POTENTIAL DIAGNOSTIC METHODS AND NUTRITIONAL CHANGES TO
COMBAT BOVINE RESPIRATORY DISEASE IN RECEIVING CATTLE

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by
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POTENTIAL DIAGNOSTIC METHODS AND NUTRITIONAL CHANGES TO

COMBAT BOVINE RESPIRATORY DISEASE IN RECEIVING CATTLE

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Graduate school has been described by predecessors in a number of ways. Some eloquently compare the challenges and rewards to the serpentine journey of a river as it flows through hills and valleys of the Missouri countryside. Others prefer the less romanticized analogy of attempting to drink water from a firehose. Depending on the time over the past year, I could have easily identified with either end of the spectrum. Regardless, the successes following hours spent treating and sampling sick calves or staring at Microsoft Excel files would not have been possible without the help and support of those around me.

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ABSTRACT

Experiments were conducted in 2 studies to investigate aspects of nutrition and animal health in receiving cattle. The first study evaluated the effects of post-ruminal amino acid supplementation and gut-modifying additives on bovine respiratory disease (BRD) occurrence and evaluated feed intake, arterial blood parameters, and acute phase proteins (APP) in receiving cattle with naturally-occurring BRD. We hypothesized receiving cattle consuming diets formulated to exceed amino acid requirements and include gut-modifying additives would present fewer BRD cases than animals fed to NRC requirements. We also hypothesized a reduction in feed intake, different arterial blood parameters, and higher concentrations of the specific APP haptoglobin (HPT) and lipopolysaccharide-binding protein (LBP) in sick calves (case) compared to healthy counterparts (control). Morbidity rates were not different between diets ($P = 0.16$). Case DMI ($P < 0.01$), $pO_2$ ($P = 0.02$), and $sO_2$ ($P = 0.03$) were lesser and APP concentrations ($P < 0.01$) were greater compared to controls. The second study compared performance and vaccination-induced antibody response in receiving cattle consuming diets with post-ruminal amino acid inclusions at or above NRC requirements and with or without gut-modifying additives. We hypothesized that diets formulated to exceed amino acid requirements and include gut-modifying additives would produce greater animal performance and novel antibody response in receiving calves. ADG was not affected by
diet ($P = 0.40$), while DMI tended to be different ($P = 0.06$). DMI with excess AA and additives (AAXS; 5.62 kg) was greater ($P = 0.03$) than excess AA alone (AA; 5.23 kg), but was not greater than controls meeting requirements without additives (CONTROL; $P = 0.71$). CONTROL DMI (5.55 kg) tended to be greater than AA ($P = 0.07$). G:F was different between diets ($P < 0.01$), as AA (0.37) was greater ($P < 0.01$) than AAXS (0.32) and tended to be greater ($P = 0.08$) than CONTROL (0.34), while AAXS and CONTROL were not different ($P = 0.12$). Diet did not affect antibody concentration or titer ($P > 0.24$). Intake, blood chemistry analysis, and HPT and LBP provided patterns with possible complementarity for more accurate objective BRD diagnosis. Post-ruminal amino acid and additive supplementation did not impact humoral response to vaccination or morbidity, though exceeding amino acid requirements could benefit production through increased feed efficiency.
CHAPTER 1

LITERATURE REVIEW

BOVINE RESPIRATORY DISEASE

Overview

Modern beef production relies heavily on maximized efficiency. This desired phenotypic efficiency depends on the interaction of genetics and environment. Though genetic homogenization like the swine and poultry industries is not practiced in beef cattle, many techniques for altering some aspect of environmental influence have been researched and adopted at each production stage. As the U.S. beef industry resembles a funnel, where 0.4% of feedlots account for nearly 50% of the total cattle on feed (USDA-APHIS, 2016), practices employed at these high-volume locations can greatly influence industry efficiency by impacting many cattle at once. One area providing potential for production and economic gain is animal health management. And, in discussing feedlot health aggravators, bovine respiratory disease (BRD) sits atop the list of common suspects.

Bovine respiratory disease is a general title used for cattle pneumonia attributable to a certain pathogen or complex of pathogens commonly cultured from respiratory cases (Dabo et al., 2008), hence the additional and arguably more accurate label, bovine respiratory disease complex. By this definition, BRD can encompass more specific diseases caused by 1 or more of the identifying pathogens. Examples include enzootic calf pneumonia (Nikunen et al., 2007) and shipping fever (Yates, 1982), which typically
differ in predominating pathogens and calf age at presentation. Calf pneumonia usually presents in dairy calves 2-4 months old (Nikunen et al., 2007), while shipping fever occurs in cattle between 5-8 months old (Snowder et al., 2006). Because cattle typically enter feeding systems around 5-8 months old, BRD and shipping fever are commonly discussed synonymously at this stage in beef production.

The timing of BRD occurs due to coinciding factors providing pathogens the opportunity to proliferate. Feeder calves are subject to numerous stressors, such as weaning, vaccination, castration, and dehorning (Taylor et al., 2010). In addition, calves may travel to sale barns, comingle, and ship distances averaging over 600 miles (Self and Gay, 1972) to a feedlot. Encountered stressors promote endocrine changes that suppress immune function (Coutinho and Chapman, 2011). Additionally, cattle are exposed to numerous pathogens, including those responsible for BRD. Interaction of these variables plays a pivotal role in BRD development, as peak morbidity occurs within the first 27 d of these insults (Buhman et al., 2000), commonly known as the receiving period in a feedlot. These outbreaks change with weather like extreme temperatures, severe temperature variations, and drought, but and are most numerous when stress and respiratory trauma from cold weather happen during fall and winter (Lalman and Smith, 2001).

Feedlot morbidity related to BRD was reported at 16% in 2011 (USDA-APHIS, 2011). Research has shown ranges from 5% to 44% with mortality usually less than 10% (Snowder et al., 2006), with some studies reporting morbidity as high as 80% (Storz et al., 2000). Disease rates like these increase production costs, with estimates of national
costs ranging from $500 million (Miles, 2009) to $1 billion (Mohamed and Abdelsalam, 2008). Precise calculations and data utilized were not included in these sources, providing some skepticism in figure accuracy. However, when probable inputs are considered, like treatment costs and performance losses, dollars can add up quickly.

The USDA-APHIS (2011) reported average single-treatment costs per calf at $23, which would only account for pharmaceutical interventions like antimicrobials, likely excluding anti-inflammatory drugs. Beyond medical costs, research has shown BRD-affected calves experience decreased ADG of 0.04 kg/d or greater compared to healthy counterparts over a 200-d feeding period (Lalman and Smith, 2001; Snowder et al., 2006). Even without considering labor costs associated with treatment, applying these morbidity and dollar values to the 24.6 million cattle on feed (USDA-APHIS, 2016) provides some validation for cost estimates.

Despite high incidence and known peak times, accurate diagnosis of BRD remains elusive. As prey animals, cattle can disguise or hide clinical signs until serious infection overwhelms their system. In addition, the clinical signs of BRD are about as nondescript as the disease definition itself. Experienced personnel, typically pen riders in a feedlot setting, evaluate calves for dull or depressed demeanor, anorexia, weakness or altered gait, elevated respiration rates and difficulty breathing, coughing, and excessive or mucopurulent ocular and nasal discharge (Berry et al., 2004; Snowder et al., 2006). Rectal temperatures greater than 39.7-40°C signify infectious processes and serve as a final qualification for treatment (Frank and Duff, 2000; Berry et al., 2004). A textbook case of BRD would present all signs; however, calves rarely follow the book and instead
present any combination of these hallmarks. To further complicate diagnosis, the more prevalent signs (like respiration changes, depression, and anorexia) are common in many pathologies. For example, these signs align with ruminal acidosis, which is a reasonable and frequent differential diagnosis in cattle adjusting to high-concentrate feedlot diets. Consequently, BRD diagnosis can be based less on presence of signs and more on the elimination of other possible diseases (Cusack, 2004). Bovine respiratory disease accounts for over 70% of feedlot morbidity (Edwards, 2010); with overlaps in clinical signs, this number could be lower than true BRD occurrence or even elevated via misdiagnosis of less common disorders as BRD (White and Renter, 2009). This ambiguity has fueled numerous experimental ventures towards discovering more effective diagnostic techniques in recent yr.

**Pathology and Pathogens**

Clinical signs of disease occur because of insult-induced alterations of homeostasis. Reactions or bodily responses can then go beyond normal physiological correction to be defined as pathological. In the case of BRD, the discussed signs are results of pathological changes in the respiratory system of affected calves. Morphological diagnoses present BRD as a cranioventrally-distributed fibrinous to fibrinopurulent bronchopneumonia (Andrews et al., 1985; Gagea et al., 2006; Mohamed and Abdelsalam, 2008). Interpreted, this description means BRD-affected cattle typically show bronchi involvement and lung consolidation primarily near the front of the lungs and towards the sternum, or rather, the lowest point of the organ in the standing animal. Fibrin-rich exudate accompanies in early stages, sometimes with pus as immune cells
migrate and chronicity increases. This presentation is characteristic of BRD and its defining pathogens.

Previously mentioned, BRD is the result of pathogenic interactions between a complex of microorganisms. Primary infections are attributed to viruses, namely strains of bovine viral diarrhea virus (BVDV), bovine respiratory syncytial virus (BRSV), bovine herpesvirus-1 or infectious bovine rhinotracheitis (BHV-1/IBR), and multiple strains of parainfluenza virus (PI-3; Salt et al., 2007). Viral infections are considered primary because they typically occur first in BRD pathogenesis, but produce milder symptoms when acting without other opportunistic pathogens (van der Poel et al., 1996; Salt et al., 2007). Each virus induces different disease processes, but overall, they are known to cause some form of mucosal injury to the respiratory tract or suppress protective immune functions. For these reasons, respiratory vaccines used in the industry are comprised of some variation of these viruses to develop immunity against their deleterious effects.

If the usual viral suspects are considered primary infectious agents, but are not responsible for the pathological manifestations of BRD, other factors must play a role. The immunosuppressive actions of some viruses, in addition to suppression through stress as previously discussed, opens the door for bacteria to take hold and colonize the respiratory system as a secondary infection (Griffin et al., 2010). While many different bacterial species could seize this opportunity, cultures have repeatedly found 3 gram-negative bacteria to be responsible for stereotypical presentations: *Mannheimia haemolytica, Pasteurella multocida,* and *Histophilus somni* (Dyson et al., 1981; Singer et
al., 1998; Catry et al., 2006; Dabo et al., 2008). These bacteria are commensal residents in the healthy respiratory tract that, when immune barriers are let down due to cell injury or suppression, overgrow and incite the damaging changes seen in BRD (Jasni et al., 1991).

**Mannheimia haemolytica**

Earlier, it was stated that BRD in feedlot cattle is used synonymously with “shipping fever.” These terms are used interchangeably because the most common pathogen cultured from receiving cattle with BRD is *Mannheimia haemolytica*, the etiological agent in shipping fever (Yates, 1982; Fodor et al., 1984; Welsh et al., 2004). This species is notorious for fibrinous bronchopneumonia in feeder cattle (Mohamed and Abdelsalam, 2008). Cattle with *M. haemolytica* overgrowths exhibit short disease courses, recovering or dying within 2 to 3 d of clinical presentation. Those that recover risk chronic respiratory issues responsible for BRD-associated performance losses.

*Mannheimia* induces these changes by the virulence factors it possesses. *M. haemolytica*, a gram-negative bacterium, has a protective capsule that permits adherence to the respiratory epithelial surface in addition to lipopolysaccharide (LPS) capable of causing extensive vascular damage and subsequent inflammatory responses (Breider et al., 1990). Additionally, this species produces leukotoxin, an exotoxin that specifically affects neutrophils and monocytes (Chang et al., 1986; Clinkenbeard et al., 1989). At lower concentrations, perhaps in early stages of infection, leukotoxin alters function in targeted cells. Higher concentrations are cytotoxic (Clinkenbeard et al., 1989). Consequently, pro-inflammatory cytokine release is stimulated, generating an immune
response. With increasing immune response, more cells are recruited to combat the bacteria and, in turn, encounter deadly leukotoxin. As more and more cells die, reactive oxygen species and enzymatic contents are released to cause further tissue damage and heightened inflammatory response; the immune response itself overreacts and becomes the source of pathological changes.

**Pasteurella multocida**

Only a matter of yr ago, *Pasteurella multocida* and *Mannheimia haemolytica* were both members of the *Pasteurella* genus (Dyson et al., 1981); therefore, it makes sense that disease presentations are fairly similar. Gagea et al. (2006) describe *P. multocida* similarly to the other 2 bacterial agents, presenting alongside a respiratory virus or *Mycoplasma bovis* and producing an edematous, fibrinous bronchopneumonia. Historically, *P. multocida* has not been regarded as a dominant pathogen in receiving-aged cattle, but rather more prevalent in calf pneumonia like that seen in young dairy calves (Nikunen et al., 2007). However, diagnostics over the last 15 yr have shown a shift in feedlot BRD cases to include *P. multocida* as a more frequent predominant bacterium (Welsh et al., 2004).

Also a gram-negative bacterium, *P. multocida* possesses similar virulence factors to *M. haemolytica*. Its capsule serves as camouflage, utilizing molecular mimicry to blend in with host tissues and evade immune defenses (DeAngelis, 1996). Lipopolysaccharide or endotoxin from *P. multocida*, as opposed to leukotoxin, serves as the primary virulence factor. Lipopolysaccharide causes endothelial damage and is highly antigenic. This action generates a strong inflammatory response through immune cell interleukin
release (Iovane et al., 1998), the importance of which will be discussed in detail later in this review; however, this response is not as intense or self-perpetuating as *M. haemolytica* (Tsuji and Matsumoto, 1988).

**Histophilus somni**

*H. somni*, the third gram-negative bacterium typically isolated in BRD cases, is less researched than the other 2. Nevertheless, studies show disease presents as a fibrinous to fibrinopurulent bronchopneumonia, like *M. haemolytica* and *P. multocida*. Andrews et al. (1985) reported multiple pulmonary abscesses and cranioventral lung consolidation, with 5-80% of the lung field affected in cases where only *H. somni* was isolated. *H. somni* has also been found to cause or progress to systemic infections affecting multiple systems, causing diseases like polyarthritis and thromboembolic meningoencephalitis if unnoticed or left untreated (Dabo et al., 2008).

*Histophilus* achieves infectious properties through LPS-induced vascular damage, just as the other 2 species (Inzana et al., 1988). Unique to this bacterium, though, is its ability to bind and inactivate immunoglobulins at the cell surface (Widders et al., 1989). This binding capacity aids in immune system avoidance and promotes bacterial persistence.

Pathogenic evaluation provides essential information regarding BRD development. Noting multiple similarities in causative agents, both bacterial and viral, helps to understand the reasoning behind defining BRD as a complex of pathogens. Additionally, knowledge of virulence factors aids in relating the tangible disease to the
corresponding immunological processes (detailed in the following sections) responsible for the disease presentation. In integrating this information, investigators can and have begun to formulate quicker, more accurate methods of BRD detection.

**ACUTE PHASE RESPONSE**

Life exists within a range of physiological parameters. Consequently, maintenance of homeostasis requires constant effort from animal systems to adjust for endogenous and exogenous factors causing deviations in these measures. In the case of BRD or other agents impacting animal health, highly coordinated immune responses take place to stop and remove the disruption. The rapid, generalized initial response is commonly referred to as the innate immune response. Following the innate is the adaptive response, characterized by agent-specified humoral and cell-mediated actions (Hirano et al., 1985; Helle et al., 1988). Both responses can be measured to gain information on immune system activity and function. For livestock health and diagnostics, however, the indications of inflammation provided with innate immunity measures have predominated in research (Dinarello 1984; Murata and Miyamoto, 1993; Godson et al., 1996; Carter et al., 2002; Jain et al., 2011; Piñeiro et al., 2013) as producers and veterinarians attempt to provide higher quality, more accurate interventions. More specifically, many researchers have investigated different components of the “first responder” events collectively known as the acute phase response.

The acute phase response (APR) consists of those changes, down to the cellular and endocrine levels, occurring both locally and systemically as the immediate reactions
to stress, trauma, or infection (Kushner, 1982; Baumann and Gauldie, 1994; Moshage, 1997). The APR is quantifiable within a matter of hours (Dinarello 1984). Typically, the APR presents with fever, altered metabolic function, mineral concentration changes, and immune protein synthesis (Beisel, 1977; Moshage, 1997).

Observed changes result from a cascade of events following insult. The inciting agent acts upon resident macrophages and circulating monocytes to stimulate differentiation into additional active macrophages; these cells act as sentinels in innate immunity and are primarily responsible for secretion of the cytokines interleukin-1 (IL-1), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNFα) which are necessary to initiate the APR (Koj, 1996). Many experiments focus on interleukin function, which will receive focus over TNF-α in this review; however, it is important to note that most immunology text and research explain IL-1 and TNF-α actions together due to similar behavior and effects during inflammatory responses (Saigusa, 1990; Moshage, 1997).

Marshall et al. (1999) and Plaut et al. (1989) also noted the importance of mast cells as additional sources of IL-6, TNFα, and other immune cell attractants. Not only do IL-1, IL-6, and TNF-α attract additional immune cells (leukocytes), they also impact thermoregulation at the hypothalamus to cause fever (Dinarello et al., 1988; Fey and Gauldie, 1990; Dinarello et al., 1991) and activate other pathways, like the arachidonic acid cascade, to promote supplementary inflammatory responses (Slauson and Cooper, 2002). For example, phospholipases are stimulated by circulating cytokines to act upon arachidonic acid in cell membranes and begin forming inflammatory substances like prostaglandins, thromboxanes, and leukotrienes responsible for vascular tonicity changes.
and pain stereotypically accompanying inflammation (Slauson and Cooper, 2002). Cytokines also influence gene expression for immune protein synthesis (Baumann and Gauldie, 1994) and secondary cytokine waves (Matsushima and Oppenheim, 1989).

While all the mentioned changes are regularly observed, the variations in metabolism, blood nutrient concentrations, and protein synthesis remain primary interests. With its involvement in each of these variables, the liver is a primary target for IL-1, IL-6, and TNF-α (Fey and Gauldie, 1990). Hepatocytes present large numbers of interleukin receptors and thus are believed to possess high sensitivities to those cytokines (Baumann et al., 1987). Under the influence of the secondary cytokine wave of the APR, the liver synthesizes specific immune proteins known as acute phase proteins (APP) (Kishimoto and Hirano, 1988), which will be detailed in sections to follow. To support the immunological processes, the liver also alters metabolic pathways and body mineral concentrations. For most diseases, including BRD, symptoms typically include anorexia; this means the animal must rely heavily on substrates and building blocks stored in tissues to meet the increased nutrient demands of fever and inflammation. Inflammatory conditions also leave the animal in a negative nitrogen balance (Dinarello et al., 1988). Tissue protein catabolism occurs, especially in skeletal muscle, to provide substrates in this state (Gruys et al., 2005). Additionally, alanine is directed away from protein synthesis and towards gluconeogenesis or branched-chain amino acid formation and tryptophan is shunted towards degradative pathways (Rapoport et al., 1970; Beisel, 1977). Chronic inflammation can permit processes to continue to a pathological state known as cachexia, characterized by reduced bone, muscle, and fat mass to provide
substrates for essential processes and immune cell proliferation (Fong et al., 1989). To counter these changes, some investigators in human medicine have found recovery success by providing high protein diets to trauma patients (Alexander et al., 1980).

Alterations in mineral stores and circulating concentrations occur alongside these protein changes, as some produced APP function in mineral binding and conservation (Ceciliani et al., 2012). The liver itself aids in conservation by holding minerals like iron in their stored forms (Cartwright et al., 1946). Other researchers have repeated this, finding decreased serum iron and zinc concentrations in subjects experiencing endotoxin challenge (Pekarek and Beisel, 1969). Mineral manipulations are categorized as protective mechanisms by reducing oxidative damage from circulating free radicals like iron and decreasing mineral availability to pathogenic microbes in attempts to starve the infection of nutrients (Eaton et al., 1982; Halliwell and Gutteridge, 1990; Alayash, 2011).

Pathological overreactions make the need for APR regulation apparent. The effects of IL-1, IL-6, and TNF-α are counteracted or reduced by other hormonal actions. Stress hormones like glucocorticoids have been shown to increase APP production while also reducing the functionality of necessary immune cells (Baumann et al., 1987). Insulin has also been noted to interrupt IL-1 effects on APP production (Campos et al., 1994). Checks and balances, provided the inciting agent is removed and stimulus does not reach a chronic state, typically permit APR resolution within 2-5 d of onset (Fey and Gauldie, 1990; Baumann and Gauldie, 1994).
Interleukin Functions

The major cytokines involved in the APR have many functions, sharing some impacts and possessing unique effects as well. Both IL-1 and IL-6 work as pyrogens, acting at the hypothalamus to alter thermoregulatory set points in efforts to restrict pathogen proliferation (Dinarello 1984; Fey and Gauldie, 1990; Saigusa, 1990). While fever can reach detrimental levels and cause caloric strain on the body, it is essential for effective infection clearance (Dinarello et al., 1988). Endocrine changes are also observed early in the acute phase, as IL-1 and IL-6 cause corticotropin-releasing hormone (CRH) secretion from the hypothalamus, subsequent adrenocorticotropic hormone (ACTH) release from the pituitary and related glucocorticoid increases downstream (Sapolsky et al., 1987; Naitoh et al., 1988; Nishio et al., 1993). Glucocorticoid release has been associated with increased interleukin receptors, heightening IL sensitivity (Baumann et al., 1987; Baybutt and Holsboer, 1990; Jain et al., 2011). IL-1 and IL-6 both attract and stimulate immune cells and modify gene expression in different cells, like monocytes, fibroblasts, and endothelial cells, to name a few. However, the timing of their influence in the APR and the specifics of what each interleukin targets helps to distinguish the cytokines and elucidate their roles.

Interleukin-1

IL-1 is recognized as a major component in the first cytokine wave in the APR, produced by stimulated macrophages to act on other cells and upregulate the immune response, especially through increasing IL-6 production in the second cytokine wave (Dinarello et al., 1991; Zheng et al., 1995). IL-1 has also been found to have more
widespread effects on inflammation, stimulating prostaglandin production in the nervous system (for fever induction) and different cell types, like monocytes, fibroblasts, and endothelial cells (Bernheim and Dinarello, 1985; Dayer et al., 1986; Dinarello et al., 1987). Beyond inflammation, IL-1 causes altered synthesis of certain APP known as Type I APP. These APP are mostly or completely dependent on IL-1 receptor stimulation for response (Moshage, 1997).

Like most physiological processes, IL-1 actions are subject to regulation. Negative feedback mechanisms have been shown to interact with IL-1 or its receptors. Insulin acts on IL-1 signaling to reduce its APP effects (Campos et al., 1994). This role could help explain the utility of anorexia during infectious processes. Other hormones like glucocorticoids, found to increase IL-1 sensitivity at the beginning of the APR, work to decrease IL-1 activity and lymphocyte proliferation as the APR duration increases, exemplifying their immunosuppressive effects as well (Staruch and Wood, 1985; Murata and Miyamoto, 1993; Coutinho and Chapman, 2011).

**Interleukin-6**

IL-1 possesses wide-reaching stimulatory effects for initiating innate immune system responses. IL-6 has been shown to intensify and prolong those responses while also functioning to integrate innate and adaptive immune responses. IL-6 concentrations increase through pathogen stimulation, like IL-1 and TNF-α; however, IL-6 also increases with administration of the latter 2 cytokines, clarifying its predominance in the second APR cytokine wave (Ray et al., 1989; Dinarello et al., 1991).
IL-6 in these APR cytokine waves targets numerous organs and cell types to induce changes. Mentioned previously, IL-6 promotes inflammatory processes and induces fever much like IL-1. However, IL-6 effects are more targeted, acting specifically on the nervous system to release prostaglandins for fever generation rather than causing generalized prostaglandin production in other somatic cells (Dinarello et al., 1991). Although IL-6 does not stimulate prostaglandin release systemically, it does act as an alarm molecule to stimulate immune cells, especially amid gram-negative bacterial infections (Helfgott et al., 1987). Innate immune cells are primed for protective actions, like reactive oxygen species release from neutrophils to combat infection, while specialized adaptive immune cells are also influenced (Kishimoto and Hirano, 1988; Heinrich et al., 1990). Specifically, B lymphocytes and cytotoxic T cells react to IL-6. B cells are prompted to mature and produce immunoglobulins to opsonize pathogens (Hirano et al., 1985; Lotz et al., 1988). Cytotoxic T cell differentiation and activation occurs under the influence of IL-6, causing cell-mediated immune action against the insult (Helle et al., 1988). The diverse target cells for IL-6 and its dependence on IL-1 for maximal response gives an idea about the importance of interleukins in acute immune responses, even though the true extent of their actions remains to be determined.

Outside of immune cell targets, IL-6 has received research attention related to APP production. IL-6 is regarded as the chief stimulant in APP synthesis, stemming from repeated research results noting widespread and generalized APP increases following IL-6 infusion (Castell et al., 1988; Gabay and Kushner, 2001; Jain et al., 2011). This generalized action becomes apparent in research showing IL-1-stimulated Type I APP
also change with IL-6 infusion; Type II APP, however, are only stimulated by IL-6 with no synergistic activation by IL-1 (Petersen et al., 2004). On a cellular basis, this relationship could be attributed to unidirectional overlaps in cytokine signaling pathways, allowing IL-1/IL-6 synergy in 1 pathway and IL-6 specificity in another (Sadowski et al., 1992). Additionally, IL-6 has a profound effect on APP synthesis at the liver because hepatocytes express high numbers of IL-6 receptors, increasing sensitivity and potential effect (Castell et al., 1988). Such a pronounced role in hepatic immune protein synthesis even earned IL-6 the name of “hepatocyte stimulating factor” (HSF) when first discovered (Gabay and Kushner, 1999).

The APR is a complex cascade of events. Common activators in many signaling pathways, messengers serve as potential backups for each other as insurance comes with redundancy in physiological systems. The cytokines also possess multiple unique roles, all essential in a successful APR. While the manifestations of completed signals in the form of cell-mediated attacks or altered protein synthesis are how the system regains homeostasis, the importance of the signaling molecules themselves cannot be ignored.

**Acute Phase Proteins**

Given their title, APP are a hallmark of the acute phase response recognized in research as early as the 1930s (Tillett and Francis, 1930; MacLeod and Avery, 1941). Experimental results and general APP information define an APP as a protein increasing or decreasing in concentration with inflammation. This dynamic nature of APP has led to varying classification methods. One method has already been discussed in which an APP is identified by its stimulating cytokine. This categorization is species-dependent, which
makes widespread use impractical (Petersen et al., 2004). A more common categorization coincides with the general definition, sorting APP based on if they increase or decrease during inflammation. Positive APP show increased synthesis, while negative APP present decreased synthesis (Birch and Schreiber, 1986). Even further, proteins can be divided into major, moderate, and minor APP based on the concentration change magnitude; like Type I/II, these subclassifications can change by species. The profile of these APP changes can vary by disease or causative agent, as different agents cause different inflammatory responses and intensities (Ceciliani et al., 2012). Just as the APR occurs with stimuli other than infectious etiologies, bovine APP concentrations have exhibited alterations with disorders like subacute ruminal acidosis (SARA) and displaced abomasum (Khafipour et al., 2009; Guzelbektes et al., 2010). Some research has noted more pronounced changes in bovine APP concentrations in bacterial infections compared to viral infections (Gruys et al., 2005; Nikunen et al., 2007). Regardless, APP changes have been shown to follow timelines of the APR, typically peaking within 24-48 h and resolving to pre-insult levels in 3 to 5 d (Fey and Gauldie, 1990; Jain et al., 2011). Considering these details, it could be discovered in future research that infectious vs. metabolic inflammation produce measurable differences in APP.

Variation in APP concentration changes related to type of injury is likely related to different bodily reactions involved with various insults, but may also be attributable to the numerous APP, their separate functions, and how those functions apply to different insults. Certain APP have been found to possess roles in microorganism opsonization and trapping, cascade initiation, and cell product scavenging, all serving to protect or restore
host functions (Gruys et al., 2005). The purposes exhibited and the changes shown with
different inflammatory agents have gained APP attention in research and application in
recent yr. Swine research has already found an APP, pig-MAP, useful for monitoring
herd health and stress, even at levels indistinguishable by visual observation (Piñeiro et
al., 2013). Similarly, cattle researchers have explored APP as options for monitoring
receiving cattle for BRD for many of the same reasons, as the disease can evade detection
and cause harm. APP detection has proven more sensitive than subjective visual
diagnosis likely due to this difficulty in observation (Godson et al., 1996; Humblet et al.,
2004; Nikunen et al., 2007; Arthington et al., 2013). Multiple APP have been utilized as
potential detection tools. However, based on typical APP changes in ruminants in
combination with the pathogens responsible for BRD, 2 proteins have gained notoriety
and will be focused upon in this review: haptoglobin (HPT) and lipopolysaccharide-
binding protein (LBP) (Sheffield et al., 1994; Idoate et al., 2015).

**Haptoglobin**

Haptoglobin is 1 of the most researched bovine APP. It is a major APP in cattle,
meaning its concentrations consistently increase 100 to 1000-fold in healthy to diseased
serum (Bremner, 1964; Goodger, 1970; Dinarello 1984; Eckersall and Bell, 2010),
making it easier to monitor and suited for diagnostic research. Additional utility comes
with HPT’s behavior with different stimuli. In transported, highly stressed cattle, research
shows only slight rises in HPT (Murata and Miyamoto, 1993). HPT concentrations also
differ between viral and bacterial respiratory infections (Godson et al., 1996; Arthington
et al., 2013). The presented traits show how HPT can fit with feedlot health monitoring. To understand these features, HPT’s function must be evaluated.

HPT binds free hemoglobin in the APR (Sheffield et al., 1994; Gabay and Kushner, 1999; Alayash, 2011). Hemoglobin (Hb), the iron-containing protein essential for oxygen transport, is released from erythrocytes when they lyse (Kristiansen et al., 2001). Haptoglobin-hemoglobin complexing is important because binding hemoglobin also binds free iron, a free radical that can oxidize tissues and cause further damage (Ceciliani et al., 2012). For this reason, HPT is considered an anti-inflammatory protein necessary for APR resolution (Gabay and Kushner, 1999). Additionally, iron is an essential nutrient for many bacterial species. Thus, HPT-Hb complexes reduce iron available for bacterial utilization, providing HPT with bacteriostatic function as well (Eaton et al., 1982). To remove Hb and iron from the circulation for good, complexes present to macrophages to stimulate endocytosis and destruction with assistance from soluble and membrane-associated CD163 (Kristiansen et al., 2001; Alayash, 2011).

Complexing, while essential for the animal, does provide an obstacle in measurement. Because developed assays measure free HPT, complexes are not bound by the antibodies and recognized as present, skewing laboratory results (Gruys et al., 2005). Therefore, ELISA kits require serum samples free of significant hemolysis. HPT provides the most information in diseases not characterized by intravascular hemolysis, hence its common use in BRD research.
Knowing HPT function led to its observation in BRD pathogen interactions. Primarily, research has focused on the magnitude of its change with different infections. In different diseases, multiple experiments have shown large increases in HPT between healthy and sick animals (Godson et al., 1996; Hirvonen et al., 1996; Jacobsen et al., 2004; Ekersall and Bell, 2010; Tothova et al., 2010). From here, HPT response to different bacterial infections was measured to see if HPT could be used to distinguish respiratory disease from others. Nikunen et al. (2007) showed increases in HPT between respiratory cases with and without *P. multocida*, a major bacterial pathogen in BRD. Other studies revealed increased HPT concentrations in *M. haemolytica* challenges, another major BRD bacteria, compared to clostridium challenge and controls (Arthington et al., 2013). Because BRD pathogenesis typically includes viral pathogens, researchers also differentiated HPT responses to common respiratory viruses from bacteria. Some studies have shown measurable differences in HPT concentration between viral and bacterial respiratory infections (Godson et al., 1996), while others did not (Heegaard et al., 2000). Godson et al. (1996) compared BHV-1 with *M. haemolytica* challenges in the same experiment, while Heegaard et al. (2000) compared BRSV to previously discovered bacterial responses; by lacking simultaneous comparison of BRSV to bacterial infections, strong counterarguments between the experiments are difficult to make. Considering this information with the common observation of more moderate disease signs under viral infections, HPT in BRD research appears to fit the hypothesis that respiratory bacterial infections invoke stronger inflammatory responses than viruses.
To establish HPT’s diagnostic utility over similar APP, comparative studies between HPT and serum amyloid A (SAA), another major bovine APP, were completed. In the face of LPS or viral challenges, HPT concentration was noted to peak later than SAA but provided a larger and more prolonged peak (Heegaard et al., 2000; Jacobsen et al., 2004). Peak timing could be attributed to HPT stimulus, being a type II APP in bovine and relying on the second cytokine wave of IL-6 to reach maximum synthesis. Logically, more rapid inflammation detection with SAA and the potential accuracy from multiple APP readings should be best for BRD diagnosis. However, from a practical standpoint, the timing and duration of HPT changes provide more achievable detection with adequate accuracy (Carter et al., 2002; Humblet et al., 2004). Others have also evaluated the utility of an APP ratio or multi-APP approach to diagnosis, though this technique has not been thoroughly researched (Toussaint et al., 1995).

Finally, researchers have examined HPT and information it provides regarding infection severity, which can help guide medical intervention decisions. Some research has questioned the reliability of HPT for measuring disease severity (Young et al., 1996). However, it should be noted that this research had limited amounts of morbidity data to work with. Numerous experiments provide strong counterevidence. In dairy cattle, Hirvonen et al. (1996) found HPT concentrations to be negatively correlated with prognosis. Higher HPT concentrations have been observed in mortality or chronic BRD cases compared to recovered cases, as well (Godson et al., 1996; Tothova et al., 2010).
Lipopolysaccharide-Binding Protein

LBP is a more recently discovered moderate, Type I APP (Wan et al., 1995; Schumann et al., 1996; Ceciliani et al., 2012), meaning it is upregulated in response to IL-1 and IL-6, but undergoes a lesser change in concentration compared to HPT. LBP can be found freely circulating on its own or in association with CD14 (a component of the LBP signaling pathway), or even associated with mononuclear cell membranes (Bannerman et al., 2003; Kopp et al., 2016). Like other APP, LBP is produced by hepatocytes (Heinrich et al., 1990), though LBP mRNA has also been observed in extrahepatic cells such as type II pneumocytes in the lungs (Dentener et al., 2000; Rahman et al., 2010).

LBP has likely received interest in researching diseases caused by gram-negative bacteria due its function. As the name implies, LBP binds the major constituent of gram-negative bacteria known as LPS (Horadagoda et al., 1995). More specifically, LBP recognizes the lipid A region and other pathogen-associated molecular patterns (PAMPs) that are highly conserved across gram-negative species (Schumann et al., 1996). LBP binds these regions with high specificity, avoiding confusion with other like molecules (Branger et al., 2005). These traits maximize its potential to identify LPS and make it functional in gram-negative bacterial infections.

Upon binding, LBP-LPS complexes interact with soluble or membrane-associated CD14 to then present to monocytes at the TLR4 receptor and transduce the signal to activate the cell and promote immune cell responses (Hailman et al., 1994; Kopp et al., 2016). It has been shown that this progression does not necessarily require LBP or other
constituent proteins, as LPS can interact with cells without LBP and LBP-LPS complexes can invoke responses without membrane receptors (Hailman et al., 1994; Kopp et al., 2016). Lipopolysaccharide can even be removed from circulation via lipoproteins (Wurfel et al., 1994). However, immune response efficiency is greatly improved when LBP is present and functioning. For example, LBP does not change CD14 affinity for LPS, but it promotes more rapid complete binding (Hailman et al., 1994). The influence of improved LPS binding and association has been exemplified when, in LBP presence, inflammatory responses have been elicited at LPS concentrations 150 to 1000-fold less than LBP-free solutions (Martin et al., 1992; Hailman et al., 1994). Also, beyond the immediate innate responses, T cell activation is reduced in the absence of LBP (Branger et al., 2005). Rapid and effective responses, like those promoted by LBP, are essential to minimize tissue damage and regain homeostasis in a timely manner.

Despite a smaller body of research compared to HPT, the LBP in feedlot health monitoring provides potential utility. Bovine respiratory disease typically involves gram-negative bacteria, so it could be deduced that LPS is also present in the system. Researchers have tested this hypothesis in recent yr with experimental and natural BRD challenges. Nikunen et al. (2007) and Idoate et al. (2015) showed increases in serum LBP concentration either with P. multocida infections in young dairy calves or naturally-occurring feedlot BRD cases, respectively. These results mirror HPT, providing some validity to LBP’s use as a potential diagnostic tool.
SUMMARY

Current industry costs associated with BRD are abundant and, compared to annual losses over previous years, have not shown substantial improvement despite advances in pharmaceuticals, technology, and management techniques. This lack of improvement involves the many challenges presented with beef production. Industry structure provides limitations that are difficult to avoid, like those associated with transportation stress and disease exposure. Bovine respiratory disease itself, as discussed, is challenging to consistently diagnose and do so accurately. However, these challenges are not insurmountable so long as innovative approaches to animal health and management are evaluated and utilized. Societal opinions and government regulations on antibiotic use in production livestock settings have provided additional incentive to go against the status quo and further alter BRD management strategies. To address these challenges, focus must be placed on improved diagnostic accuracy and treatment success. Bovine respiratory disease cases need to be discovered consistently and differentiated from similar, non-respiratory presentations and this must occur early in the course of the disease. Investigators have worked to make these improvements studying things like behavioral cues and serological parameters as innovative diagnostic approaches. A foolproof method likely does not exist and BRD elimination is not possible. But even slight advancements could provide large payoffs, both for producers’ pocketbooks and society’s views on production agriculture.
CHAPTER 2

NUTRITIONAL MEASURES AND POTENTIAL DIAGNOSTIC METHODS IN COMBATING BOVINE RESPIRATORY DISEASE

ABSTRACT

This experiment aimed to determine effects of amino acid supplementation and gut-modifying additives on occurrence of bovine respiratory disease (BRD), as well as evaluate feed intake, arterial blood parameters, and acute phase proteins (APP) in receiving cattle with naturally-occurring BRD. We hypothesized that receiving cattle consuming diets formulated to exceed amino acid requirements and include gut-modifying additives would present fewer BRD cases than animals fed to NRC requirements. We also hypothesized a greater reduction in feed intake, different arterial blood parameters, and higher concentrations of APP haptoglobin (HPT) and lipopolysaccharide-binding protein (LBP) in sick calves (case) compared to healthy counterparts (control). Three-hundred-eleven mixed breed beef steers of similar age and initial BW (IBW; 233.61 ± 20.74 kg) were randomly assigned to 1 of 6 pens for a 21-d receiving period feeding trial. One of 3 treatment diets were randomly assigned to pens: a diet without additives formulated to meet AA requirements (CONTROL, n = 104), a diet without additives formulated to exceed AA requirements by ≥ 10% (AA, n = 104), and a diet with additives formulated to exceed AA requirements by ≥ 10% (AAXS, n = 103). Twenty-two steers (CONTROL, n = 8; AA, n = 4; AAXS, n = 10) were diagnosed with BRD using a combination of previous d DMI and industry-standard visual observation of signs. A control was pulled with each case steer, when cases received treatment and
arterial and jugular blood samples were obtained for chemistry and APP analysis, respectively, for both case and control animals. Retrospective analysis was completed for feed intake differences in case and control calves. Morbidity rates were not different between diets ($P = 0.16$). Control DMI was greater compared to case DMI ($P < 0.01$). Control arterial $pO_2$ ($P = 0.02$) and $sO_2$ ($P = 0.03$) were greater compared to cases. HPT and LBP concentrations were greater in cases ($P < 0.01$). Bovine respiratory disease prevention through amino acid and additive supplementation was inconclusive, but showed numerical reductions in morbidity with amino acid supplementation. Intake, blood chemistry analysis, and HPT and LBP provided potential for improvement upon current industry diagnostic rates. Possible complementarity in these methods could help to enhance BRD diagnosis in research and the cattle industry.

**INTRODUCTION**

Accounting for up to 70% of health disruptions (USDA-APHIS, 2011) and $500$ million in associated medical and production losses (Miles, 2009), bovine respiratory disease (BRD) receives considerable attention from cattle health personnel in production and research alike. Bovine respiratory disease diagnosis traditionally involves subjective observation by experienced handlers for clinical signs; however, this method has proven inconsistent with estimated sensitivity and specificity at 62% and 63%, respectively (White and Renter, 2009). To reduce associated losses, research has aimed to address BRD more effectively through nutrition-based immune support and earlier, more accurate BRD detection through objective measures. Procedures revolve around typical BRD manifestations and their corresponding measurable changes. Symptoms like dyspnea and
anorexia have prompted research of related blood parameter and feed intake alterations (Šoltésová et al., 2015; Jackson et al., 2016). Acute phase proteins, innate immune response components, have been extensively researched for their consistent changes during BRD (Idoate et al., 2015). Further, dietary support for immune-related nutrient demand increases has shown benefit in disease recovery in other species (Alexander et al., 1980), but nutrition and its aid in disease prevention is still unclear. The current study evaluated each of these aspects.

The first objective was to evaluate morbidity in animals consuming diets of varying amino acid inclusion and gut-modifying additives, with the hypothesis that receiving cattle consuming diets formulated to exceed amino acid requirements and include gut-modifying additives would present fewer BRD cases than animals fed to NRC requirements. The second experimental objective was to investigate feed intake changes surrounding morbidity for potential diagnostic patterns. We hypothesized greater reductions in case calf feed intake on d of diagnosis, with observable patterns derived from these differences. The third experimental objective was to compare arterial blood gas and chemistry parameters in case and control calves, hypothesizing that these parameters would differ consistently between case and control animals. The final experimental objective was to analyze concentrations of the APP haptoglobin (HPT) and lipopolysaccharide-binding protein (LBP) in calves with naturally-occurring bovine respiratory disease diagnosed by standard industry methods. We hypothesized HPT and LBP concentrations would be greater in case calves compared to controls.
MATERIALS AND METHODS

Animals and experimental design

All procedures were approved by the University of Missouri Institutional Animal Care and Use Committee. Three-hundred-eleven mixed breed beef steers and bulls of similar age and initial BW (IBW; IBW=233.61 ± 20.74 kg) were purchased from a regional stockyard in November 2016. Calves were purchased in 2 groups, 1 week apart (Group 1: n = 150; IBW = 235.00 ± 19.34 kg; Group 2: n = 161; IBW = 231.38 ± 21.80 kg), and transported 214 miles to the University of Missouri Beef Research and Teaching Farm (BRTF; Columbia, MO). Upon arrival, cattle were unloaded in equal numbers to 6 pens measuring 36.6 x 23.4 m with feed bunks and water on a 6.1 x 36.6 m concrete pad and the remainder in dirt and gravel. Each pen contained 2 structures (7.6 x 9.1 m) for shelter from sunlight and inclement weather. Animals were provided ad libitum access to grass hay and water overnight. Hay was provided in concrete J-bunks. At 0700 h the next d, consecutive 2-d weights and processing commenced. Radio frequency identification tags (RFID) and visual identification tags (VID; Allflex, USA, Inc., Dallas Fort Worth, TX, USA) were placed in the left and right ear, respectively. The right ear was also notched to test for persistently-infected bovine viral diarrhea (BVD-PI) cases; no animals tested positive for BVD-PI. According to Beef Quality Assurance standards, calves received subcutaneous metaphylactic tulathromycin (Draxxin; Zoetis, Florham Park, NJ, USA) to prevent illness prior to GrowSafe adoption because feed intake was a variable of interest as a potential diagnostic test, as well as oral fenbendazole (Safe-Guard; Merck Animal Health, Omaha, NE, USA). Sex was determined for stratification. Animals were
stratified by IBW, color, and sex, and randomly assigned to 1 of 6 pens with 1 of 3 treatment diets (CONTROL; n=104, AA; n=104, AAXS; n=103) randomly assigned to pen on d 2 of the consecutive weigh d. Fifteen bulls were castrated with a Newberry knife (SyrVet, St-Alphonse-de-Granby, QC, Canada) prior to sorting on d 2. Consecutive 2-d weights were taken at study completion after animals had received respective diets for 21 d. Animals were removed from the study (n=30; Control, n=9; AA, n=7; AAXS, n=14) for crossing pen barriers and consuming a different diet, failure to adapt to the GrowSafe system, or mortality unrelated to BRD.

**Diets and feeding**

Steers returned to assigned pens following initial second-d weighing and sorting. There, they received rations *ad libitum* in automated GrowSafe feed bunks (GrowSafe Systems Ltd., Airdrie, Alberta, Canada) to monitor intake behavior for the remainder of the 21-d receiving trial. Each pen contained 5 GrowSafe bunks. Diets were provided between 0700-0800 h daily. All diets consisted of a whole shelled corn base with rye baleage and a concentrate supplement to which experimental changes were applied (Table 2.7). Formulations were completed to achieve 1.41 kg ADG with 2.10 % BW DMI using average receiving period BW. The control diet was formulated to meet or exceed growing beef animal requirements for amino acids, vitamins, and minerals (NRC, 2000), and meet requirements for effective energy (EE; Emmans, 1994). Methionine was the most limiting AA (103% of requirement), followed closely by arginine (105% of requirement). The AA diet was formulated to meet or exceed requirements for vitamins, minerals and EE like CONTROL; however, blood meal and DDGS were removed and
soybean meal reduced in the supplement and replaced by cottonseed meal, rumen-protected encapsulated lysine (AjiPro, Ajinomoto Heartland, Inc., Eddyville, IA, USA), and rumen-protected encapsulated methionine (Adisseo USA, Inc., Alpharetta, GA, USA) to increase all amino acids to a minimum of 113% of requirement, with lysine as the most limiting AA, followed by arginine (115% of requirement) and threonine (115% of requirement). The AAXS diet was formulated to meet or exceed requirements the same as the AA diet; however, a small percentage of corn in the supplement was replaced with a plant extract-based rumen modifier (XTRACT Ruminants X60-7065; Pancosma, Geneva, Switzerland) and an artificial sweetener (SUCRAM, Pancosma, Geneva, Switzerland). Ground corn served as a carrier in the concentrate supplement.

Supplements were mixed in 909 kg batches and stored in upright bins at the BRTF. Supplement, whole shelled corn, and rye baleage were mixed at time of feeding in a truck-mounted mixer.

Feed samples for proximate analysis were collected weekly from the truck-mounted feed mixer as diets were dispensed to the GrowSafe bunks. Samples were stored at -20°C until analyzed. Diet samples were initially weighed and dried at 55°C (Model 7921; Blickman Health Industries, Clifton, NJ, USA), then ground to pass through a 3-mm screen (Wiley Mill; Arthur H. Thomas Company, Philadelphia, PA, USA) and 1-mm screen (Foss Tecator Cyclotec Sample Mill, Foss North America, Eden Prairie, MN, USA) sequentially. Ground samples were analyzed for NDF and ADF (ANKOM200 Fiber Analyzer; ANKOM Technology, Macedon, NY, USA) and nitrogen content (vario Macro Cube, Elementar Americas, Mt. Laurel, NJ, USA). Samples were then dried at 105°C and
analyzed for DM (Isotemp Oven Model 255 G; Fisher Scientific, Pittsburg, PA, USA), and ash (Isotemp Muffle Furnace; Fisher Scientific, Pittsburg, PA, USA). Weekly samples were analyzed separately and results were compiled to formulate averages for each diet.

**Clinical examination**

Preceding d feed intakes were observed prior to visual appraisal of steer health. Calves presenting intakes less than 4.54 kg were listed for close in-pen inspection by animal technicians for bovine respiratory disease (BRD). Bovine respiratory disease was diagnosed by visual observation for depression, low gut fill, labored and shallow breathing, increased respiration rate, coughing, excessive salivation, nasal discharge, and ocular discharge. Steers presenting combinations of these signs with sufficient severity to satisfy BRD diagnosis definitions set forth by experienced veterinary and feedlot personnel, even if not on the low intake list, were pulled for rectal temperature. Temperatures greater than 40°C completed case animal definition and thus warranted antimicrobial treatment with enrofloxacin (Baytril 100; Bayer Animal Health, Shawnee, KS, USA); animals meeting all criteria for case definition except rectal temperature (n=3) were treated, but were not sampled or analyzed as cases. For every case calf (CONTROL, n = 8; AA, n = 4; AAXS, n = 10), a corresponding control was pulled from the same pen. Control calves could not satisfy any of the case definitions to qualify. No recurring respiratory illnesses were observed in the 21-d trial. Technicians were not blinded to diet treatments.
Intake behavior

Feed intake data surrounding morbidity events were analyzed retrospectively using individual intakes obtained from GrowSafe data. Daily intakes were converted to dry matter according to the proximate analysis of the diet consumed. Both case and control animal intakes were plotted and a polynomial regression line for average or predicted intake (Reg) was fitted to each individual’s data. Noting that cases were treated for BRD based on previous-d intake, this intake was treated as the diagnostic feed intake. Changes in residuals of this diagnostic intake (Resint) below the individual animal’s mean intake for the 21-d period were also observed for predictive patterns. Standard deviations (stddev) in Resint were calculated as an additional dimension.

Biological sampling

Arterial blood samples were obtained from the intermediate branch of the caudal auricular artery. The site for blood draw was shaved and cleansed with isopropyl alcohol-soaked gauze. Between 0.02 mL and 0.04 mL lidocaine HCl 2% were administered subcutaneously at the sampling location. Arterial blood was drawn using a sterile scalp vein butterfly set consisting of a 0.8×19 mm needle and 30.4 cm of tubing (EXELINT International, Co., Redondo Beach, CA, USA) flushed with heparinized saline prior to ateriopuncture to prevent clotting. Less than 1 mL was collected to evaluate blood components using a handheld blood analyzer (VetScan i-STAT 1; Abaxis, Inc., Union City, CA, USA). Samples were analyzed immediately following sampling and results for patient rectal temperature (temp), pH, pCO₂, pO₂, extracellular fluid base excess (BEecf),
bicarbonate concentration (HCO₃), total CO₂ (TCO₂), percent oxygen saturation of hemoglobin (sO₂), and lactate concentration (lactate) were recorded.

Blood samples for acute phase protein (APP) analysis were collected from cases and corresponding controls in 10 mL serum separator tubes (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) via jugular venipuncture, allowed to clot, and stored in ice until transported 5.9 miles to the lab for processing. Blood was then centrifuged at 1500 × g for 30 min at 4°C. Serum was harvested equally into 3 aliquots, 1-1.5 mL apiece, and stored at -20°C for later analysis.

**Acute phase protein analysis**

Serum LBP concentration was analyzed with a commercially available Multispecies-Reactive Human LBP ELISA kit (Cell Sciences Inc., Canton, MA) that recognizes human and bovine LBP. Case and control samples were initially diluted at 1:50 and adjusted according to returned concentration differences. Final case sample dilutions ranged from 1:50 to 1:400 and final control sample dilutions remained at 1:50. Serum was analyzed in duplicate, randomized across plate, and assayed according to manufacturer’s instructions. The LBP ELISA kit detection range is 0.05-2.5 μg/mL. Manufacturer-provided reference serum was utilized as an internal control. Hemolyzed samples and samples falling outside the detection range (n = 4) were removed from analysis.

Serum HPT concentration was determined using a commercially available Bovine Haptoglobin ELISA kit (Immunology Consultants Laboratory, Inc., Portland, OR, USA).
Case and control samples were initially diluted at 1:400 and 1:8000, respectively. Based on results at these initial dilutions, adjustments were made accordingly to account for concentration differences. Final control sample dilutions ranged from 1:50 to 1:1000 and final case sample dilutions ranged from 1:1000 to 1:24,000. Samples were analyzed in duplicate, randomized across plate, and assayed according to manufacturer’s instructions. The HPT ELISA kit detection range is 15.6-1000 ng/mL. An obtained serum sample that provided consistent duplicated readings with a low coefficient of variation (CV) was utilized as an internal control. Hemolyzed samples and samples falling outside the detection range (n=7) were removed from analysis.

**Diagnostic test comparison**

Data from feed intake and APP analyses were further evaluated for diagnostic capabilities. Individual case and control values for each measurement were plotted in a scatterplot with animal identification on the x-axis and the variable of interest on the y-axis. Diagnostic thresholds for feed intake and APP serum concentration were determined using confidence intervals based upon observed patterns and differences in the data. Correct and incorrect diagnoses, using the described diagnostic procedure as the gold-standard test, were used to calculate test accuracy (percent of correctly diagnosed animals), sensitivity (percent of disease-positive animals that tested positive), and specificity (percent of disease-negative animals that tested negative).

**Statistical analysis**

Data were analyzed as a completely randomized design with individual animal as the experimental unit. Analysis 1 pertaining to morbidity differences between diets
consisted of a chi-square test using the GENMOD procedure of SAS (SAS 9.4; SAS Inst. Inc., Cary, NC), fitting diet as a categorical variable and percent morbidity as a dependent variable. Percent morbidity was calculated as the number of case animals in diet divided by total animals in diet. Significance was set at $\alpha \leq 0.05$ and tendencies were set at $\alpha \leq 0.10$. For analysis 2 related to intake behavior, an analysis of variance (ANOVA) was conducted with health status (case vs. control) fitted as the independent variable and DMI, Reg, Resint, and stddev fitted as dependent variables. Least squares means for dependent variables were obtained and analyzed using the LSMEANS statement within the GLM procedure of SAS 9.4. Significance was set at $\alpha \leq 0.05$ and tendencies were set at $\alpha \leq 0.10$. For arterial blood parameters and APP, data were determined to not be normally distributed; therefore, a rank transformation using the RANK procedure of SAS was conducted according to Conover and Iman (1981) to normalize data; all differences were determined using the rank data and were presented alongside actual data means. Arterial blood parameters were evaluated as analysis 3 using GLM with health status as the independent variable and temp, pH, pCO$_2$, pO$_2$, BEecf, HCO$_3$, TCO$_2$, sO$_2$, and lactate as dependent variables. Significance was set at $\alpha \leq 0.05$ and tendencies were set at $\alpha \leq 0.10$. The fourth analysis utilized GLM and LSMEANS to compare APP concentrations. Health status was set as the independent variable and serum APP concentration was set as the dependent variable. This procedure was conducted for both HPT and LBP. Significance was set at $\alpha \leq 0.001$ based on similar research.
RESULTS

Morbidity

Though numerically different, morbidity rates were not statistically different among diets ($P = 0.16$). Results are shown in Table 2.2.

Intake Behavior

Intake behavior measurements at d of BRD diagnosis are reported in Table 2.3. Significant differences were shown in all observed variables. Control DMI was greater compared to case DMI ($P < 0.0001$). Control Reg was also greater than case ($P = 0.01$). Residual intake was lesser in case animals than controls ($P = 0.006$). Both categories showed changes in stddev below the mean, but cases presented a greater stddev change below the mean ($P = 0.002$).

Individual DMI at diagnosis is plotted in Figure 2.1 as a percent of control average DMI, which functions as the normal, entire group average DMI. 60% of the group average DMI was used as a threshold for BRD diagnosis because it was determined as the upper limit for the 95% confidence interval in case DMI data. Corresponding sensitivity, specificity, and test accuracy were 75%, 71%, and 72%, respectively.

Arterial Blood Parameters

Clinical reference ranges of all utilized arterial blood parameters and experimental results for blood parameters at BRD diagnosis are presented in Table 2.4, with $P$-values from rank transformation analysis presented alongside means and standard errors of
actual measured data. Differences were not observed for pH, pCO₂, BEecf, HCO₃, TCO₂, or lactate ($P > 0.49$). Animal rectal temperature was different between categories, with case temperature greater than control ($P < 0.0001$). Control pO₂ was greater than case pO₂ ($P = 0.02$). Also, sO₂ was greater in controls compared to cases ($P = 0.03$).

**Acute phase proteins**

Comparison of LBP concentration is reported in Table 2.5, with $P$-values from rank transformation analysis presented alongside means and standard errors of actual measured data. LBP concentration was greater in case calves compared to controls ($P < 0.0001$).

Individual LBP concentration is plotted in Figure 2.2. The upper limit of the 95% confidence interval for control LBP concentration (1446 ng/mL) was used as the threshold for a positive test. Corresponding sensitivity, specificity, and test accuracy were 74%, 100%, and 87.5%, respectively.

HPT concentration analysis results are also shown in Table 2.5. HPT concentration was increased in case calves over control calves ($P < 0.0001$).

Individual HPT concentration is plotted in Figure 2.3. The upper limit of the 95% confidence interval for control HPT concentration (0.46 mg/mL) was used as the threshold for a positive test. Corresponding sensitivity, specificity, and test accuracy were 83%, 100%, and 92%, respectively.
DISCUSSION

Morbidity

Morbidity presented numerical differences between diets, with lowest morbidity in AA and highest in AAXS, control being intermediate. Differences approached a tendency, but still failed to reject the null hypothesis in this study. Findings in human medicine by Alexander et al. (1980) showed improved recovery rates in burn victims receiving increased protein supplementation. While obvious species differences exist, the accompanying inflammatory immune response has been shown to encompass infectious and traumatic injury and is highly conserved across species (Cray et al., 2009). It is possible that protein supplementation prior to insult, like the current study, does not provide the same immune-supporting benefits expressed during recovery. Unfortunately, study duration and illness patterns in these cattle prevented evaluation of chronically sick calves or recovery success with treatment related to the different diets.

In cattle, Waggoner et al. (2009) observed correction of decreased plasma nitrogen in immune-challenged calves supplemented with bypass protein, though subsequent effects on morbidity or disease severity were not included in their study. This study aimed to fill this void and bridge information between immunity and nutrition, as amino acids have been shown as essential substrates in immune response (Li et al., 2007).

Because this connection between nutrition and immunity is not well-established or defined, it is possible these results reflect misunderstandings about substrate utilization during immune responses. Dietary amino acids may not be absorbed and assimilated in a BRD-positive calf as they are in a healthy animal, just as many systemic metabolic
changes coincide with inflammation (Beisel, 1977). Assuming Waggoner et al. were correct in their conclusions and dietary substrates are available to and employed by the immune response, it is possible that experimental treatments deviated from expectations. Delivered diets were not identical to formulations; though this is to be expected in feeding trials, the slight deviation could have skewed nutrient differences between diets from the expected values and influenced results. Additionally, though studies have shown glucose transporter upregulation and assumed enhanced absorption capabilities with artificial sweeteners (Moran et al., 2014), research aimed at quantifying absorption increases has yet to be completed, especially in ruminant animals with minimal glucose to delivery to the small intestine under normal conditions.

Despite the possibilities of experimental error in treatment administration, perhaps the more important consideration resides in overall study morbidity. With industry BRD morbidity averaging 16% (USDA-APHIS, 2011) and ranging from 5% - 44% or higher (Snowder et al., 2006), this experiment was not exemplary of industry norms, presenting near the low end of this range. True, the overall morbidity rate does not necessarily impact treatment differences, but the low power provided with only 22 BRD-positive calves and treatments presenting as few as 4 hd presents a greater likelihood of Type II error in this analysis. Added repetitions for increased observations could be beneficial in this regard.

**Intake**

Statistical analysis showed case animals ate less than healthy counterparts, which is neither surprising nor groundbreaking. But, taking this information and applying it
provided useful results. Feed intake as a diagnostic test performed more accurately in this instance compared to current subjective methods most commonly used (White and Renter, 2009), but validation of the specific methods used in this study is difficult. Feed intake and intake behaviors have been extensively studied over the last decade in attempts to improve feedlot diagnostics, yet searches return few experiments using the same techniques. Many of these studies have attempted to find intake patterns for earlier diagnosis and have done so with apparent success, but focus has been placed upon data inflections rather than quantity-centered evaluation like this study. Improvements in this study agree with other studies using various intake monitoring strategies (Quimby et al., 2001; Jackson et al., 2016), but these experiments provided greater accuracy compared to the current.

González et al. (2008) observed decreased DMI in cows acutely infected with mastitis as a potential parameter, like the current study. They found intake changes were variable and unreliable as a stand-alone test, similar to our results. More importantly, DMI changes only occurred on or closely surrounding d of diagnosis, reducing the predictive capabilities of this method and providing some question to the validity of other studies focused multiple d prior to diagnosis. Granted, mastitis is not a comparable infection to BRD in terms of affected system or pathogenesis, but the corresponding acute phase response and resulting physiological changes could be expected to be similar. With this information, it could be argued that infections associated with BRD or mastitis (as opposed to metabolic disorders) progress too rapidly to allow anorexia to present with sufficient diagnostic time prior to clinical signs for consistent results.
Other intake measures performed could be used as well, though issues arise in accuracy for all. Reg differences between case and control were likely related to the observed DMI differences, which would have altered the 21-d regression. Similarly, different Resint would correspond with noted DMI changes because case animals ate less than control animals. Stddev was different between cases and controls, but only by approximately 0.50 standard deviations. These differences could be exemplary of disease symptoms as desired in this experiment, but could also be explained by normal DMI variation commonly observed in feedlot cattle. Because regular DMI differences d to d can be between 2 – 3.5 kg over an entire feeding period (Schwartzkopf-Genswein et al., 2011), it is reasonable to assume receiving cattle feed intake would be even more variable, clouding the diagnostic accuracy of these measures.

**Blood parameters**

Trends in results for sO₂ and pO₂ agreed with previously reported results (Nagy et al., 2006; Šoltésová et al., 2015). However, actual values did not coincide with previous research. Oxygen saturation was greater in case animals in this study compared to Šoltésová et al., nearly equivalent to their analyses in healthy animals (96%). Values for pO₂ in the current study were also much greater compared to previous research, both in cases and controls. Explanation for these differences is elusive. This experiment used different analytical equipment, but variations of the observed magnitude due to equipment error seem unlikely. More probable, cold weather limited the ability to immediately run arterial samples outside at the sampling location with the portable chemical analyzer. Time to analysis remained within manufacturer instructions, but it is
likely samples were exposed to air at some point between blood draw and analysis. This could account for the oxygen differences between experiments. It should be noted that, even though reference ranges could have been derived from cattle dissimilar to receiving steers, \( pO_2 \) returned values above the reference range and potentially outside normal physiological and atmospheric possibilities, indicating possible issues in sampling. Furthermore, \( sO_2 \) values for both case and control animals remained within clinically normal ranges, so statistical significance in this measure appeared to have little biological significance even if collection and analysis procedures are assumed flawless. It could be argued that use of specific values for parameters is minimally important and greater focus should be placed upon patterns instead; regardless, the variation in results and poorly defined reference range criteria (ie – animals used to generate ranges, etc.) further justifies skepticism in the current experimental results and warrants additional research.

Temperature differences between cases and controls was expected due to fever associated with infection. However, in these data, this is an artifact of our predetermined case definition; only animals within the stated rectal temperature ranges could qualify as a case or control, so little weight should be placed upon these results.

**Acute Phase Protein**

HPT and LBP concentrations in case and control calves showed an association with naturally-occurring BRD, in agreement with previous research by Idoate et al. (2015) at the same location. APP concentrations also agreed with preceding findings in BRD diagnosed in field conditions or experimental challenges (Godson et al., 1996), especially pertaining to levels shown in healthy animals (Idoate et al., 2015). Other
experimental challenges with various BRD pathogens have shown more intense concentration changes for both LBP and HPT (Heegaard et al., 2000; Schroedl et al., 2001). These inter-experimental differences are likely due to natural vs. experimental infection, with inoculation doses between infection types possibly resulting in varied immune response intensities. Nikunen et al. (2007) reported greater LBP concentrations and lesser HPT concentrations in calves with Pasteurella multocida-associated pneumonia compared to the current study. This study primarily used 3-4-month-old dairy calves, perhaps showing differences due to age or breed. Additionally, the age utilized more closely aligns with enzootic calf pneumonia as opposed to the more frequently observed “shipping fever” subclassification of BRD in feedlot-age calves.

Sensitivity, specificity, and test accuracy for LBP and HPT at the outlined threshold values were substantially improved over standard industry methods. However, threshold values did not agree with similar research (Idoate et al., 2015). Our optimum HPT was less and optimum LBP was greater compared to the previous study; using their cut points would alter the detection accuracy in our data. We did not produce receiver operating characteristic (ROC) curves, perhaps accounting for some difference in threshold values.

Comparing LBP and HPT scatterplots, if a case animal was diagnosed with BRD if either test returned positive results, only 2 animals would have been incorrectly diagnosed. Test statistics would again be improved accounting for this observation.
CONCLUSION

Nutritional measures for BRD prevention were inconclusive, requiring further research with greater numbers of sick cattle to make more definitive assessments. Nutrition research like this could also observe relationships between diet and recurring illness or treatment success rates to determine a more accurate timeline for when nutrients are used to improve immune system efficacy. As for BRD diagnosis, available tools were shown to have varying success, assuming personnel identified and diagnosed all cases correctly. Feed intake, though not a perfect test, provided improvements upon standard industry methods. Arterial blood parameter analysis did not return results that were both logical and clinically relevant. HPT and LBP concentrations continued to show correlation with a diseased state in receiving cattle. Unfortunately, no single test method was determined as perfect for diagnosis respiratory disease. Feed intake as a marker for BRD is limited by natural variations in receiving cattle feed consumption, and inability of most settings to monitor individual intake. But, compared to current blood tests, simple intake observation would be more easily implemented in a commercial setting. Blood parameters, either chemical or immune-protein analysis, could greatly improve diagnostic accuracy but are limited by feasibility. Not only does sample collection and analysis require impractical lengths of time and a certain degree of unique skill, but animals must still be identified as sick, which has proven problematic for personnel. Each method has its own benefits and downfalls that prevent a single test from rising above the others. However, these tools show potential complementarity. A perfect diagnostic test for BRD will be difficult to uncover and may not exist, but by utilizing the technologies available
to progress management and research, incremental improvements in the current stalemate against BRD could be possible.
Table 2.1. Diet composition for receiving period diets formulated to meet or exceed amino acid requirements and include gut-modifying additives

<table>
<thead>
<tr>
<th>Ingredient (%DM)</th>
<th>Control</th>
<th>AA</th>
<th>AAXS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn</td>
<td>66.84</td>
<td>69.38</td>
<td>69.35</td>
</tr>
<tr>
<td>Cottonseed Meal</td>
<td>-</td>
<td>14.43</td>
<td>14.43</td>
</tr>
<tr>
<td>Soybean Meal</td>
<td>8.03</td>
<td>2.27</td>
<td>2.27</td>
</tr>
<tr>
<td>Rye Baleage</td>
<td>8.11</td>
<td>8.12</td>
<td>8.12</td>
</tr>
<tr>
<td>DDGS</td>
<td>7.60</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Bloodmeal</td>
<td>4.81</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Limestone</td>
<td>1.08</td>
<td>1.50</td>
<td>1.5</td>
</tr>
<tr>
<td>Dyna K(^1)</td>
<td>0.99</td>
<td>0.90</td>
<td>0.90</td>
</tr>
<tr>
<td>CocciCurb(^2)</td>
<td>0.68</td>
<td>0.67</td>
<td>0.67</td>
</tr>
<tr>
<td>AjiPro(^3)</td>
<td>-</td>
<td>0.60</td>
<td>0.60</td>
</tr>
<tr>
<td>Smartamine(^4)</td>
<td>-</td>
<td>0.19</td>
<td>0.19</td>
</tr>
<tr>
<td>Fat</td>
<td>0.50</td>
<td>0.50</td>
<td>0.50</td>
</tr>
<tr>
<td>Salt</td>
<td>0.48</td>
<td>0.36</td>
<td>0.36</td>
</tr>
<tr>
<td>Vit. E(^5)</td>
<td>0.24</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Trace Mineral Premix(^6)</td>
<td>0.21</td>
<td>0.24</td>
<td>0.24</td>
</tr>
<tr>
<td>MgO(^7)</td>
<td>0.20</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Aureomycin(^8)</td>
<td>0.11</td>
<td>0.11</td>
<td>0.11</td>
</tr>
<tr>
<td>Urea</td>
<td>0.10</td>
<td>0.12</td>
<td>0.12</td>
</tr>
<tr>
<td>ADE(^9)</td>
<td>0.05</td>
<td>0.12</td>
<td>0.12</td>
</tr>
<tr>
<td>Sucram(^10)</td>
<td>-</td>
<td>-</td>
<td>0.02</td>
</tr>
<tr>
<td>Xtract(^10)</td>
<td>-</td>
<td>-</td>
<td>0.01</td>
</tr>
<tr>
<td>Nutrient Composition</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DM,%</td>
<td>77.74</td>
<td>78.99</td>
<td>78.60</td>
</tr>
<tr>
<td>CP,% DM</td>
<td>17.50</td>
<td>14.66</td>
<td>14.65</td>
</tr>
<tr>
<td>NDF,% DM</td>
<td>14.09</td>
<td>15.15</td>
<td>15.03</td>
</tr>
<tr>
<td>ADF,% DM</td>
<td>5.53</td>
<td>7.14</td>
<td>6.95</td>
</tr>
<tr>
<td>OM,%</td>
<td>92.76</td>
<td>92.21</td>
<td>93.11</td>
</tr>
</tbody>
</table>

1. The Mosaic Company, Plymouth MN; Contains 50% K, 46.4% Cl, 95.3% KCl
2. NutraBlend, LLC, Neosho, MO
3. AjiPro-L; Ajinomoto, Chicago, IL
4. Adisseo USA, Inc., Alpharetta, GA
5. Vitamin E= 44,000 IU/kg
6. Trace Mineral Premix= 24% Ca, 3.0% Zn, 2.5% Fe, 2.0% Mn, 1.0% Cu, 100 ppm Co, 500 ppm I, 100 ppm Se
7. Magnesium Oxide 54, Feed Products and Services Company, Madison II; 93% MgO loss free
8. Zoetis, Florham Park, NJ
9. ADE= 8,800,000 IU/kg Vitamin A, 1.100 IU/kg Vitamin E, 1,760,000 IU/kg Vitamin D
10. Pancosma, Le Grand, Saconnex, Geneva, Switzerland
Table 2.2. Morbidity differences in receiving cattle consuming diets formulated to meet or exceed amino acid requirements and include gut-modifying additives

<table>
<thead>
<tr>
<th>Item</th>
<th>AA</th>
<th>AAXS</th>
<th>Control</th>
<th>Chi-square⁴</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRD + ¹</td>
<td>4%</td>
<td>12%</td>
<td>8%</td>
<td>3.68</td>
<td>0.16</td>
</tr>
</tbody>
</table>

¹ Percent of cattle diagnosed with BRD
² Diet received;
   - AA (n = 104), provides ≥110% AA requirement
   - AAXS (n = 103), provides ≥110% AA requirement + gut-modifying additives
   - Control (n = 104), provides ≥100% AA requirement

³ Chi-square value
Table 2.3. Intake differences between case (n = 22) and control (n = 22) receiving cattle at bovine respiratory disease (BRD) diagnosis

<table>
<thead>
<tr>
<th>Item</th>
<th>Case</th>
<th>Control</th>
<th>SEM(^5)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMI, kg</td>
<td>2.65</td>
<td>5.89</td>
<td>0.45</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Reg(^1), kg</td>
<td>5.07</td>
<td>6.37</td>
<td>0.34</td>
<td>0.0101</td>
</tr>
<tr>
<td>Resint(^2), kg</td>
<td>-2.42</td>
<td>-0.47</td>
<td>0.47</td>
<td>0.0055</td>
</tr>
<tr>
<td>Stddev(^3)</td>
<td>-0.51</td>
<td>-0.06</td>
<td>0.10</td>
<td>0.0018</td>
</tr>
</tbody>
</table>

\(^1\) Reg: predicted intake from 21-d DMI regression  
\(^2\) Resint: residual intake (actual DMI – regression DMI)  
\(^3\) Stddev: number of standard deviations residual intake lies about regression DMI  
\(^4\) Diagnosis: Case = BRD-positive; Control = BRD-negative/healthy  
\(^5\) SEM: Standard error of least squares means
Table 2.4. Arterial blood gas composition at bovine respiratory disease (BRD) diagnosis in receiving cattle

<table>
<thead>
<tr>
<th>Item</th>
<th>Diagnosis(^9)</th>
<th>SEM(^{10})</th>
<th>Reference(^{11})</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Case</td>
<td>Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temp(^1, ^{1, \circ}C)</td>
<td>40.68</td>
<td>39.22</td>
<td>0.09</td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>7.44</td>
<td>7.44</td>
<td>0.02</td>
<td>7.35 - 7.50</td>
</tr>
<tr>
<td>pCO(_2)(^2, \text{mmHg})</td>
<td>37.60</td>
<td>36.05</td>
<td>1.67</td>
<td>35 - 44</td>
</tr>
<tr>
<td>pO(_2)(^3, \text{mmHg})</td>
<td>107.11</td>
<td>130.24</td>
<td>7.12</td>
<td>92</td>
</tr>
<tr>
<td>BEecf(^4, \text{mmol/L})</td>
<td>1.32</td>
<td>0.19</td>
<td>1.17</td>
<td></td>
</tr>
<tr>
<td>HCO(_3)(^5, \text{mmol/L})</td>
<td>24.56</td>
<td>23.79</td>
<td>1.06</td>
<td>20 - 30</td>
</tr>
<tr>
<td>TCO(_2)(^6, \text{mmol/L})</td>
<td>25.47</td>
<td>24.81</td>
<td>1.10</td>
<td>23 - 27</td>
</tr>
<tr>
<td>sO(_2)(^7, %)</td>
<td>95</td>
<td>98.14</td>
<td>1.03</td>
<td>95</td>
</tr>
<tr>
<td>Lactate(^8, \text{mmol/L})</td>
<td>4.68</td>
<td>4.82</td>
<td>0.70</td>
<td>&lt; 1.50</td>
</tr>
</tbody>
</table>

1 Temp: Animal rectal temperature
2 pCO\(_2\): partial pressure of carbon dioxide
3 pO\(_2\): partial pressure of oxygen
4 BEecf: base excess in extracellular fluid; monitors metabolic/non-respiratory acid-base status
5 HCO\(_3\): bicarbonate concentration
6 TCO\(_2\): total carbon dioxide accounting for pCO\(_2\) and HCO\(_3\)
7 sO\(_2\): percentage of hemoglobin fully combined with oxygen
8 Lactate: lactate concentration
9 Diagnosis: Case = BRD-positive; Control = BRD-negative/healthy
10 SEM: Standard error of least squares means
11 Arterial blood gas composition reference ranges for cattle
Table 2.5. Serum lipopolysaccharide-binding protein (LBP) and haptoglobin (HPT) concentrations at bovine respiratory disease (BRD) diagnosis in receiving cattle

<table>
<thead>
<tr>
<th>Item</th>
<th>Diagnosis</th>
<th>SEM</th>
<th>TRT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Case</td>
<td>Control</td>
<td></td>
</tr>
<tr>
<td>LBP concentration¹, ng/mL</td>
<td>4527.89</td>
<td>288.70</td>
<td>578.52</td>
</tr>
<tr>
<td>HPT concentration¹, mg/mL</td>
<td>2.14</td>
<td>0.04</td>
<td>0.21</td>
</tr>
</tbody>
</table>

¹ Concentration: Serum concentration  
² Diagnosis: Case = BRD-positive; Control = BRD-negative/healthy  
³ SEM: Standard error of least squares means
Figure 2.1. Individual DMI as a percent of group average on d of bovine respiratory disease (BRD) diagnosis in receiving cattle

* Case and control DMI 95% confidence intervals; shaded box, 2 standard deviations about the mean case (T) DMI; unshaded box, 2 standard deviations about the mean control (C) DMI
**Figure 2.2.** Serum lipopolysaccharide-binding protein (LBP) concentration at diagnosis of bovine respiratory disease (BRD) in receiving cattle

* Case and control LBP concentration 95% confidence intervals; shaded box, 2 standard deviations about the mean case (T) concentration; unshaded box, 2 standard deviations about the mean control (C) concentration
Figure 2.3. Serum haptoglobin (HPT) concentration at diagnosis of bovine respiratory disease (BRD) in receiving cattle

* Case and control HPT concentration 95% confidence intervals; shaded box, 2 standard deviations about the mean case (T) concentration; unshaded box, 2 standard deviations about the mean control (C) concentration
CHAPTER 3

FEEDING POSTRUMINAL AMINO ACIDS TO EXCEED REQUIREMENTS AND IMPACTS ON PERFORMANCE AND IMMUNE FUNCTION IN RECEIVING CATTLE

ABSTRACT

A two-faceted experiment was conducted to determine the effects of amino acid supplementation and gut-modifying additives on performance and vaccination-induced antibody response in receiving cattle. We hypothesized that diets formulated to exceed amino acid requirements and include gut-modifying additives would produce greater animal performance and novel antibody response in receiving calves. Three-hundred-eleven mixed breed beef steers and bulls of similar age and initial BW (IBW; 233.61 ± 20.74 kg) were randomly assigned to 1 of 6 pens for a 21-d receiving period feeding trial. One of 3 treatment diets were randomly assigned to pens: a diet without additives formulated to meet AA requirements (CONTROL, n = 104), a diet without additives formulated to exceed AA requirements by ≥ 10% (AA, n = 104), and a diet with additives formulated to exceed AA requirements by ≥ 10% (AAXS, n = 103). All calves were administered rabies vaccinations and blood samples for titer analysis. Serum subsamples were collected at study onset and completion from randomly selected steers (CONTROL, n = 20; AA, n = 21; AAXS, n = 20) and all bulls castrated at arrival (CONTROL, n = 5; AA, n = 5; AAXS, n = 5). ADG was not affected by diet (P = 0.40). DMI tended to be different between diets (P = 0.06). AAXS DMI (5.62 kg) was greater (P = 0.03) than AA (5.23 kg) but not CONTROL (P = 0.71), while CONTROL (5.55 kg) tended to be greater.
than AA ($P = 0.07$). G:F was different between diets ($P < 0.01$). AA (0.37) was greater ($P < 0.01$) than AAXS (0.32) and tended ($P = 0.08$) to be greater than CONTROL (0.34) while AAXS and CONTROL were not different ($P = 0.12$). Diet did not affect rabies antibody concentration ($P > 0.24$). Providing amino acids to exceed requirements in receiving cattle diets did not appear to increase humoral response to vaccination, but could be beneficial through improved feed efficiency.

**INTRODUCTION**

Methods for improved feedlot cattle efficiency have been thoroughly researched to support the increasing global population and producers’ profitability. Many investigations have focused on major ration inputs to define requirements more accurately and minimize wasteful feed provision (Veira et al., 1980; Emmans, 1994) in healthy, growing animals. However, requirements in feedlot calves facing stress and illness remain less defined and thus more difficult to efficiently address. Disease and immune challenges typically present in 5-8 month old calves in their first 21-28 d in the feedlot, known as the receiving period (Snowder et al., 2006). Associated immune responses promote catabolic processes and increase amino acid demands to support immune cell function and humoral protein upregulation (Beisel, 1977; Iyer et al., 2012). Waggoner et al. (2009) showed decreased evidence of muscle catabolism in sick calves through increased bypass protein supplementation, helping to illustrate the mentioned deviations and potential solutions. In immune-challenged cattle, compounded with stereotypically low feed intakes, it could be beneficial for immune function and overall performance to provide diets balanced to address the physiological changes observed
during the receiving period. To evaluate this, our first experimental objective was to compare performance in receiving cattle consuming diets with different amino acid inclusions, at or above NRC requirements, and with or without gut-modifying additives. We hypothesized that diets formulated to exceed amino acid requirements and include gut-modifying additives would produce greater animal performance. Our second objective was to assess novel antibody response as a measure of immune function in receiving cattle consuming diets with different amino acid inclusions, at or above NRC requirements, and with or without gut-modifying additives. We hypothesized that diets formulated to exceed amino acid requirements and include gut-modifying additives would result in greater novel antibody response in receiving calves.

**MATERIALS AND METHODS**

**Animals and Experimental Design**

All procedures were approved by the University of Missouri Institutional Animal Care and Use Committee. Three-hundred-eleven mixed breed beef steers and bulls of similar age and initial BW (IBW; 233.61 ± 20.74 kg) were purchased from a regional stockyard in November 2016. Calves were purchased in 2 allotments (Group), 1 week apart (Group 1: n = 150, IBW = 235.00 ± 19.34 kg; Group 2: n = 161, IBW = 231.38 ± 21.80 kg), and transported 214 miles to the University of Missouri Beef Research and Teaching Farm (BRTF; Columbia, MO). Upon arrival, cattle were unloaded in equal numbers to 6 pens measuring 36.6 x 23.4 m with feed bunks and water on a 6.1 x 36.6 m concrete pad and the remainder in dirt and gravel. Each pen contained 2 structures (7.6 x 9.1 m) for shelter from sunlight and inclement weather. Animals were provided ad
libitum access to grass hay and water overnight. Hay was provided in concrete J-bunks. At 0700 h the next d, consecutive 2-d weights and processing commenced. Radio frequency identification tags (RFID) and visual identification tags (VID; Allflex, USA, Inc., Dallas Fort Worth, TX, USA) were placed in the left and right ear, respectively. The right ear was also notched to test for persistently-infected bovine viral diarrhea (BVD-PI) cases; no animals tested positive for BVD-PI. All calves received: subcutaneous metaphylactic tulathromycin (Draxxin; Zoetis, Florham Park, NJ, USA) to prevent illness prior to GrowSafe adoption because feed intake was a variable of interest as a potential diagnostic test in a coinciding study; intramuscular killed rabies vaccination (IMRAB LA, Merial Inc., Duluth, GA, USA) for a novel, measurable antibody response representing immune function; and oral fenbendazole (Safe-Guard; Merck Animal Health, Omaha, NE, USA), all according to Beef Quality Assurance standards. Sex was determined for stratification. Animals were stratified by IBW, color, and sex, and randomly assigned to 1 of 6 pens with 3 treatment diets randomly assigned to pens (Control, n=104; AA, n=104; AAXS, n=103) on d 2 of the consecutive weigh d. Jugular blood samples were taken from randomly selected steers (CONTROL, n = 20; AA, n = 21; AAXS, n = 20) and all bulls castrated at arrival (CONTROL, n = 5; AA, n = 5; AAXS, n = 5), for baseline rabies antibody titer determination. Bulls were castrated with a Newberry knife (SyrVet, St-Alphonse-de-Granby, QC, Canada) prior to sorting on d 2. Consecutive 2-d weights and were taken at study completion after animals had received respective diets for 21 d. Study-completion jugular blood samples were also obtained from the same animals per pen remaining on study (CONTROL, n = 23; AA, n = 26;
AAXS, n = 23) to determine antibody titer change over the study. Animals were removed from the study (n=30; Control, n=9; AA, n=7; AAXS, n=14) for crossing pen barriers and consuming a different diet, failure to adapt to the GrowSafe system, or mortality unrelated to BRD.

**Diets and Feeding**

Steers returned to assigned pens following initial second-d weighing and sorting. There, they received rations *ad libitum* in automated GrowSafe feed bunks (GrowSafe Systems Ltd., Airdrie, Alberta, Canada) to monitor intake behavior for the remainder of the 21-d receiving trial. Each pen contained 5 GrowSafe bunks. Diets were provided between 0700-0800 h daily. All diets consisted of a whole shelled corn base with rye baleage and a concentrate supplement to which experimental changes were applied (Table 3.1). Formulations were completed to achieve 1.41 kg ADG with 2.10% BW DMI using average receiving period BW. The control diet was formulated to meet or exceed growing beef animal requirements for amino acids, vitamins, and minerals (NRC, 2000), and meet requirements for effective energy (EE; Emmans, 1994). Methionine was the most limiting AA (103% of requirement), followed closely by arginine (105% of requirement). The AA diet was formulated to meet or exceed requirements for vitamins, minerals and EE like CONTROL; however, blood meal and DDGS were removed and soybean meal reduced in the supplement and replaced by cottonseed meal, rumen-protected encapsulated lysine (AjiPro, Ajinomoto Heartland, Inc., Eddyville, IA, USA), and rumen-protected encapsulated methionine (Adisseo USA, Inc., Alpharetta, GA, USA) to increase all amino acids to a minimum of 113% of requirement, with lysine as...
the most limiting AA, followed by arginine (115% of requirement) and threonine (115% of requirement). The AAXS diet was formulated to meet or exceed requirements the same as the AA diet; however, a small percentage of corn in the supplement was replaced with a plant extract-based rumen modifier (XTRACT Ruminants X60-7065; Pancosma, Geneva, Switzerland) and an artificial sweetener (SUCRAM, Pancosma, Geneva, Switzerland). Ground corn served as a carrier in the concentrate supplement. Supplements were mixed in 909 kg batches and stored in upright bins at the BRTF. Supplement, whole shelled corn, and rye baleage were mixed at time of feeding in a truck-mounted mixer.

Performance measures were calculated using averages of consecutive 2-d weights from study onset and completion with average daily intakes from GrowSafe data, converted to dry matter using proximate analysis results.

Feed samples for proximate analysis were collected weekly from the truck-mounted feed mixer as diets were dispensed to the GrowSafe bunks. Samples were stored at -20°C until analyzed. Diet samples were initially weighed and dried at 55°C (Model 7921; Blickman Health Industries, Clifton, NJ, USA), then ground to pass through a 3-mm screen (Wiley Mill; Arthur H. Thomas Company, Philadelphia, PA, USA) and 1-mm screen (Foss Tecator Cyclotec Sample Mill, Foss North America, Eden Prairie, MN, USA) sequentially. Ground samples were analyzed for NDF and ADF (ANKOM200 Fiber Analyzer; ANKOM Technology, Macedon, NY, USA) and nitrogen content (vario Macro Cube, Elementar Americas, Mt. Laurel, NJ, USA). Samples were then dried at 105°C and analyzed for DM (Isotemp Oven Model 255 G; Fisher Scientific, Pittsburg, PA, USA),
and ash (Isotemp Muffle Furnace; Fisher Scientific, Pittsburg, PA, USA). Weekly samples were analyzed separately and results were compiled to formulate averages for each diet.

**Biological Sampling**

Blood samples for rabies titer analysis were collected at study onset and completion from 10 randomly selected steers per pen and all bulls (Group 1: n = 3; Group 2: n = 12) castrated at arrival in 10 mL serum separator tubes (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) via jugular venipuncture, allowed to clot, and stored in ice until transported 5.9 miles to the lab for processing. Blood was then centrifuged at 1500 × g for 30 min at 4°C. Serum was harvested equally into 3 aliquots, 1-1.5 mL apiece, and stored at -20°C for later analysis. Beginning and end samples were sent to an outside laboratory for final endpoint titer analysis with the Rapid Fluorescent Foci Inhibition Test (RFFIT; Kansas State University Rabies Laboratory, Manhattan, KS, USA). Antibody concentrations greater than 0.5 IU/mL were indicative of a robust immune response to vaccine. Animals were removed from the rabies titer study (n=14; Control: n=4; AA: n=2; AAXS: n=8) for morbidity, crossing pen barriers and consuming a different diet, and failure to adopt to the GrowSafe system.

**Statistical Analysis**

Performance data were analyzed as a completely randomized design with individual animal as the experimental unit. Initial BW (IBW), end BW (EBW), ADG, DMI, G:F, and DMI as a percent of BW (%BW) were fitted as dependent variables and diet was set as the independent variable following determination that sex did not have an
effect. Differences were determined, pending significant F-test ($\alpha \leq 0.05$), using the Least Significant Difference test from the LSMEANS statement in the GLM procedure of SAS (SAS 9.4; SAS Inst. Inc., Cary, NC). This procedure was first completed independently for each group of cattle, defined by their arrival date outlined previously, to ensure variances were equivalent across group. As variances were determined equivalent for all variables, all data were pooled and analyzed for the same dependent variables with diet, group, and interaction (diet*group) fitted as independent variables. Significance was set at $\alpha \leq 0.05$ and tendencies were set at $\alpha \leq 0.10$.

Antibody titer data were analyzed as a completely randomized design with animal as the experimental unit. Tests for normality returned non-normal distributions in results, so rank transformation using the RANK procedure of SAS was conducted according to Conover and Iman (1981) to normalize data. Ranks of initial concentration (IUon), initial titer (TITERon), end concentration (IUoff), end titer (TITERoff), concentration change ($\Delta$IU), and titer change ($\Delta$TITER) were dependent variables while sex, group, sex*group and sex, diet, and sex*diet were fitted as independent variables in separate models. All differences were analyzed using Least Significant Difference in the LSMEANS statement in PROC GLM. Significance was set at $\alpha \leq 0.05$ and tendencies were set at $\alpha \leq 0.10$. These effects were not significant for any trait ($P > 0.44$), so all animals were pooled and analyzed together. With the same traits fitted as dependent variables and diet, group, and their interaction (diet*group) fitted as independent variables.
RESULTS

Performance

Results for diet and group effects on animal performance are shown in Table 3.3. Interaction between diet and group was not significant for any variable ($P > 0.19$). Neither IBW ($P = 0.49$) nor EBW ($P = 0.92$) were affected by diet. ADG ($P = 0.40$) and %BW ($P = 0.11$) were not affected by diet. DMI tended to be different between diets ($P = 0.06$). AAXS DMI was greater than AA ($P = 0.03$) but was not different from CONTROL ($P = 0.71$). CONTROL DMI tended to be greater than AA ($P = 0.07$). G:F was different between diets ($P = 0.005$). AA G:F was greater than AAXS ($P = 0.001$) and tended to be greater than CONTROL ($P = 0.08$) while AAXS and CONTROL G:F were not different ($P = 0.12$).

Differences existed in performance measures between groups. IBW ($P = 0.03$) and EBW ($P = 0.0002$) were greater in group 1. Group 1 also showed greater ADG ($P = 0.0004$), DMI ($P < 0.0001$), and %BW ($P = 0.003$). G:F was not different between groups ($P = 0.68$).

Antibody Titer

Antibody titer results are presented in Table 3.4, with $P$-values from rank transformation analysis presented alongside means and standard errors of actual measured data instead of ranks. Diet by group interaction was not significant for any measure ($P > 0.19$). Diet also did not affect any measurement ($P > 0.19$). Group differences were not significant for IUon or TITERon ($P > 0.22$). Group 2 was greater
than group 1 in IUoff ($P = 0.01$), TITERoff ($P = 0.01$), ΔIU ($P = 0.01$), and ΔTITER ($P = 0.01$).

DISCUSSION

Performance

Being a week apart in a short-term study, group differences were not surprising and likely due to animal variation associated with numerous factors including but not limited to genetics, previous management practices and temperament.

The current study showed a tendency for DMI differences between diets, with the AAXS being greatest, followed numerically by the CONTROL diet (balanced to meet requirements and excluding additives) and significantly by the AA diet (balanced to exceed AA requirements excluding additives). ADG was not different among diets, thus resulting in opposite ordering of treatments for G:F, as cattle on AA were more efficient than AAXS and tended to be more efficient than Control. Diets providing both additives in combination have not been found in research archives. However, regarding sweetener inclusion, these results agreed with McMeniman et al. (2006) who showed tendencies for DMI increases with sweetener inclusion. This aligns with the primary marketing strategy that artificial sweetener inclusion increases palatability and appeal in animals adapting to a new diet. Plant extracts in ruminant diets, especially in receiving cattle, have presented variable results regarding comparable performance results. This could be attributed to inconsistent inclusions and combinations of specific essential oils across experiments; the product utilized in the current study provides minimal comparative research in growing cattle. Some research has shown increased DMI with certain shared compounds, like
capsicum, in beef animals on high-concentrate diets (Cardozo et al., 2006), agreeing with the current study. More commonly, investigation for efficiency measures have occurred and found similar results between plant extracts and ionophores, showing reduced acetate:propionate ratios and methane production to improve efficiency (Geraci et al., 2012). Though ionophores were not included in any diet, and essential oils and other modifiers were only included in the AAXS diet, this information fueled our hypothesis that extract inclusion would improve animal efficiency. However, the data did not support the hypothesis or previous research. Variability in plant extract products has been noted and potentially responsible for this disagreement. It is also possible that the intake-stimulating actions of the added sweetener were responsible for increased consumption beyond effective energy requirements, consequently sacrificing efficiency compared to the AA diet.

This speculation is strengthened considering remaining efficiency results aligned with expectations. Animals consuming the AA diet tended to be more efficient than controls. This agrees with Fluharty and Loerch (1995), who showed an improvement in G:F with greater inclusions of ruminally-undegradable protein (RUP). While the current study focused on estimating rather than actually measuring AA delivery to the small intestine, this estimation is still worth acknowledging as a different strategy than formulating for RUP. These AA adjustments stemmed from the perceived immune and metabolic changes experienced by receiving cattle in the face of stress and common illness. Waggoner et al. (2009) observed decreased plasma amino acids in immune-challenged calves, likely supporting immune protein synthesis for inflammatory
responses. These amino acids are generally thought to be sourced from skeletal muscle catabolism (Beisel, 1977). More importantly to this study, Waggoner also showed increased nitrogen retention in non-challenged animals and reduced nitrogen loss in challenged calves with increased RUP inclusion, concluding increased post-ruminal protein delivery mitigated amino acid losses to support immune function by possibly providing AA needed during the inflammatory response. By providing AA to exceed requirements, it is possible that the current study accomplished similar results and reduced stress-induced catabolic losses, translating into improved efficiency. Conversely, diet comparisons could have been affected by differences in expected and delivered diet composition (Table 3.2). Proximate analyses were not conducted on individual feedstuffs, providing room for error in assumptions made about typical feedstuff composition at formulation. Regardless, diets may not have provided nutrients exactly as expected, but performance differences were shown in support of our hypotheses.

**Antibody Titers**

No differences existed between treatments for any antibody measurement. Both concentrations and titers were reported for further information and ease in comparison to other research and medical information. It was expected that concentrations and titers from the same period (onset or completion) would return similar statistical differences because they report the same information in a different format.

Interestingly, group differences were substantial and cannot be explained. By simple subjective analysis of the 2 groups, we would have expected a more robust immune response out of Group 1, as these calves appeared healthier and well-managed. It
is possible that a greater antibody response, at a certain level, is not beneficial and could indicate other issues. Because the list of possibilities could grow almost indefinitely, this finding could justify additional research for more accurate and substantiated assessments.

Outside of group differences, rabies antibody levels at study onset were not different between treatments and at levels considered negligible to nonexistent, as expected. Rabies vaccine was used for this reason. Because case fatality rates are reported at 100%, no antibodies from natural disease exposure would be expected in these cattle. Lack of differences at study completion failed to reject the null hypothesis. Though research specifically measuring dietary effects on rabies titer changes is limited, studies have evaluated nutrition and adaptive immune responses, especially in other animal models. *In vivo* murine research by Petro and Bhattacharjee (1981) found decreased antibody synthesis in protein-deficient mice compared to those receiving requirements, which is similar to human malnourishment studies (Mathews et al., 1972); however, because no diets in the current study were deficient, comparisons of results do not provide much validation. *In vitro* experiments have shown improved antibody production with amino acid-enriched standard media (Mahboudi et al., 2013), driving our hypothesis that supplementation would improve antibody synthesis. Application of this research (supplementation above normal levels) is limited *in vivo*. Additionally, cell culture research did not relate supplementation to animal requirements like the current study.

Perhaps more explanatory for our results, research focused on branched-chain amino acids found minimal lymphocyte activity changes with supplementation above
requirements (Calder, 2006). It is possible that antibody production was sufficiently supported by all diets in this study and further benefit would not be obtained through provision beyond the requirement diet. The argument could also be made that, knowing the timing of adaptive immune responses, excess amino acids were not sufficient to support potentially interfering innate responses to stress or infection in addition to antibody production.

Sweetener inclusion was expected to provide glucose support to the immune system beyond just protein supplementation in the AA diet. This expectation revolved around research showing glucose transporter upregulation in the small intestine and, subsequently, increased capacity for glucose absorption in both cattle and pigs (Moran et al., 2010; Moran et al., 2014). However, results did not support this expectation. Understanding ruminant digestive physiology, it is possible this upregulation, if it truly occurs, does not provide additional glucose for animal use. The rapidly fermented carbohydrates absorbed at these transporters may be completely degraded for VFA production, effectively eliminated the potential for increased intestinal absorption despite increased transporter presence. Research to confirm assumptions about increased glucose absorption are necessary, especially in ruminant species. If assumptions are correct and sufficient quantities of glucose do reach the small intestine for absorption, it is possible that whatever additional glucose was absorbed was utilized prior to adaptive response to vaccination and was therefore unavailable for lymphocytes, much like our previously stated speculations for amino acids.
CONCLUSION

Providing amino acids to exceed requirements in receiving cattle diets returned improvements in feed efficiency. These improvements could be negated with addition of gut-modifying additives, like sweeteners, that promote increased feed intake; however, the effects of plant extracts and sweeteners could not be separated with the current experimental design. These diets did not improve vaccine response in the form of protective antibodies, leading to the conclusion that amino acids were utilized elsewhere outside of lymphocytes. The source of improved efficiency remains unidentified. Based on this research, it could be beneficial to provide excess amino acids to receiving cattle, if diet changes are not cost-prohibitive, to reduce feed inputs for equivalent gain.
Table 3.1. Diet composition for receiving period diets formulated to meet or exceed amino acid requirements and include gut-modifying additives

<table>
<thead>
<tr>
<th>Ingredient (%DM)</th>
<th>Control</th>
<th>AA</th>
<th>AAXS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn</td>
<td>66.84</td>
<td>69.38</td>
<td>69.35</td>
</tr>
<tr>
<td>Cottonseed Meal</td>
<td>-</td>
<td>14.43</td>
<td>14.43</td>
</tr>
<tr>
<td>Soybean Meal</td>
<td>8.03</td>
<td>2.27</td>
<td>2.27</td>
</tr>
<tr>
<td>Rye Baleage</td>
<td>8.11</td>
<td>8.12</td>
<td>8.12</td>
</tr>
<tr>
<td>DDGS</td>
<td>7.60</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Bloodmeal</td>
<td>4.81</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Limestone</td>
<td>1.08</td>
<td>1.50</td>
<td>1.5</td>
</tr>
<tr>
<td>Dyna K&lt;sup&gt;1&lt;/sup&gt;</td>
<td>0.99</td>
<td>0.90</td>
<td>0.90</td>
</tr>
<tr>
<td>CocciCurb&lt;sup&gt;2&lt;/sup&gt;</td>
<td>0.68</td>
<td>0.67</td>
<td>0.67</td>
</tr>
<tr>
<td>AjiPro&lt;sup&gt;3&lt;/sup&gt;</td>
<td>-</td>
<td>0.60</td>
<td>0.60</td>
</tr>
<tr>
<td>Smartamine&lt;sup&gt;4&lt;/sup&gt;</td>
<td>-</td>
<td>0.19</td>
<td>0.19</td>
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<tr>
<td>Fat</td>
<td>0.50</td>
<td>0.50</td>
<td>0.50</td>
</tr>
<tr>
<td>Salt</td>
<td>0.48</td>
<td>0.36</td>
<td>0.36</td>
</tr>
<tr>
<td>Vit. E&lt;sup&gt;5&lt;/sup&gt;</td>
<td>0.24</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Trace Mineral Premix&lt;sup&gt;6&lt;/sup&gt;</td>
<td>0.21</td>
<td>0.24</td>
<td>0.24</td>
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<tr>
<td>MgO&lt;sup&gt;7&lt;/sup&gt;</td>
<td>0.20</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Aureomycin&lt;sup&gt;8&lt;/sup&gt;</td>
<td>0.11</td>
<td>0.11</td>
<td>0.11</td>
</tr>
<tr>
<td>Urea</td>
<td>0.10</td>
<td>0.12</td>
<td>0.12</td>
</tr>
<tr>
<td>ADE&lt;sup&gt;9&lt;/sup&gt;</td>
<td>0.05</td>
<td>0.12</td>
<td>0.12</td>
</tr>
<tr>
<td>Sucram&lt;sup&gt;10&lt;/sup&gt;</td>
<td>-</td>
<td>-</td>
<td>0.02</td>
</tr>
<tr>
<td>Xtract&lt;sup&gt;10&lt;/sup&gt;</td>
<td>-</td>
<td>-</td>
<td>0.01</td>
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Nutrient Composition

<table>
<thead>
<tr>
<th>DM,%</th>
<th>Control</th>
<th>AA</th>
<th>AAXS</th>
</tr>
</thead>
<tbody>
<tr>
<td>77.74</td>
<td>78.99</td>
<td>78.60</td>
<td></td>
</tr>
<tr>
<td>17.50</td>
<td>14.66</td>
<td>14.65</td>
<td></td>
</tr>
<tr>
<td>14.09</td>
<td>15.15</td>
<td>15.03</td>
<td></td>
</tr>
<tr>
<td>5.53</td>
<td>7.14</td>
<td>6.95</td>
<td></td>
</tr>
<tr>
<td>92.76</td>
<td>92.21</td>
<td>93.11</td>
<td></td>
</tr>
</tbody>
</table>

<sup>1</sup>The Mosaic Company, Plymouth MN; Contains 50% K, 46.4% Cl<sub>-</sub>, 95.3% KCl
<sup>2</sup>NutraBlend, LLC, Neosho, MO
<sup>3</sup>AjiPro-L; Ajinomoto, Chicago, IL
<sup>4</sup>Adisseo USA, Inc., Alpharetta, GA
<sup>5</sup>Vitamin E= 44,000 IU/kg
<sup>6</sup>Trace Mineral Premix= 24% Ca, 3.0% Zn, 2.5% Fe, 2.0% Mn, 1.0% Cu, 100 ppm Co, 500 ppm I, 100 ppm Se)
<sup>7</sup>Magnesium Oxide 54, Feed Products and Services Company, Madison II; 93% MgO loss free
<sup>8</sup>Zoetis, Florham Park, NJ
<sup>9</sup>ADE= 8,800,000 IU/kg Vitamin A, 1.100 IU/kg Vitamin E, 1,760,000 IU/kg Vitamin D
<sup>10</sup>Pancosma, Le Grand, Saconnex, Geneva, Switzerland
Table 3.2. Expected and actual composition for receiving period diets formulated to meet or exceed amino acid requirements and include gut-modifying additives

<table>
<thead>
<tr>
<th>Item</th>
<th>CONTROL 2</th>
<th>AA</th>
<th>AAXS</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM, %</td>
<td>79.95</td>
<td>77.74</td>
<td>79.81</td>
</tr>
<tr>
<td>CP, %</td>
<td>18.55</td>
<td>17.50</td>
<td>16.13</td>
</tr>
<tr>
<td>NDF, %</td>
<td>14.32</td>
<td>14.09</td>
<td>16.07</td>
</tr>
<tr>
<td>OM, %</td>
<td>92.54</td>
<td>92.76</td>
<td>91.19</td>
</tr>
</tbody>
</table>

1 Proximate analysis variable
2 Diet; CONTROL = provides ≥100% AA requirement AA = provides ≥110% AA requirement; AAXS = provides ≥110% AA requirement + gut-modifying additives
3 Expected: expected diet composition; Actual: delivered diet composition
<table>
<thead>
<tr>
<th>Item</th>
<th>Group 1</th>
<th>Group 2</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CONTROL</td>
<td>AA</td>
<td>AAXS</td>
</tr>
<tr>
<td>IBW&lt;sup&gt;1&lt;/sup&gt;, kg</td>
<td>234.14</td>
<td>235.89</td>
<td>240.43</td>
</tr>
<tr>
<td>EBW&lt;sup&gt;2&lt;/sup&gt;, kg</td>
<td>276.91</td>
<td>276.98</td>
<td>280.80</td>
</tr>
<tr>
<td>ADG, kg</td>
<td>2.04</td>
<td>1.96</td>
<td>1.92</td>
</tr>
<tr>
<td>DMI, kg</td>
<td>5.71&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.59&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.09&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>DMI, %BW</td>
<td>2.20</td>
<td>2.20</td>
<td>2.30</td>
</tr>
<tr>
<td>G:F</td>
<td>0.36&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.36&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.32&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>1</sup> IBW: initial BW
<sup>2</sup> EBW: BW at study completion
<sup>3</sup> Diet;
- AA = provides ≥110% AA requirement
- AAXS = provides ≥110% AA requirement + gut-modifying additives
- Control = provides ≥100% AA requirement
<sup>4</sup> Group: variable accounting for loads of cattle arriving 1 week apart
<sup>5</sup> SEM: Standard error of least squares means

<sup>a,b</sup> Diet least squares means within row without a common superscript differ (P < 0.05)
<table>
<thead>
<tr>
<th>Item</th>
<th>CONTROL 7</th>
<th>AA</th>
<th>AAXS</th>
<th>CONTROL</th>
<th>AA</th>
<th>AAXS</th>
<th>SEM 9</th>
<th>P-value</th>
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</thead>
<tbody>
<tr>
<td>IUon</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
<td>0.12</td>
<td>0.10</td>
<td>0.002</td>
<td>0.19</td>
</tr>
<tr>
<td>TITERon</td>
<td>2.60</td>
<td>2.17</td>
<td>2.20</td>
<td>2.27</td>
<td>5.67</td>
<td>2.57</td>
<td>0.45</td>
<td>0.68</td>
</tr>
<tr>
<td>IUoff</td>
<td>3.44</td>
<td>2.43</td>
<td>2.55</td>
<td>11.41</td>
<td>15.34</td>
<td>17.56</td>
<td>2.25</td>
<td>0.48</td>
</tr>
<tr>
<td>TITERoff</td>
<td>368.60</td>
<td>257.75</td>
<td>265.80</td>
<td>1273.18</td>
<td>1722.17</td>
<td>1987.86</td>
<td>258.47</td>
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<tr>
<td>ΔIU</td>
<td>3.34</td>
<td>2.33</td>
<td>2.45</td>
<td>11.31</td>
<td>15.23</td>
<td>17.46</td>
<td>2.25</td>
<td>0.48</td>
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<td>ΔTITER</td>
<td>366.00</td>
<td>255.58</td>
<td>263.60</td>
<td>1270.91</td>
<td>1716.50</td>
<td>1985.29</td>
<td>258.50</td>
<td>0.46</td>
</tr>
</tbody>
</table>

1 IUon: Antibody concentration at study onset
2 TITERon: Reciprocal antibody titer at study onset
3 IUoff: Antibody concentration at study completion
4 TITERoff: Reciprocal antibody titer at study completion
5 ΔIU: Change in antibody concentration over study
6 ΔTITER: Change in reciprocal antibody titer over study
7 Diet:
   - Control (n=21) provides ≥100% AA requirement
   - AA (n=24): provides ≥110% AA requirement
   - AAXS (n=17): provides ≥110% AA requirement + gut-modifying additives
8 Group: variable accounting for loads of cattle arriving 1 week apart
9 SEM: Standard error of least squares means
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