 4.5). These variables similarly increased among treatments following initial vaccination (Table 4.6), suggesting similar vaccine efficacy and subsequent immune protection against these pathogens in AAC, INR and CON cattle during preconditioning and feedlot receiving (Callan, 2001). Contrary to our findings, George et al. (1997) reported that heifers supplemented with organic complexed Co, Cu, Mn and Zn had improved antibody titer response to infectious bovine rhinotracheitis virus vaccination compared with heifers supplemented with inorganic sources of these trace elements. However, George et al. (1997) did not evaluate liver status of these trace minerals, and perhaps the organic complexed treatments evaluated by these authors impacted liver Co, Cu, Zn and Mn status differently than herein, such as by replenishing hepatic deficiencies.

Plasma cortisol and haptoglobin concentrations peaked on days 47 and 53 of the experiment, respectively, as expected based on the neuroendocrine stress response and acute-phase protein reaction elicited by transport and feedlot entry (Cooke et al., 2011; Cooke et al., 2013c). However, elevated cortisol has been positively associated with plasma haptoglobin concentrations (Cooke et al., 2012; Cooke et al., 2013c), while the greater mean plasma cortisol concentration in CON cattle from days 45 to 60 did not yield a similar haptoglobin response. These outcomes suggest that Co, Cu, Zn and Mn supplementation to feeder cattle during preconditioning, either as sulfate or organic complexed sources, alleviated the neuroendocrine stress response elicited by transport and feedlot entry without impacting the resultant acute-phase protein reaction (Carroll and Forsberg, 2007).

As previously mentioned, Dorton et al. (2006) reported increased feedlot receiving ADG when beef cattle were supplemented with organic complexed Zn, Cu, Mn and Co during a 30-day preconditioning. Based on these outcomes, we hypothesized that supplementing cattle with an organic complexed Zn, Cu, Mn and Co during a 45-day pre-conditioning program would yield similar or greater benefits as reported by Dorton et al. (2006), mainly due to increased supplementation length. However, receiving performance and health parameters were similar among CON, INR and AAC cattle (Table 4.7), which should also be attributed to proper liver status of Co, Cu, Zn and Mn in all treatment groups during the preconditioning period. Nonetheless, Dorton et al. (2006) did not evaluate liver status of these trace minerals, and it is unknown if outcomes reported by these authors are related to treatment effects on replenishing hepatic deficiencies of these trace minerals. Dorton et al. (2006) also

maintained different Co, Cu, Zn and Mn supple-mentation strategies during the 28-day receiving period, while in the present experiment all cattle were offered the same feedlot receiving diet (Table 4.2). Thus, receiving performance may also be impacted when Co, Cu, Zn and Mn supplementation is altered during preconditioning and receiving period, as in Dorton et al. (2006).

Morbidity during the receiving period in this experiment (Table 4.7) was not as prevalent compared with values from research conducted at commercial receiving yards (Snowder et al., 2006; Marques et al., 2016). In fact, calves utilized herein were subjected to the stress of weaning, transportation, as well as exposure to cattle from other sources in a novel environment during feedlot receiving (Arthington et al., 2008; Step et al., 2008; Cooke et al., 2013c). Hence, reduced morbidity during feedlot receiving was unexpected but can be attributed to an effective 45-day preconditioning program (Faber et al., 1999; Duff and Galyean, 2007) independent of Co, Cu, Zn and Mn supplementation, which may have also contributed to the lack of treatments effects on receiving performance and health variables.

Conclusions

Collectively, results from this experiment indicate that supplementing beef cattle with an inorganic or organic complexed source of Co, Cu, Mn and Zn during a 45-day preconditioning program increased liver concentrations of Co, Zn and Cu through preconditioning and reduced plasma cortisol concentrations during the period comprising transport and feedlot entry, but did not impact cattle performance and health responses during preconditioning and a 58-day receiving period. It is important to note,

how-ever, that cattle evaluated herein had adequate liver status of Co, Cu, Mn and Zn at the beginning of the experiment. Hence, additional research is warranted to further assess the impacts of inorganic or organic complexed sources of Co, Cu, Mn and Zn on performance and health responses of cattle preconditioned for 45 days, particularly cattle deficient in these trace minerals and experiencing elevated morbidity rates during feedlot receiving.

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Table 4.1. Ingredient composition and nutrient profile of orchardgrass (Dactylis glomerata L.), long-stem hay and concentrates containing no (**CON**; n = 6), inorganic (**INR**; n = 6) or organic complexed (**AAC**; n = 6) sources of supplemental Cu, Co, Mn and Zn, and offered to cattle during a 45-day preconditioning

		Preconditioning Concentrate		
Item	**Grass Hay**	**CON**	**INR**	**AAC**
Ingredients (as-fed basis) (kg/day)	-			
Ground corn (kg/day)	-	2.27	2.27	2.27
Soybean meal (kg/day)	-	0.36	0.36	0.36
Macromineral mix[1] (g/day)	-	31.8	31.8	31.8
Inorganic trace mix[2] (g/day)	-	-	4.32	-
Organic trace mix[3] (g/day)	-	-		
Nutrient profile (dry matter basis basis)				
Net energy for maintenance[4] (Mcal/kg)	1.19	2.23	2.23	2.23
Net energy for growth[4] (Mcal/kg)	0.62	1.46	1.46	1.46
CP (%)	12.1	14.6	14.6	14.6
Ca (%)	0.35	0.29	0.29	0.29
P (%)	0.30	0.44	0.44	0.44
Mg (%)	0.21	0.12	0.12	0.12
K (%)	3.55	0.60	0.60	0.60
Na (%)	0.01	0.10	0.10	0.10
S (%)	0.25	0.16	0.18	0.16
Co (mg/kg)	0.32	0.12	5.77	6.31
Cu (mg/kg)	7.00	3.50	58.9	56.9
Fe (mg/kg)	442	85.8	86.0	85.5
Mn (mg/kg)	212	10.86	98.6	89.0
Se (mg/kg)	0.02	2.56	2.56	2.56
Zn (mg/kg)	16.5	19.67	177.6	171.8

[1]Containing (dry matter basis) 571.1 g/kg $CaHPO_4$, 190 g/kg NaCl, 164.1 $CaCO_3$, 31.3 g/kg MgO, 16.8 g/kg Na_2O_3Se 1%, 15 g/kg KCl, 10 g/kg $MgCl_2$, 0.8 g/kg vitamin A 1000, 0.6 g/kg vitamin E 50%, 0.2 g/kg vitamin D 500 and 0.1 g/kg $C_2H_{10}I_2N_2$ 79.5%.
[2]Containing (dry matter basis) 500 g/kg of ground corn, 231 g/kg $ZnSO_4$, 147 g/kg $MnSO_4$, 114 g/kg $CuSO_4$ and 8 g/kg of $CoSO_4$.
[3]Availa®4 (Zinpro Corporation, Eden Prairie, MN, USA), which contained (dry matter basis) 5.15% Zn from 1 : 1 Zn and amino acid complex, 2.86% Mn
from 1 : 1 Mn and amino acid complex, 1.80% Cu from 1 : 1 Cu and amino acid complex and 0.18% Co from Co glucoheptonate.
[4]Calculations for net energy for maintenance and growth were calculated with the equations proposed by the NRC (2000).

Table 4.2. Ingredient composition (as-fed basis) of receiving diets offered (free-choice) to cattle

	Receiving diets[1]	
Ingredients (% as-fed)	**CON**	**INR**
Alfalfa hay	55.0	9.0
Canola meal	12.0	0.0
Triticale	0.0	15.0
Corn silage	0.0	25.0
High-moisture corn	29.0	18.0
Wheat	0.0	17.0

[1]Diet A was offered for 24 days upon arrival; diet B was offered for 33 days after diet A.

[2]Customized blend of minerals, vitamins and feed additives (Performix Nutrition Systems, Nampa, ID, USA), which contained 1/3 of Zn, Mn and Cu as metal:amino acid complexes (Zinpro Corporation, Eden Prairie, MN, USA) and 2/3 as sulfate sources.

Table 4.3. Intake, performance and health responses during a 45-day preconditioning from beef cattle receiving a pre-conditioning concentrate containing no (**CON**; n = 6), inorganic (**INR**; n = 6) or organic complexed (**AAC**; n = 6) sources of supplemental Cu, Co, Mn and Zn[1,2]

Item	CON	INR	AAC	SEM	P-value
Intake parameters (DM basis)					
Hay (kg/day)	5.28	5.20	5.36	0.11	0.62
Concentrate (kg/day)	2.09	2.09	2.09	0.002	0.47
Total (kg/day)	7.37	7.29	7.45	0.11	0.64
Co (mg/day)	1.91[a]	13.69[b]	14.86[c]	0.04	<0.01
Cu (mg/day)	44.3[a]	159.5[b]	156.4[c]	0.9	<0.01
Mn (mg/day)	1144[a]	1309[b]	1323[b]	23	<0.01
Zn (mg/day)	128[a]	457[b]	447[c]	2	<0.01
Performance parameters					
Initial BW (kg)	256	258	257	4	0.89
Final BW (kg)	308	309	311	5	0.86
Average daily gain (kg/day)	1.16	1.12	1.21	0.04	0.17
Feed efficiency (g/kg)	164	160	169	4	0.30
Health parameters[3]					
Morbidity (%)	16.7	33.3	16.7	9.0	0.29
Mortality (%)	–	–	–	–	–

[1]INR and AAC cows received the same amount of Cu, Co, Mn and Zn from sulfate sources or Availa®4 (Zinpro Corporation, Eden Prairie, MN, USA).

[2]Concentrate, hay and total dry matter intake were evaluated daily from each pen by collecting and weighing refusals daily, divided by the number of animals within each pen, and expressed as kg per calf/day. Daily intake of Co, Cu, Mn and Zn were estimated by hay and concentrate intake of each pen, in addition to trace mineral content of hay and concentrate. Calf average daily gain was calculated based on initial preconditioning BW (average from days −1 and 0) and final preconditioning BW (average from days 45 and 46). Feed efficiency was calculated based on total BW gain (g) divided by total dry matter intake (kg) from day 0 to 45.

[3]Calves were observed daily for morbidity according to the subjective criteria described by Berry et al. (2004), and received 0.1 ml/kg of BW of Hexasol LA Solution (Norbrook® Inc. USA, Overland Park, KS, USA) when symptoms were observed.

[a,b,c]Within rows, Means with different superscript letters differ ($P \leq 0.05$).

Table 4.4. Liver concentrations of Co, Cu, Mn and Zn in beef cattle receiving concentrate containing no (**CON**; n = 6), inorganic (**INR**; n = 6) or organic complexed (**AAC**; n = 6) sources of supplemental Cu, Co, Mn and Zn during a 45-day preconditioning program[1,2]

Item	CON	INR	AAC	SEM	P-value
Co (ppm)	0.102[a]	0.871[b]	0.963[b]	0.044	<0.01
Cu (ppm)					
Day 22	34.8[a]	134.1[b]	119.8[b]	9.1	<0.01
Day 45	40.0[a]	194.1[b]	176.4[b]	9.1	<0.01
Mn (ppm)	9.6	10.0	10.3	0.3	0.23
Zn (ppm)	248[a]	273[b]	272[b]	8	0.03

[1]INR and AAC cows received the same amount of Cu, Co, Mn and Zn from sulfate sources or Availa®4 (Zinpro Corporation, Eden Prairie, MN, USA).

[2]Liver samples were collected at the beginning (day 0), and on days 22 and 45 of the preconditioning period via needle biopsy (Marques et al., 2016). Values on day 0 served as independent covariate. Concentrations of CO, Cu, Mn and Zn were determined by the Michigan State University's Diagnostic Center for Population & Animal Health (Lansing, MI, USA).

[a,b,c]Within rows, Means with different superscript letters differ ($P \leq 0.05$). A treatment×day interaction was detected for liver Cu concentrations ($P < 0.01$), but not for liver Co, Mn and Zn concentrations ($P \geq 0.72$).

Table 4.5. Plasma concentrations of antibodies against *Mannheimia haemolytica* (**MH**; ng/antibody bound), bovine viral diarrhea viruses (**BVDV**; sample : positive control ratio as in Gonda et al., 2012), as well as cortisol (ng/ml) and haptoglobin (µg/ml) in beef cattle receiving concentrate containing no (**CON**; n = 6), inorganic (**INR**; n = 6) or organic complexed (**AAC**; n = 6) sources of supplemental Cu, Co, Mn and Zn during a 45-day preconditioning program[1,2,3]

Item	CON	INR	AAC	SEM	P-value
MH	0.532	0.528	0.549	0.055	0.96
BVDV	0.946	1.024	0.929	0.060	0.48
Haptoglobin	0.139	0.143	0.137	0.025	0.98
Cortisol	36.5[a]	31.3[b]	31.4[b]	1.7	0.04

[1]INR and AAC cows received the same amount of Cu, Co, Mn and Zn from sulfate sources or Availa®4 (Zinpro Corporation, Eden Prairie, MN, USA).

[2]Calves were preconditioned from day 0 to 45 of the experiment, and trans-ported for 192 km to a commercial feedlot on day 46, where they remained for 58 days (day 46 to 103 of the experiment). Cattle were vaccinated against Clostridium (One Shot Ultra 7; Zoetis, Florham Park, NJ, USA), parainfluenza virus, infectious bovine rhinotracheitis virus, BVDV types 1 and 2, and MH (Bovi-Shield Gold One Shot; Zoetis) on days 15 and 29. On day 46, cattle were vaccinated against Clostridium (Ultrabac 8; Zoetis), parainfluenza virus, infectious bovine rhinotracheitis virus and MH (Pyramid 5 + Presponse; Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, USA).

[3]Blood samples were collected for MH and BVDV analyses on days 15, 29, 45 and 60, and for cortisol and haptoglobin analyses on days 45, 47, 49, 53 and 60 of the experiment.

[a,b]Within rows, means with different superscript letters differ ($P < 0.05$).

Table 4.6. Plasma concentrations of antibodies against *Mannheimia haemolytica* (**MH**; ng/antibody bound), bovine viral diarrhea virus (**BVDV**; sample : positive control ratio as in Gonda et al., 2012), as well as cortisol (ng/ml) and haptoglobin (μg/ml) in beef cattle during a 45-day preconditioning and 58-day feedlot receiving period[1,2,3]

	MH	BVDV	Haptoglobin	Cortisol
Day				
15	0.183[a]	0.903[a]	-	-
29	0.685[b]	0.942[b]	-	-
45	0.695[b]	1.008[c]	0.119[a]	31.5[a]
47	-	-	0.118[a]	38.3[b]
49	-	-	0.151[a]	35.6[c]
53	-	-	0.182[b]	33.5[ac]
60	0.581[c]	1.013[c]	0.129[a]	26.3[d]
SEM	0.040	0.039	0.025	1.4
P-value	<0.01	<0.01	0.05	<0.01

[1]Calves were preconditioned from day 0 to 45 of the experiment, and trans-ported for 192 km to a commercial feedlot on day 46, where they remained for 58 days (days 46 to 103 of the experiment).

[2]Cattle were vaccinated against Clostridium (One Shot Ultra 7; Zoetis, Florham Park, NJ, USA), parainfluenza virus, infectious bovine rhinotracheitis virus, BVDV types 1 and 2, and MH (Bovi-Shield Gold One Shot; Zoetis) on days 15 and 29. On day 46, cattle were vaccinated against Clostridium (Ultrabac 8; Zoetis), parainfluenza virus, infectious bovine rhinotracheitis virus and MH (Pyramid 5 + Presponse; Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT, USA).

[3]Blood samples were collected for MH and BVDV analyses on days 15, 29, 45 and 60, and for cortisol and haptoglobin analyses on days 45, 47, 49, 53 and 60 of the experiment.

[a,b,c,d]Within columns, means with different superscript letters differ ($P < 0.05$).

Table 4.7. Performance and health responses during a 58-day feedlot receiving from beef cattle offered a concentrate containing no (**CON**; n = 6), inorganic (**INR**; n = 6) or organic complexed (**AAC**; n = 6) sources of supplemental Cu, Co, Mn and Zn only during a 45-day preconditioning[1,2,3]

Item	CON	INR	AAC	SEM	P-value
Performance parameters					
Average daily gain (kg/day)	0.95	0.98	0.96	0.05	0.44
Final receiving BW (kg)	367	366	367	5	0.51
Health parameters					
Morbidity (%)	0.0	3.0	0.0	2.1	0.37
Mortality (%)	0.0	3.0	0.0	2.1	0.37

[1]INR and AAC cows received the same amount of Cu, Co, Mn and Zn from sulfate sources or Availa®4 (Zinpro Corporation, Eden Prairie, MN, USA).

[2]Calves were preconditioned from day 0 to 45 of the experiment, and trans-ported for 192 km to a commercial feedlot (Lighting Feeders, Nyssa, OR, USA) on day 46, where they remained for 58 days (days 46 to 103 of the experiment). Calves were observed daily for morbidity according to the DART system (Zoetis, Florham Park, NJ, USA) and received medication according to the management criteria of the commercial feedlot.

[3]Calf average daily gain was calculated based on final preconditioning BW (average from days 45 and 46) and final receiving BW (average from days 102 and 103).

CHAPTER V

PHYSIOLOGIC, HEALTH, AND PERFORMANCE RESPONSES OF BEEF STEERS SUPPLEMENTED WITH AN IMMUNOMODULATORY FEED INGREDIENT DURING FEEDLOT RECEIVING [1]

Introduction

Feedlot receiving is one of the most critical phases of the beef production cycle, when cattle are ex-posed to a multitude of stress and health challenges that directly impact their welfare and productivity (Duff and Galyean, 2007). As examples, receiving cattle often experience long road transport and are immediately subjected to commingling with different animals and exposure to novel diets and environments, which are known to impair their immune system and growth metabolism (Cooke, 2017). Accordingly, the incidence of BRD is elevated during feedlot receiving, despite vaccination against BRD pathogens and efforts to minimize the aforementioned stressors (Kirkpatrick et al., 2008).[3]

Prophylactic medication with feed-grade antimicrobials is often effective in mitigating BRD incidence during feedlot receiving (Wilson et al., 2017). However, with increased regulations regarding the use of feed-grade antimicrobials in livestock systems (U.S. Food and Drug Administration, 2015), alternative dietary strategies that enhance the immune function of receiving cattle are warranted. These include the use of nonantibiotic feed ingredients with immunomodulatory properties, such as OmniGen-AF (**OMN**; Phibro Animal Health Corp., Teaneck, NJ) and Immune Primer

[1] This is a copy-edited, author-produced version of an article accepted for publication in the Journal of Animal Science. The version of record K. D. Lippolis et al. Physiologic, health, and performance responses of beef steers supplemented with an immunomodulatory feed ingredient during feedlot receiving. *Journal of Animal Sciences* (2017) 95 (11): 4945-4957 is available at: https://doi.org/10.2527/jas2017.1837

formulas (2 oral capsules of Stocker Immune Primer [Ramaekers Nutrition, Santa Cruz, CA] on d 0 + 15 g/steer daily [as-fed basis] of Stocker Preconditioned Premix [Ramaekers Nutrition] from d 7 to 30 [**IPF**]). The OMN was recently shown to improve milk production and innate immunity parameters in transition dairy cows (Brandão et al., 2016). The products contained in the IPF treatment are based on transfer factor proteins and lactate-producing probiotics, which are associated with improved immunity in humans and cattle (Fudenberg and Fudenberg, 1989; Krehbiel et al., 2003). Based on this information, we hypothesized that OMN or IPF are dietary alternatives to improve cattle immunocompetence and productivity during feedlot receiving. Therefore, this experiment evaluated the effects of supplementing OMN or IPF products on performance, health, and physiological responses of receiving cattle.

Materials and Methods

This experiment was conducted at the Oregon State University – Eastern Oregon Agricultural Research Center (Union station; Union, OR). All animals were cared for in accordance with acceptable practices and experimental protocols reviewed and approved by the Oregon State University Institutional Animal Care and Use Committee (number 4851).

Animals and Treatments

One hundred eight Angus × Hereford steers were purchased from a commercial auction yard (Producers Livestock Marketing Association, Vale, OR) and used in this experiment (d 0 to 80). Steers originated from 7 cow–calf operations located in eastern

and central Oregon, and no health or management history of the steers was available at the time of purchase (d −2). Steers were loaded into a double-deck commercial livestock trailer (Legend 50-foot [15.24 m] cattle liner; Barrett Trailers, LLC, Purcell, OK) at the auction yard (d −2; 1800 h) and transported for 800 km to elicit the stress of a long haul (Cooke et al., 2013c). During transport, the driver stopped once after 6 h of driving to rest for 60 min, whereas total transport time was 12 h. Steers remained in the truck throughout the 12-h transportation period. Minimum, maximum, and average environmental temperatures during transport were −1, 23, and 11°C, respectively, whereas average humidity was 37% and no precipitation was observed.

On d −1, steers were unloaded (0600 h) at the Eastern Oregon Agricultural Research Center, immediately weighed (220 ± 2 kg initial shrunk BW), and maintained as a single group in a drylot pen (80 by 40 m) with ad libitum access to orchardgrass (*Dactylis glomerata* L.) hay, water, and a commercial mineral mix (described in Table 1) for 24 h. On d 0, steers were ranked according to source and shrunk BW and allocated to 1 of 18 drylot pens (35 by 15 m; 6 steers/pen) in a manner such that pens had equivalent initial shrunk BW and steers from 3 different sources to stimulate the stress of commingling (Step et al., 2008). Pens were assigned to receive 1 of 3 treatments: 1) no immuno-modulatory ingredient supplementation during feedlot receiving (**CON**; $n = 6$), 2) supplementation with OMN (22 g/steer daily, as-fed basis; Phibro Animal Health Corp.) from d 0 to 30 ($n = 6$), or 3) administration of IPF products (Ramaekers Nutrition), which was 2 oral capsules of Stocker Immune Primer on d 0 + 15 g/steer daily (as-fed basis) of Stocker Preconditioned Premix from d 7 to 30 ($n = 6$). Pens were assigned to treatments in a manner such that the OMN,

IPF, and CON were balanced for initial shrunk BW and calf source and they contained steers from each of the 7 cow–calf sources.

According to the manufacturer, OMN contains a mixture of active dried *Saccharomyces cerevisiae*, dried *Trichoderma longibrachiatum* fermentation product, niacin, vitamin B_{12}, riboflavin-5-phosphate, d-calcium pantothenate, choline chloride, biotin, thiamine monohydrate, pyridoxine hydrochloride, menodione dimethylpyrimidinol bisulfate, folic acid, calcium aluminosilicate, sodium aluminosilicate, diatomaceous earth, calcium carbonate, rice hulls, and mineral oil (the full formulation is proprietary). Both IPF products, Stocker Immune Primer capsules and the Stocker Preconditioned Premix, have similar composition and are based on transfer factor proteins extracted from bovine colostrum and egg yolks, plant-derived heteropolysaccharides, lactate-producing probiotics, vitamins, and minerals. The inclusion and administration rate of OMN and IPF products were according to manufacturer's recommendations for growing cattle.

From d 0 to 80, steers had free-choice access to orchardgrass (*D. glomerata* L.) hay and water and received a corn-based concentrate (Table 5.1). Hay and concentrate were offered (0800 h) separately in different sections of the feed bunk (d 0 to 80). The OMN (d 0 to 30) and IPF Stocker Preconditioned Premix (d 7 to 30) were mixed daily with the concentrate, whereas IPF oral capsules of Stocker Immune Primer were administered (d 0) using a bolus applicator provided by the manufacturer (Ramaekers Nutrition). From d 31 to 80, all steers received diets without the addition OMN or IPF (Table 1). On d 0, steers were vaccinated against *Clostridium* and *M. haemolytica* (One Shot Ultra 7; Zoetis Inc., Florham Park, NJ) and infectious bovine rhinotracheitis,

bovine viral diarrhea complex, parainfluenza-3 virus (**PI3**), and bovine respiratory syncytial virus (**BRSV**; Bovi-Shield Gold 5; Zoetis Inc.) and were administered an anthelmintic (Dectomax; Zoetis Inc.). On d 21, steers were revaccinated against *Clostridium* (Ultrabac 8; Zoetis Inc.) and infectious bovine rhinotracheitis virus, bovine viral diarrhea complex, PI3, and BRSV (Bovi-Shield Gold 5; Zoetis Inc.).

Sampling

Samples of hay and concentrate ingredients were collected weekly, pooled across all weeks, and analyzed for nutrient content by a commercial laboratory (Dairy One Forage Laboratory, Ithaca, NY). All samples were analyzed by wet chemistry procedures for concentrations of CP (method 984.13; AOAC, 2006), ADF (method 973.18, modified for use in an Ankom 200 fiber analyzer [ANKOM Technology Corp., Fairport, NY]; AOAC, 2006), and NDF (Van Soest et al., 1991; modified for use in an Ankom 200 fiber analyzer [ANKOM Technology Corp.]). Calculations for TDN used the equation proposed by Weiss et al. (1992), whereas NEm and NEg were calculated with the equations proposed by the NRC (1996). The hay nutritional profile was (DM basis) 57% TDN, 59.7% NDF, 38.1% ADF, 1.12 Mcal/ kg of NEm, 0.57 Mcal/kg of NEg, and 11.0% CP. The nutrient profile of the concentrate is described in Table 5.1.

Steer full BW was recorded on d 0, 3, 7, 10, 14, 21, 31, 42, 73, and 80 of the experiment at 0700 h, prior to the hay and concentrate feeding of the day. Shrunk BW was recorded on d 81, after 16 h of water and feed withdrawal. Shrunk BW values from d −1 and 81 were used to calculate steer ADG during the experiment. Concentrate, hay, and total DMI were evaluated daily from d 0 to 80 from each pen by collecting and

weighing offered and nonconsumed feed. All samples were dried for 96 h at 50°C in forced-air ovens for DM calculation. Hay, concentrate, and total daily DMI of each pen were divided by the number of steers within each pen and expressed as kilograms per steer per day. Total BW gain and DMI of each pen were used for G:F calculation.

Steers were observed daily for BRD signs according to the DART system (Zoetis Inc.) and received anti-microbial treatment as described by Wilson et al. (2015). Moreover, IPF steers diagnosed with BRD also received 2 oral capsules of Stocker Immune Primer (Ramaekers Nutrition) concurrently with each antimicrobial administration, as recommended by the manufacturer.

Blood samples were collected from all steers, concurrently with full BW evaluation from d 0 to 73, into commercial blood collection tubes (Vacutainer, 10 mL; Becton, Dickinson and Company, Franklin Lakes, NJ) containing no additive or containing freeze-dried sodium heparin for serum and plasma collection, respectively. Blood samples were also collected from 3 steers/pen, which were randomly selected on d −1, into PAXgene tubes (BD Diagnostic Systems, Sparks, MD) for whole-blood RNA extraction. These samples were collected on d 0, 3, 7, 10, and 14 for mRNA expression analysis of innate immunity genes (Table 5.2) to assess such response during the initial 2 wk of feedlot receiving, when cattle are coping with the stressors associated with feedlot entry (Duff and Galyean, 2007; Cooke, 2017; Wilson et al., 2017).

Blood Laboratorial Analyses

Plasma and Serum Samples. After collection, all blood samples were immediately placed on ice, centrifuged (2,500 × *g* for 30 min at 4°C) for plasma or serum harvest, and stored at −80°C on the same day of collection. Plasma samples collected from d 0 to 31 were analyzed for cortisol (Immulite 1000; Siemens Medical Solutions Diagnostics, Inc., Los Angeles, CA) and haptoglobin (Cooke and Arthington, 2013) concentrations, given that adrenocortical and acute-phase protein responses in receiving cattle return to baseline levels within 4 wk after feedlot entry (Cooke, 2017). Plasma samples collected on d 0, 21, 42, and 73 were analyzed for IGF-I concentrations (Immulite 1000; Siemens Medical Solutions Diagnostics, Inc.) to metabolically assess steer nutritional status throughout the experimental period (Hess et al., 2005). The intra- and interassay CV for haptoglobin were 2.4 and 8.0%, respectively. Plasma IGF-I and cortisol were analyzed within single assays, and the intra-assay CV were 3.1 and 4.1%, respectively. Serum samples collected on d 0, 10, 21, 31, and 42 were analyzed for antibody titers against BRSV, bovine herpesvirus-1 (**BHV-1**), bovine viral diarrhea virus-1 (**BVD-1**), and PI3 using virus neutralization tests and for antibodies against *M. haemolytica* using a quantitative agglutination test (Texas A&M Veterinary Medical Diagnostic Laboratory, Amarillo, TX).

PAXgene Samples. Total RNA was extracted using the PAXgene Blood RNA Kit (QIAGEN Inc., Valencia, CA). Quantity and quality of isolated RNA were assessed using UV absorbance (NanoDrop Lite; Thermo Fisher Scientific Inc., Wilmington, DE) at 260 nm and a 260:280 nm ratio, respectively (Fleige and Pfaffl, 2006). Extracted RNA (120 ng) was reverse transcribed using the High Capacity cDNA Reverse

Transcription Kit with random hexamers (Applied Biosystems, Inc., Foster City, CA). Real-time reverse-transcription PCR was completed using the Fast SYBR Green Master Mix (Applied Biosystems, Inc.) and gene-specific primers (20 p*M* each; Table 2) with the StepOne Real-Time PCR System (Applied Biosystems, Inc.), according to procedures described by Cooke et al. (2008). At the end of each reverse-transcription PCR, amplified products were subjected to a dissociation gradient (95°C for 15 s, 60°C for 30 s, and 95°C for 15 s) to verify the amplification of a single product by denaturation at the anticipated temperature. Responses were quantified based on the threshold cycle ($\mathbf{C_T}$), the number of PCR cycles required for target amplification to reach a predetermined threshold. Responses from genes of interest were quantified based on the C_T and normalized to the geometrical mean of the C_T values from β2-microglobulin and β-actin (Vandesompele et al., 2002). The CV for the geometrical mean of the β2-microglobulin and β-actin C_T values across all samples was 2.0%. Results are expressed as relative fold change ($2^{-} C_T$) as described by Ocón-Grove et al. (2008).

Statistical Analysis

Pen was considered the experimental unit for all analyses. Quantitative data were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC), whereas binary data were analyzed using the GLIMMIX procedure of SAS with a binomial distribution and logit link function. All data were analyzed using Satterthwaite approximation to determine the denominator degrees of freedom for tests of fixed effects, with pen(treatment) and steer(pen) as random variables, except for DMI and

G:F, which used pen(treatment) as a random variable. The model statement for initial and final BW, ADG, G:F, and morbidity-related and mortality results contained the effects of treatment. The model statement for DMI, cumulative BRD incidence, full BW change, and blood variables contained the effects of treatment, day, and the resultant interaction, in addition to results from d 0 as an independent covariate only for blood variables. Steer source was also included as an independent covariate for mRNA expression analysis of innate immunity genes, given that steers were randomly selected within each pen for blood mRNA sampling. The specified term for all repeated statements was day, with pen(treatment) as the subject for DMI and steer(pen) as the subject for all other analyses. The covariance structure used was first-order autoregressive, which provided the smallest Akaike information criterion and, hence, the best fit for all variables analyzed. All results are reported as least squares means except for blood variables, which are reported as covariately adjusted least squares means. Significance was set at $P \leq 0.05$ and tendencies were determined if $P > 0.05$ and ≤ 0.10. Results are reported according to main effects if no interactions were significant or according to the highest-order interaction detected.

Results

Performance and Health Variables

A treatment effect was detected ($P < 0.01$) for ADG, which was greater ($P \leq 0.05$) in CON steers than in IPF and OMN steers and greater ($P < 0.01$) in IPF steers than in OMN steers (Table 5.3). Accordingly, a treatment effect was also detected ($P < 0.01$) for final shrunk BW (d 81), which was greater ($P \leq 0.05$) in CON steers than

in IPF and OMN steers and greater ($P < 0.01$) in IPF steers than in OMN steers (Table 5.3). No treatment effects were detected ($P \geq 0.77$) for hay, concentrate, or total DMI (Table 5.3). Based on the concentrate intake of each pen, IPF steers consumed a mean of 14.5 g/d (SE 0.1) of Stocker Preconditioned Premix from d 7 to 30, whereas OMN was consumed at a mean of 20.2 g/d (SE 0.3) from d 0 to 30 by OMN steers. A treatment effect was detected ($P = 0.01$) for G:F, which was less ($P \leq 0.04$) in OMN steers than in CON and IPF steers (Table 5.3) but did not differ ($P = 0.33$) between IPF steers and CON steers, despite differences in ADG and equivalent DMI between these latter treatments (Table 5.3).

It should be noted, however, that full BW did not differ ($P \geq 0.69$) among treatments until d 56 and differed ($P \leq 0.09$) among treatments only on d 73 and 80 (treatment × day interaction, $P < 0.01$; Fig. 5.1a). Moreover, DMI parameters did not differ among treatments throughout the experiment (treatment × day interaction, $P \geq 0.94$; Fig. 5.1b). Hence, G:F from d 56 to 80 was reduced ($P < 0.01$) in OMN steers compared with CON and IPF steers (50, 230, and 176 g/kg [SEM 27], respectively) and also tended to be less ($P = 0.09$) in IPF steers than in CON steers.

No treatment differences were detected ($P \geq 0.55$) for BRD incidence (Table 5.4), whereas BRD signs were observed only during the initial 30 d of feedlot receiving (Fig. 2; day effect, $P < 0.01$). No treatment differences were detected ($P = 0.36$) for other morbidity reasons (i.e., bloat), number of antimicrobial treatments required on BRD diagnosis, and percentage of cattle that required ≥1 antimicrobial treatment on BRD diagnosis as well as mortality rate during the experiment (Table 5.4).

Physiological Variables

A treatment effect was detected for plasma cortisol ($P = 0.02$), given that mean plasma cortisol concentration was greater ($P = 0.01$) in CON steers than in IPF and OMN steers and did not differ ($P = 0.93$) between IPF steers and OMN steers (Table 5). A treatment × day interaction was detected ($P = 0.05$; Fig. 5.3) for plasma haptoglobin concentration, which tended ($P = 0.10$) to be greater in CON steers than in IPF steers on d 3, was greater ($P = 0.04$) in IPF steers than in CON steers on d 7, and tended ($P \leq 0.10$) to be less in OMN steers than in IPF and CON steers on d 21 of the experiment (Fig. 5.3). No treatment effects were detected ($P = 0.41$) for plasma IGF-I concentrations (Table 5.5). Moreover, day effects were detected ($P \leq 0.01$) for all plasma and serum variables (Fig. 5.3; Table 5.6),

No treatment effects were detected ($P = 0.21$) for serum titers against *M. haemolytica*, PI3, BRSV, BVD-1, and BHV-1 (Table 5.5), whereas day effects were detected ($P \leq 0.03$) for all these variables (Table 5.6). Tendencies for treatment × day interactions were detected ($P \leq 0.08$; Fig. 5.4) for blood mRNA expression of *interleukin 8* and *tumor necrosis-α.* Blood mRNA expression of *interleukin 8* was greater ($P \leq 0.05$) in OMN and IPF steers than in CON steers on d 3 and greater in OMN steers than in CON and IPF steers on d 14 (Fig. 5.4a). Blood mRNA expression of *tumor necrosis-α* was greater ($P \leq 0.05$) in OMN and IPF steers than in CON steers on d 10 (Fig. 5.4b). No treatment effects were detected ($P \geq 0.24$) for blood mRNA expression of *chemokine ligand 5*, *cyclooxygenase 2*, *interleukin 8 receptor*, and *L-selectin* (Table 5.5). Day effects were also detected ($P \leq 0.05$) for blood mRNA expression variables (Fig. 5.4; Table 5.6).

Discussion

As is common in commercial feedlot operations, the management history of steers prior to the initiation of this experiment was not fully known; therefore, they were considered high risk (Wilson et al., 2017). Furthermore, steers experienced the stress of weaning, auction, transportation, vaccination, and feedlot entry within a 72-h period, whereas the combination of these stressors are known to stimulate neuroendocrine and inflammatory responses that impact cattle immunocompetence and performance (Cooke, 2017). Accordingly, day effects observed for plasma cortisol and haptoglobin corroborate that steers experienced an adrenocortical and subsequent acute-phase protein response elicited by transport, vaccination, and feedlot entry (Cooke et al., 2011). Day effects detected for mRNA expression of whole-blood genes suggest immune activation on feedlot entry, given that *interleukin 8*, *tumor necrosis-α*, *chemokine ligand 5*, *cyclooxygenase 2*, *interleukin 8 receptor*, and *L-selectin* are key inflammatory components of the innate immune system (Abbas and Lichtman, 2007). Collectively, these stress-induced inflammatory processes are linked with the BRD complex in receiving cattle (Berry et al., 2004; Cooke, 2017), supporting the substantial incidence of BRD observed in the present experiment, which is comparable to research efforts conducted at commercial receiving yards (Snowder et al., 2006; Marques et al., 2016).

Contrary to our hypothesis, however, the OMN-and IPF-based products failed to mitigate BRD incidence and improve steer receiving performance. These ingredients were tested due to 1) their immunomodulatory potential (Fudenberg and Fudenberg, 1989; Krehbiel et al., 2003; Brandão et al., 2016), 2) the need for alternative dietary

strategies to enhance immunocompetence of receiving cattle, and 3) the negative association among BRD incidence and cattle productivity (Snowder et al., 2006; Schneider et al., 2009). Moreover, OMN- and IPF-based products were administered to cattle during the initial 30 d of feedlot receiving, when the majority of BRD is observed in feedlot cattle (Snowder et al., 2006). The IPF products are based on transfer factor proteins extracted from bovine colostrum and egg yolks as well as lactate-producing probiotics. Transfer factor is a component of dialyzable leukocyte extracts produced in small quantities by T-lymphocytes (Rozzo and Kirkpatrick, 1992) that, once extracted and administered into the recipient, is able to transfer cell-mediated immunity for specific pathogens (Fudenberg and Fudenberg, 1989). Treatment with transfer factor has been shown to provide delayed hypersensitivity to *Eimeria bovis* in calves (Klesius and Kristensen, 1977). Published research examining its use in cattle have been scarce; however, Montgomery et al. (2008) investigated using transfer factor delivered as an oral drench in receiving heifers compared with treatment with tilmicosin phosphate. Similar to the results seen in the current study, treatment with transfer factor did not improve DMI, ADG, or feed efficiency during a 36-d receiving period; however, the incidence of BRD was increased in heifers receiving transfer factor (Montgomery et al., 2008). Hence, these authors concluded that transfer factor was not as effective as tilmicosin phosphate in preventing incidence of BRD and attributed this outcome to extensive rumen degradation of transfer factor. Alternatively, a direct-fed microbial such as lactic acid–producing bacteria have been shown to improve performance and decrease morbidity in receiving cattle (Krehbiel et al., 2003; McDonald et al., 2005); therefore, the lactic acid–producing bacteria in the IPF-based

products were also expected to mitigate BRD incidence and enhance steer performance in the present experiment.

The OMN is based on components that have been shown to modulate the innate immune system, particularly yeast-based ingredients. More specifically, yeast products contain pathogen-associated molecular patterns (**PAMPs**) such as β-glucans that are recognized by the immune system trigger innate immune responses by binding to Toll-like receptors and preparing the immune system against pathogens (Heine and Ulmer, 2005; Broadway et al., 2015). Feeding yeast cell wall components to newly received feedlot heifers alleviated inflammatory and serum cortisol responses to lipopolysaccharide administration (Burdick Sanchez et al., 2013). Accordingly, OMN supplementation has been shown to impact leukocytes gene expression related to the inflammatory response in dairy cows (Wang et al., 2009; Nace et al., 2014). These include improved leukocyte function, surface L-selectin concentration, and phagocytosis of extracellular pathogens in addition to fewer incidents of udder edema and mastitis following OMN supplementation (Wang et al., 2009; Ryman et al., 2013; Nace et al., 2014). In beef cattle, Armstrong et al. (2016) observed altered mRNA expression in a variety of genes relevant to innate immune function, suggesting that OMN regulates antigen presentation and signal transduction in cattle. However, no research evaluating the impacts of OMN supplementation on performance and immunocompetence of high-risk receiving cattle had been conducted to date.

Corroborating their immunomodulatory properties, supplementing OMN or IPF products to receiving steers herein impacted blood parameters that indicate enhanced innate immunity. More specifically, treatment differences in whole-blood

mRNA expression of *interleukin 8* and *tumor necrosis-a* suggest heightened inflammation, particularly in OMN steers, as previously observed by others (Burdick et al., 2012; Burdick Sanchez et al., 2014; Brandão et al., 2016). Treatment effects on plasma haptoglobin concentrations, an acute-phase protein whose synthesis is stimulated by proinflammatory cytokines (Carroll and Forsberg, 2007), also suggest an altered acute-phase response typical of feedlot receiving (Cooke, 2017) in OMN and IPF steers. These outcomes also can be associated with treatment differences in plasma cortisol concentrations, which serves as an effector molecule on proinflammatory and acute-phase reactions (Steiger et al., 1999; Carroll et al., 2009; Cooke et al., 2012). In turn, a potential enhancement in innate immunity from OMN and IPF supplementation may have contributed to a lessened adrenocortical response to the stress and immune challenges associated with feedlot receiving (Carroll and Forsberg, Cooke, 2017).

Others have also reported reduced circulating cortisol concentrations when OMN was supplemented to dairy cows exposed to heat-stress conditions or receiving lipopolysaccharide administration (Hall et al., 2014; McBride et al., 2016). Moreover, pathological conditions such as BRD elicit inflammatory and innate immune reactions (Ackermann et al., 2010). Given that BRD incidence did not differ among treatments throughout the experiment, treatment differences observed in plasma haptoglobin, cortisol, and blood mRNA expression of interleukin 8 and tumor necrosis-a were not caused by morbidity differences among OMN, IPF, and CON steers.

Heightened innate immunity often results in enhanced acquired immunity on vaccination (Durum and Muegge, 1996). However, supplementing OMN and IPF did not improve acquired humoral immunity against BRD pathogens, which likely

contributed to the similar incidence of BRD among treatments (Callan, 2001). Perhaps treatment differences in innate immune parameters were not sufficient to improve acquired humoral responses to vaccinated antigens. It should be noted that serum antibody titers against BRD pathogens increased across all treatments during the experiment, indicating that steers effectively acquired humoral immunity against these pathogens on vaccination and revaccination (Howard et al., 1989; Bolin and Ridpath, 1990; Richeson et al., 2008). Moreover, vaccination against BRD pathogens is also known to further contribute to the adrenocortical and inflammatory responses reported herein (Rodrigues et al., 2015; Lippolis et al., 2016), and it is typically performed on feedlot arrival and a few weeks later due to lack of health history in high-risk receiving cattle (Richeson et al., 2008; Edwards, 2010). Therefore, and as previously mentioned, vaccination against BRD also contributed to the increase in cortisol, haptoglobin, and whole-blood mRNA expression parameters on feedlot arrival observed across treatments.

Supplementing OMN and IPF also failed to improve feedlot receiving performance. In fact, both treatments impaired steer growth and feed efficiency during the 80-d receiving period compared with CON steers, with a greater disadvantage to steers receiving OMN. Nevertheless, treatment differences in BW were noted only after d 56 of the receiving period, despite similar DMI throughout the experiment. Hence, treatment differences on ADG and final BW should be mainly associated with the impaired G:F of IPF and OMN steers after d 56. Yet the OMN and IPF treatments were administered until d 30 of feedlot receiving, when cattle are constantly exposed to a multitude of stressors that directly impair their growth metabolism (Duff and Galyean,

2007; Cooke, 2017). Moreover, BRD symptoms were observed only during the initial 30 d of feedlot receiving (Snowder et al., 2006) and did not differ among OMN, CON, and IPF steers and, therefore, should not be associated with treatment differences on BW and ADG (Schneider et al., 2009). Differences in ADG and BW between treatments were also not reflected by plasma IGF-I concentrations, which is positively associated with cattle growth rates (Bishop et al., 1989; Ellenberger et al., 1989; Elsasser et al., 1989). Hence, the exact reasons why OMN and IPF steers experienced reduced growth rates compared with CON steers after d 56 of the experiment cannot be properly elucidated, particularly because all steers were receiving the same overall and nutritional management during this period.

In summary, this experimental model fully represented the stress and health challenges that commercial feeder cattle experience during feedlot receiving, resulting in elevated BRD incidence and morbidity. However, neither OMN nor IPF supplementation were capable of mitigating BRD incidence and improving receiving performance, despite their impacts on adrenocortical and inflammatory responses on feedlot entry. As previously mentioned, published literature investigating the inclusion of OMN or IPF products into feedlot receiving diets is extremely limited. Based on the results from Montgomery et al. (2008), perhaps the transfer factor contained in the IPF products was extensively degraded in the rumen, whereas the lactic acid–producing bacteria failed to improve immunocompetence and productive traits of receiving cattle (Krehbiel et al., 2003). Alternatively, OMN supplementation elicited immunomodulatory effects in cattle administered lipopolysaccharide when offered for 28 d prior to the challenge (Burdick Sanchez et al., 2014). In fact, research suggests

cattle should be adapted to OMN supplementation for several weeks prior to the stress or immune insult (Nace et al., 2014; Ryman et al., 2013). Hence, one can speculate that the OMN supplementation failed to improve health and performance of receiving cattle due to the lack of a previous adaption period, such as during a preconditioning program (Pritchard and Mendez, 1990). Consequently, additional research is warranted to further evaluate the use of OMN and IPF products as well as identify other nonantibiotic feed ingredients that enhance cattle immunocompetence and productivity during feedlot receiving.

Table 5.1. Ingredient composition and nutrient profile of concentrate offered during the experiment (d 0 to 80)[1]

Item	Day 0 to 7	Day 8 to 19	Day 20 to 30	Day 31 to 80
Ingredient (as-fed basis)				
Whole corn, kg/d	0.91	2.27	4.09	5.23
Soybean meal, kg/d	0.36	0.36	0.55	0.68
Mineral mix,[2] kg/d	0.05	0.05	0.05	0.05
Nutrient profile[3] (DM basis)				
TDN, %	83	86	87	87
NEm, Mcal/kg	2.11	2.14	2.16	2.16
NEg, Mcal/kg	1.46	1.47	1.49	1.49
NDF, %	8.4	8.1	8.1	8.1
ADF, %	4.6	4.1	4.0	4.0
CP, %	20.3	14	13.7	13.5

[1]Steers had free-choice access to orchardgrass (*Dactylis glomerata* L.) hay and water throughout the experimental period. Hay and concentrate were offered separately in different sections of the feed bunk.

[2]Cattleman's Choice (Performix Nutrition Systems, Nampa, ID) containing 14% Ca, 10% P, 16% NaCl, 1.5% Mg, 3,200 mg/kg Cu, 65 mg/kg I, 900 mg/kg Mn, 140 mg/kg Se, 6,000 mg/kg Zn, 136,000 IU/kg vitamin A, 13,000 IU/kg vitamin D_3, and 50 IU/kg vitamin E.

[3]Based on nutritional profile of each ingredient, which were analyzed using wet chemistry procedures by a commercial laboratory (Dairy One Forage Laboratory, Ithaca, NY). Calculation for TDN used the equation proposed by Weiss et al. (1992), whereas NEm and NEg were calculated using the equations proposed by the NRC (1996).

Table 5.2. Primer sequences, accession number, and reference for all gene transcripts analyzed by real-time reverse-transcription PCR

Target gene	Primer sequence	Accession no.	Source
Cyclooxygenase-2			
Forward	AATCATTCACCAGGCAAAGG	AF031699	Silva et al. (2008)
Reverse	TAGGGCTTCAGCAGAAAACG		
Tumor necrosis factor α			
Forward	AACAGCCCTCTGGTTCAAAC	NM_173966	Riollet et al. (2000)
Reverse	TCTTGATGGCAGACAGGATG		
L-selectin			
Forward	GACACTTCCCTTCAGCCGTAC	NM_174182.1	Playford et al. (2014)
Reverse	AGTTCTTTGCTTCTTCAGTGAGAG		
Interleukin-8			
Forward	ACACATTCCACACCTTTCCAC	NM_173925.2	Kliem et al. (2013)
Reverse	ACCTTCTGCACCCACTTTTC		
Interleukin-8 receptor			
Forward	CGGGTCATCTTTGCTGTCG	NM_174360.3	Playford et al. (2014)
Reverse	ATGAGGGTGTCCGCGATC		
CCL5			
Forward	GCCCTGCTGCTTTGCCTATAT	NM_175827.2	Buza et al. (2003)
Reverse	TCCACCCTAGCTCAACTCCAA		
β-actin			
Forward	CTGGACTTCGAGCAGGAGAT	AY141970	Gifford et al. (2007)
Reverse	GGATGTCGACGTCACACTTC		
β2-microglobulin			
Forward	GGGCTGCTGTCGCTGTCT	NM_173893	Silva et al. (2008)
Reverse	TCTTCTGGTGGGTGTCTTGAGT		

Table 5.3. Performance parameters during an 80-d feedlot receiving from beef steers supplemented or not (no immunomodulatory ingredient supplementation during feedlot receiving [CON]; $n = 6$) with immunostimulant ingredients (IPF[1] [$n = 6$] and OMN[2] [$n = 6$]) from d 0 to 30 of the receiving period[3]

Item	CON	IPF	OMN	SEM	*P*-value
Initial BW (d −1), kg	219	220	220	7	0.99
Final BW (d 81), kg	320[a]	307[b]	282[c]	4	<0.01
ADG, kg/d	1.23[a]	1.06[b]	0.76[c]	0.06	<0.01
DMI, kg/d					
Hay	3.19	3.06	3.10	0.25	0.93
Concentrate	4.64	4.63	4.69	0.06	0.77
Total	7.83	7.69	7.79	0.30	0.94
G:F,[4] g/kg	173[a]	152[a]	107[b]	14	0.01

[a–c]Values within rows with different superscripts differ ($P \leq 0.05$).

[1]IPF = 2 oral capsules of Stocker Immune Primer on d 0 + 15 g/ steer daily (as-fed basis) of Stocker Preconditioned Premix (Ramaekers Nutrition, Santa Cruz, CA) from d 7 to 30.

[2]OMN = supplementation with OmniGen-AF (22 g/steer daily, as-fed basis, from d 0 to 30; Phibro Animal Health Corp., Teaneck, NJ).

[3]Steer shrunk BW was obtained after road transport (800 km for 12 h) on d −1 and after 16 h of water and feed withdrawal on d 81. Steer ADG was calculated using initial and final BW. Feed intake was recorded daily from d 0 to 80 by measuring feed offered and refusals from each pen, divided by the number of steers within each pen, and expressed as kilograms per steer per day.

[4]Calculated using total DMI from d 0 to 80 and BW gain of each pen from d −1 to d 81.

Table 5.4. Morbidity and mortality parameters during a 80-d feedlot receiving from beef steers supplemented or not (no immunomodulatory ingredient supplementation during feedlot receiving [**CON**]; n = 6) with immunostimulant ingredients (**IPF**[1] [n = 6] and **OMN**[2] [n = 6]) from d 0 to 30 of the receiving period[3]

Item	CON	IPF	OMN	SEM	*P*-value
Incidence of bovine respiratory disease symptoms, %	69.4	61.1	69.4	9.1	0.76
Number of antimicrobial treatments required	1.13	1.32	1.31	0.13	0.55
Calves that required ≥ 1 antimicrobial treatment,%	13.1	23.3	27.3	9.9	0.59
Other morbidity reasons, %	8.3	8.3	2.8	4.1	0.55
Mortality, %	2.8	5.5	0.0	2.7	0.36

[1]IPF = 2 oral capsules of Stocker Immune Primer on d 0 + 15 g/steer daily (as-fed basis) of Stocker Preconditioned Premix (Ramaekers Nutrition, Santa Cruz, CA) from d 7 to 30.

[2]OMN = supplementation with OmniGen-AF (22 g/steer daily, as-fed basis, from d 0 to 30; Phibro Animal Health Corp., Teaneck, NJ).

[3]Steers were observed daily for bovine respiratory disease symptoms according to the DART system (Zoetis Inc., Florham Park, NJ) and received antimicrobial treatment as described by Wilson et al. (2015).

[4]All non–bovine respiratory disease–related morbidity was due to bloat, with steers receiving 60 mL (oral drench, mixed with 500 mL of water) of Therabloat (Zoetis Inc.) when bloat was detected (Meyer and Bartley, 1972).

Table 5.5. Metabolic, humoral, and gene expression responses during an 80-d feedlot receiving in beef steers supplemented or not (no immunomodulatory ingredient supplementation during feedlot receiving [**CON**]; n = 6) with immunostimulant ingredients (**IPF**[1] [n = 6] and **OMN**[2] [n = 6]) from d 0 to 30 of the receiving period[3,4]

Item	CON	IPF	OMN	SEM	*P*-value
Metabolic variables					
Plasma cortisol, ng/mL	21.89[a]	18.74[b]	18.63[b]	0.92	0.02
Plasma IGF-I, ng/mL	178	166	175	7	0.41
Serum antibody variables, titer log 2					
Mannheimia haemolytica	10.44	10.41	10.23	0.23	0.79
Parainfluenza-3 virus	6.65	5.99	6.18	0.51	0.66
Bovine respiratory syncytial virus	5.59	5.47	4.81	0.43	0.39
Bovine viral diarrhea virus-1	5.77	6.19	6.16	0.51	0.81
Bovine herpesvirus-1	3.53	3.02	4.15	0.43	0.21
Blood mRNA expression					
Chemokine ligand 5	4.47	3.22	2.89	0.61	0.24
Cyclooxygenase 2	3.93	3.34	3.88	0.53	0.69
Interleukin 8 receptor	4.86	4.73	5.44	0.97	0.85
L-selectin	1.83	1.78	1.75	0.12	0.91

[a–b]Values within rows with different superscripts differ ($P \leq 0.05$).

[1]IPF = 2 oral capsules of Stocker Immune Primer on d 0 + 15 g/ steer daily (as-fed basis) of Stocker Preconditioned Premix (Ramaekers Nutrition, Santa Cruz, CA) from d 7 to 30.

[2]OMN = supplementation with OmniGen-AF (22 g/steer daily, as-fed basis, from d 0 to 30; Phibro Animal Health Corp., Teaneck, NJ).

[3]On d 0, steers were vaccinated against *Clostridium* and *Mannheimia haemolytica* (One Shot Ultra 7; Zoetis Inc., Florham Park, NJ) and infectious bovine rhinotracheitis, bovine viral diarrhea complex, parainfluenza-3 virus, and bovine respiratory syncytial virus (Bovi-Shield Gold 5; Zoetis Inc.) and were administered an anthelmintic (Dectomax; Zoetis Inc.). On d 21, steers were revaccinated against *Clostridium* (Ultrabac 8; Zoetis Inc.) and infectious bovine rhinotracheitis virus, bovine viral diarrhea complex, parainfluenza-3 virus, and bovine respiratory syncytial virus (Bovi-Shield Gold 5; Zoetis Inc.).

[4]Blood samples were collected on d 0, 3, 7, 10, 14, 21, 31, 42, and 73 and analyzed for cortisol (d 0 to 31), IGF-I (d 0, 21, 42, and 73), serum antibody variables (d 0, 10, 21, 31, and 42), and whole-blood mRNA expression (d 0 to 14).

Table 5.6. Concentrations of plasma cortisol (ng/mL) and IGF-1 (ng/ml), serum titers against *Mannheimia haemolytica* (MH), parainfluenza-3 virus (PI3), bovine respiratory syncytial virus (BRSV), bovine viral diarrhea virus-1 (**BVD-1**), and bovine herpesvirus-1 (**BHV-1**); and whole-blood mRNA expression of *chemokine ligand 5 (CCL5), cyclooxygenase 2 (COX2), interleukin 8 receptor (IL8r),* and *L-selectin (SELL)* in beef steers during an 80-d feedlot receiving[1,2]

	Plasma variables		Serum antibody titers					Blood mRNA expression			
Day	**Cortisol**	**IGF-1**	**MH**	**PI3**	**BRSV**	**BVD-1**	**BHV-1**	**CCL5**	**COX2**	**IL8R**	**SELL**
0	22.98^{a}	113^{c}	7.44^{d}	5.1^{c}	0.55^{d}	3.78^{dc}	0.77^{d}	5.21^{a}	3.59^{b}	5.13^{b}	1.68^{b}
3	21.35^{ab}	-	-	-	-	-	-	3.41^{bc}	5.18^{a}	7.05^{a}	1.96^{a}
7	19.46^{bc}	-	-	-	-	-	-	2.90^{c}	2.51^{c}	3.89^{b}	1.54^{b}
10	18.80^{c}	-	8.89^{c}	6.38^{a}	2.78^{c}	5.17^{c}	3.72^{b}	3.82^{b}	3.64^{b}	4.93^{b}	1.82^{ab}
14	19.95^{bc}	-	-	-	-	-	-	3.93^{b}	3.48^{b}	4.15^{b}	1.81^{ab}
21	20.09^{bc}	132^{b}	11.05^{a}	5.83^{b}	5.39^{b}	6.17^{b}	2.77^{c}	-	-	-	-
31	18.87^{c}	-	11.28^{a}	6.55^{a}	6.67^{a}	6.00^{b}	4.44^{a}	-	-	-	-
42	-	192^{a}	10.22^{b}	6.33^{ab}	6.33^{a}	6.83^{a}	3.33^{b}	-	-	-	-
73	-	196^{a}	-	-	-	-	-	-	-	-	-
SEM	0.97	5	0.21	0.38	0.30	0.38	0.28	0.52	0.43	0.87	0.12
P-value	0.01	< 0.01	<0.01	< 0.03	< 0.01	< 0.01	< 0.01	<0.01	<0.01	0.05	0.05

$^{a–d}$Values within columns with different superscripts differ ($P \leq 0.05$).

[1]On d 0, steers were vaccinated against *Clostridium* and *Mannheimia haemolytica* (One Shot Ultra 7; Zoetis Inc., Florham Park, NJ) and infectious bovine rhinotracheitis, bovine viral diarrhea complex, PI3, and BRSV (Bovi-Shield Gold 5; Zoetis Inc.) and were administered an anthelmintic (Dectomax; Zoetis Inc.). On d 21, steers were revaccinated against *Clostridium* (Ultrabac 8; Zoetis Inc.) and infectious bovine rhinotracheitis virus, bovine viral diarrhea complex, PI3, and BRSV (Bovi-Shield Gold 5; Zoetis Inc.).

[2]Blood samples were collected on d 0, 3, 7, 10, 14, 21, 31, 42, and 73 and analyzed for cortisol (d 0 to 31), IGF-I (d 0, 21, 42, and 73), serum antibody variables (d 0, 10, 21, 31, and 42), and whole-blood mRNA expression

Figure 5.1. Body weight (panel A) and DMI (hay + concentrate; panel B) during a 80-d feedlot receiving from beef steers supplemented or not (no immunomodulatory ingredient supplementation during feedlot receiving [**CON]**; n = 6) with 1) 2 oral capsules of Stocker Immune Primer on d 0 + 15 g/steer daily (as-fed basis) of Stocker Preconditioned Premix (Ramaekers Nutrition; Santa Cruz, CA; n = 6) from d 7 to 30 (IPF) or 2) supplementation with Omnigen-AF (**OMN**; 22 g/steer daily, as-fed basis, from d 0 to 30; Phibro Animal Health Corp., Teaneck, NJ; n = 6). A treatment × day interaction was detected ($P < 0.01$) for BW but not for DMI ($P = 0.97$). Within day, letters indicate the following treatment differences; [a]CON vs. OMN ($P < 0.01$), [b]IPF vs. OMN ($P \leq 0.04$), and [c]IPF vs. CON ($P = 0.09$).

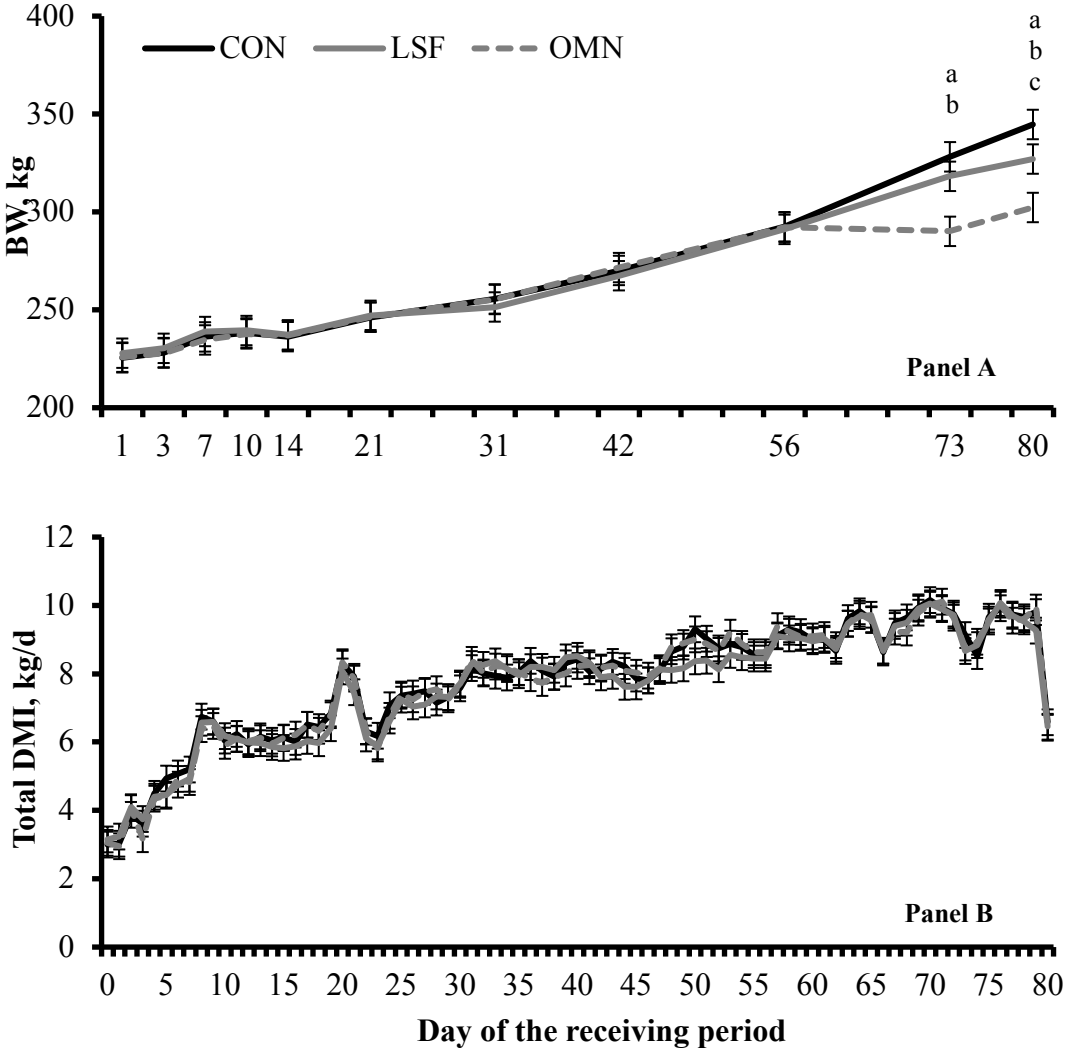


Figure 5.2. Cumulative incidence of bovine respiratory disease (**BRD**) symptoms during a 80-d feedlot receiving from beef steers supplemented or not (no immunomodulatory ingredient supplementation during feedlot receiving [**CON]**; n = 6) with 1) 2 oral capsules of Stocker Immune Primer on d 0 + 15 g/steer daily (as-fed basis) of Stocker Preconditioned Premix (Ramaekers Nutrition; Santa Cruz, CA; n = 6) from d 7 to 30 (IPF) or 2) supplementation with Omnigen-AF (**OMN**; 22 g/steer daily, as-fed basis, from d 0 to 30; Phibro Animal Health Corp., Teaneck, NJ; n = 6). Steers were observed daily for BRD symptoms according to the DART system (Zoetis Inc., Florham Park, NJ) and received medication as described by Wilson et al. (2015). No treatment or treatment × day interaction were detected ($P \geq 0.59$), whereas a day effect was significant ($P < 0.01$).

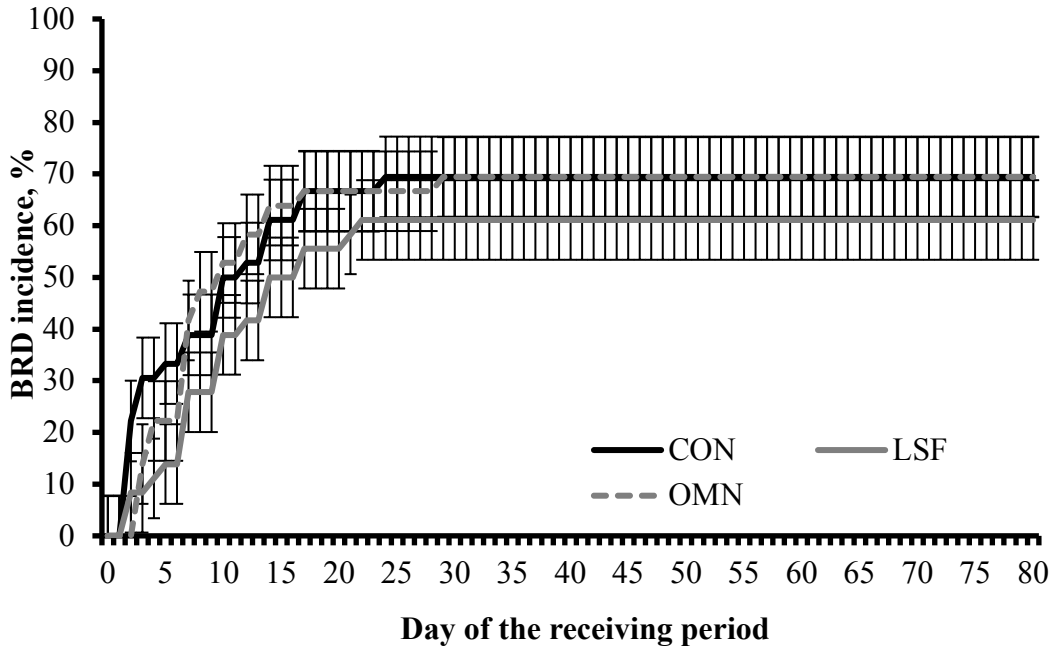


Figure 5.3. Plasma haptoglobin concentrations in feedlot receiving steers supplemented or not (no immunomodulatory ingredient supplementation during feedlot receiving [CON]; $n = 6$) with 1) 2 oral capsules of Stocker Immune Primer on d 0 + 15 g/steer daily (as-fed basis) of Stocker Preconditioned Premix (Ramaekers Nutrition, Santa Cruz, CA; $n = 6$) from d 7 to 30 (IPF) or 2) supplementation with OmniGen-AF (OMN; 22 g/steer daily, as-fed basis**,** from d 0 to 30; Phibro Animal Health Corp., Teaneck, NJ; $n = 6$). A treatment × day interaction was detected ($P \leq 0.05$). Blood samples collected on d 0 were a significant covariate ($P = 0.02$) but did not differ among treatments (0.21, 0.25, and 0.28 mg/mL [SEM 0.04] in CON, IPF, and OMN steers, respectively). Within day, letters indicate the following treatment differences: [a]IPF vs. CON ($P \leq 0.10$), [b]OMN vs. CON ($P = 0.10$), and [c]OMN vs. IPF ($P = 0.09$).

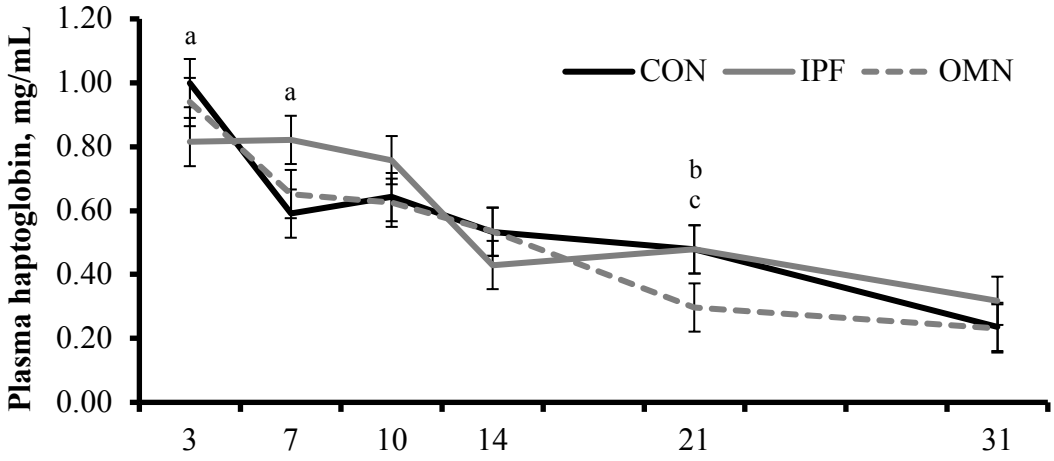
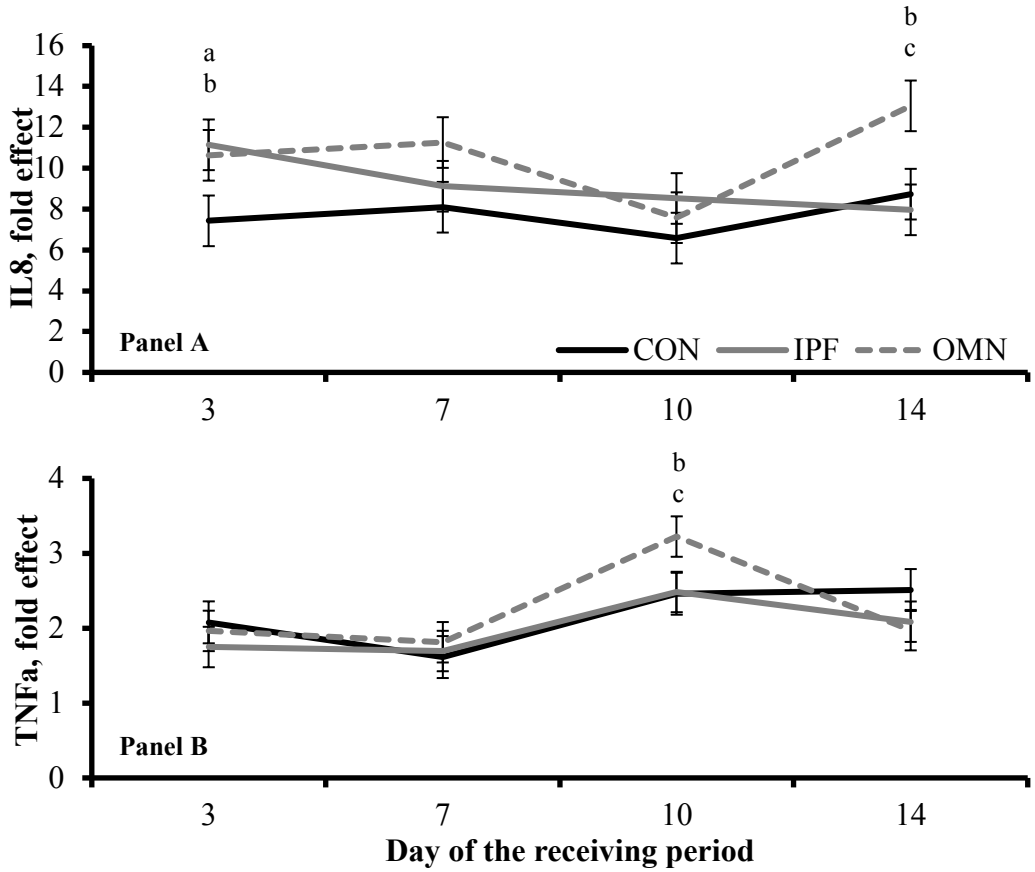


Figure 5.4. Whole blood mRNA expression of *interleukin 8* (**IL8**; Panel A) and *tumor necrosis-α* (**TNFa**; Panel B) in feedlot receiving steers supplemented or not (**CON**; n = 6) with: 1) **IPF** = 2 oral capsules of Stocker Immune Primer on d 0 + 15 g/steer daily (as-fed basis) of Stocker Preconditioned Premix (Ramaekers Nutrition; Santa Cruz, CA; n = 6)) from d 7 to 30, or 2) **OMN** = supplementation with Omnigen-AF (22 g/steer daily, as-fed basis; Phibro Animal Health, Teaneck, NJ) from d 0 to 30 (n = 6). Blood samples collected on d 0 were significant covariates ($P \leq 0.04$) but did not differ among CON, IPF, and OMN steers (8.1, 7.3, and 7.4 fold effect for *interleukin 8* mRNA expression, SEM = 1.7; 3.6, 3.5, and 3.3 fold effect for *tumor necrosis-α* mRNA expression, SEM = 0.3; respectively). Treatment × day interactions were detected ($P \leq 0.08$). Within day, letters indicate the following treatment differences; a = IPF vs. CON ($P = 0.05$), b = OMN vs. CON ($P \leq 0.05$), c = OMN vs. IPF ($P \leq 0.01$).



CHAPTER VI

OVERALL CONCLUSIONS FOR ALL EXPERIMENTS

In experiment 1, anticipating initial and booster vaccination prior to feedlot entry decreased ADG pre-weaning. However, there was no difference in weaning weight and EARLY calves exhibited increased ADG during feedlot receiving. Additionally, EARLY calves had greater *M. haemolytica* plasma antibody concentration than CON at feedlot entry, and greater BVDV plasma antibody concentration than DELAYED calves at feedlot entry. Unfortunately, experiment 1 lacks detail on additional physiologic and metabolic responses due to vaccination strategy throughout the preconditioning and feedlot receiving periods due to failing to collect serum. Nonetheless, anticipating initial and booster vaccination against respiratory pathogens to provide both doses prior to feedlot entry appears to be a valid strategy to enhance cattle health and performance during feedlot receiving.

In experiment 2, supplementation of organic and inorganic trace minerals during a 45-d preconditioning period increased mean liver concentrations of Co, Zn, and Cu. No differences were observed for performance during preconditioning or feedlot receiving. There was no difference in antibody response to vaccination or plasma haptoglobin. Plasma cortisol concentration was lower for INR and AAC compared to CON. The cumulative impact of these preconditioning strategies on the entire beef production system could not be elucidated in either experiment 1 or 2 due to not following cattle past the feedlot and collecting carcass data. Therefore, while INR and AAC increased liver concentrations of Co, Zn, and Cu through

preconditioning, supplementation did not impact cattle performance or immune responses during preconditioning or feedlot receiving.

In experiment 3, supplementation of immunomodulatory ingredients resulted in decreased ADG and final BW compared to CON calves. No differences were observed for BRD incidence or DMI. However, mean plasma cortisol concentration was greater for CON than OMN or IPF. Blood mRNA expression of IL-8 was greater in OMN and IPF than CON on d 3, and greater in OMN than IPF and CON on d 14. Blood expression of TNFα was greater in OMN and IPF steers than CON. The immunomodulatory feed ingredients evaluated herein impacted adrenocortical and innate immune responses but failed to mitigate BRD incidence and improve performance of receiving cattle.

Collectively, these results detail several stressors experienced by different sources of feeder cattle, including their impact on physiological, adrenocortical, and innate immune responses. Preconditioning was effective at minimizing incidence of morbidity during feedlot receiving compared to that seen in the literature and at commercial feedyards, and inclusion of an anticipated vaccination protocol improved overall response to vaccination and performance during feedlot receiving. While experiment 2 did not improve performance or morbidity rates, it should be noted that calf liver mineral status of Co, Cu, Zn, and Mn were adequate at the beginning of the trial. Therefore, this strategy may be more beneficial for calves deficient or of unknown mineral status. Finally, experiment 3 was able to impact adrenocortical responses and measures of the innate immune response but did not improve performance or morbidity of receiving cattle. Instead, supplementation with OMN or IPF in the feedlot receiving

period may be detrimental to cattle performance. Hence, the effects of the experiments discussed herein should be considered when determining management strategies of preconditioning or high-risk feeder cattle.

CHAPTER VII

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