STRATEGIES AND TECHNOLOGIES FOR THE MANAGEMENT AND MITIGATION OF BOVINE RESPIRATORY DISEASE

A Dissertation

by

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ABSTRACT

Opportunities exist to improve treatment success rates and animal welfare standards through use of remote sensor technologies for early detection of bovine respiratory disease (BRD), which is the most prevalent disease affecting beef cattle. Remote sensor technologies can monitor real-time deviations in physiological and behavioral responses of individual animals, that are not biased by the presence of pen riders, to enable more objective and accurate assessments of an animal's health status. The objectives of this study were to evaluate behavioral, physiological, and immune alterations in cattle diagnosed with BRD, or in cattle experimentally challenged with BRD pathogens. In Trial 1, physical activity data collected from leg-attached accelerometers from BRD-diagnosed and healthy calves were analyzed using Shewhart statistical process control (SPC) procedures to determine if the activity sensors could detect onset of BRD prior to feedlot personnel. High-risk crossbred steers and bulls were fitted with accelerometers and evaluated for 56 d at a commercial feedlot. Univariate SPC models had moderate sensitivity (40 to 57%) and specificity (23 to 81%), and signaled up to 2 d prior to visual diagnosis. Multivariate SPC models had moderate sensitivity (43.9 to 57.4%) and low specificity (29.2 to 37.2%), and signaled 2 d prior to visual diagnosis. The moderate diagnostic accuracies of the SPC models reported in this study may be due to the relatively high within-animal daily variation in physical activity, and minimal time for early model training. In Trial 2, behavioral, physiological, and immune alterations of steers that mounted substantial or minimal haptoglobin (HPT)

responses following an experimental challenge with *Mannheimia haemolytica* (MH) were compared with PBS-challenged controls. The HPT-responsive steers had greater post-challenge concentrations of neutrophils and lymphocytes, rumen temperature, DMI, and day-to-day variation in feeding behavior than HPT non-responsive steers, but differences in metabolite profiles were not detected between HPT responsive phenotypes. Research has shown lower basal cortisol and increase variation in behavior are associated with decreased disease resilience. Trial 3 was conducted to determine if pulse oximetry could accurately predict SO_2 in hypoxic conditions in anesthetized cattle. As graded levels of hypoxia were induced, heart rate and blood pressure were increased, SpO_2 levels were decreased, but lactate and pH were minimally affected. Trial 4 was conducted to determine time-series deviations in physiological, behavioral and immunological responses following a combined viral (bovine herpes virus-1; BHV-1) and bacterial (MH) challenge (VB) in steers to further explore the use of pulse oximetry for preclinical detection of BRD. The VB-challenged steers exhibited elevated leukocyte, acute phase protein, and febrile responses, and decreased DMI, feeding behavior patterns and physical activity. However, blood gas analysis and pulse oximetry were minimally altered by the VB challenge, indicating that sensors to monitor blood SpO_2 would have more utility as a prognostic than diagnostic tool. Future research should investigate multifactorial algorithms that incorporate multiple sensors to monitor activity, temperature and feeding behavior to improve the accuracy of preclinical detection of BRD detection and animal welfare status in beef cattle.

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NOMENCLATURE

BHV-1	Bovine herpes virus-1
BRD	Bovine respiratory disease
BRSV	Bovine respiratory syncytial virus
BV	Bunk visit
BVDV	Bovine viral diarrhea virus
CBC	Complete blood count
CIS	Clinical Illness score
CON	Control
CUSUM	Cumulative summation
FiO_2	Inspiratory fraction of oxygen
F: G	Feed: gain
FN	False negative
FP	False positive
HD	Head down
HPT	Haptoglobin
LPS	Lipopolysaccharide
MH	Mannheimia haemolytica
NON	Haptoglobin non-responsive
PBS	Phosphate-buffered saline
PCA	Principal component analysis

PCO_2	Partial pressure of CO_2
PFU	Plaque-forming units
PO_2	Partial pressure of O_2
RES	Haptoglobin responsive
RMSE	Root mean square error
SO_2	Blood O_2 saturation
SPC	Statistical process control
SpO_2	Peripheral O_2 saturation
TN	True negative
TP	True positive
TTB	Time to bunk
VB	Viral-bacterial

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CHAPTER I

INTRODUCTION

Bovine Respiratory Disease

As the biggest threat to the health of weaned cattle, bovine respiratory disease (BRD) has dominated ruminant health research for years and yet an effective solution remains elusive. Nearly 75% of feedlot morbidity and 50% of feedlot mortality is attributed to BRD, and an excess of $1 billion USD is spent on treatment and mitigation strategies annually (Edwards, 1996; Smith, 1998; Aich et al., 2009; Burciaga-Robles et al., 2009; Cernicchiaro et al., 2013; Blakebrough-Hall et al., 2020). Costs of BRD are both direct in the form of medication, and indirect in production losses and decreased carcass value from cattle that are treated multiple times for BRD (Stovall et al., 2000; Reinhardt et al., 2012; Griffen, 2014; Blakebrough-Hall et al., 2020a). Even BRD early in life can have lasting effects; Holland et al. (2010) stated heifers treated for BRD during preconditioning had lighter BW at the start of feedlot finishing and took more days on feed to finish. In an analysis of nearly 18,000 feedlot steers, Reinhardt et al. (2012) concluded that increasing number of BRD treatments were associated with decreasing ADG, final BW, and carcass quality. Cernicchiaro et al. (2013) reported higher net returns from feedlot cattle that were never treated for BRD compared to cattle treated even only once, due to weight and quality grade differences. Meanwhile, public perception of antimicrobial therapy use and animal welfare, particularly in feedlots, has led to legislation which increases pressure even more to improve treatment success and

reduce animal morbidity (e.g., FDA Veterinary Feed Directive 2017). Due to the ubiquitous nature of BRD-causative pathogens and stress-inducing marketing process most beef calves experience, preventative measures are likely to never eradicate the condition of BRD, and research should instead focus on identification of disease resilient phenotypes and early detection of disease (Cusack et al., 2003). Emerging research in sensor technology has shown great promise in improving BRD detection, thereby improving animal welfare and efficacy of antimicrobial treatment. Commercial chute-side sensors have been developed to identify newly-arrived feedlot cattle at greater risk for BRD-development in an effort to tailor metaphylactic antimicrobial administration to only those individuals more likely to benefit from it (von Konigslow et al., 2019). Other sensors are remote and record traits such as physical activity (Belaid et al., 2019a), rumination (Marchesini et al., 2018), rumen temperature (Timset et al., 2011), and computerized feeding behavior (Wolfger et al., 2015; Kayser et al., 2020). These remote sensors have been shown to accurately detect BRD prior to detection by feedlot personnel. Development of these chute-side, remote body-mounted, and in-pen technologies relies on extensive knowledge of the shifts in biological systems associated with BRD. This review will discuss what is known of disease resiliency, the known immunologic, physiologic, and behavioral changes associated with onset of BRD, effect of temperament on those shifts, the timeline in which the changes typically present, and outline where future research should focus.

Disease Resilience

With ever-increasing public scrutiny on the use of antimicrobial technology in agriculture, identification of animals that are naturally more disease resilient would be valuable (König and May, 2018). Disease resilient animals are those that maintain production performance even with infection pressure present, and are able return rapidly to pre-infection state following pathogen exposure (Colditz and Hine, 2016; Putz et al., 2019). Resilient animals could be managed differently, requiring fewer health interventions. Recently, utility of day-to-day variation in feed intake and feeding duration as potential indicators of disease resiliency in pigs was investigated (Putz et al., 2019). In a natural-disease challenge model, infection pressure was maintained at the feeding facility by continually introducing infected pigs from other facilities, resulting in 26% overall mortality rate. Daily variances in both DMI and feeding duration were found to be moderately heritable (0.21 – 0.26), and had a positive genetic correlation (0.37 to 0.62) with mortality and morbidity rates in pigs. Putz et al. (2019) concluded that decreased day-to-day variance may be indicative of resilience. Similarly, lower variance in daily milk production was related to improved health and longevity in dairy cows (Elgersma et al., 2018). In geriatric humans reporting daily self evaluations for physical, mental, and social health, greater day-to-day variation was associated with decreased disease resilience (Gijzel et al., 2017). Resilience to heat stress has also been evaluated. Day-to-day variance in vaginal temperature of heifers was negatively associated with heat stress resilience (Dikmen and Mateescu, 2019). These results suggest that increases in day-to-day variance of production and behavior traits may be

able to lead to development of sensor technology which can identify resilient phenotypes, and that these phenotypes may be used for selection of animals with genetic merit for disease resilience. However, care must be taken when selecting for resilient animals. Carabaño et al. (2017) suggested that level of production and disease resilience may be mildly antagonistic, as the resilient animals are potentially diverting more physiological resources to immune function. Future research using sensor technologies in feedlot cattle at risk for BRD should evaluate variance in production and behavior traits surrounding diagnosis events.

Immunological Changes

Knowledge of common shifts in immunologic parameters associated with BRD onset will allow for development of remote sensor and chute-side risk-assessment technologies which can aid in preclinical diagnostics.

Hematology

Neutrophils are a phagocytic leukocyte, and are the first leukocyte to respond in immune insult or injury. As the first immune cell to respond, an important responsibility of neutrophils is release of chemokines, which in turn recruit acute phase proteins such as haptoglobin (Malech et al. 2014). In nearly all studies that measure differential leukocytes in BRD-diagnosed cattle, concentration of neutrophils is reported to increase compared to healthy cohorts (Burciaga-Robles et al., 2010; Hanzlicek et al., 2010; Kayser et al., 2019a; Kayser et al., 2019b).

Another frequently observed alteration in blood differentials of BRD-diagnosed cattle is decreased lymphocyte concentration. Lymphocytes are primarily involved in

viral immunity, and have cell types involved in both innate and adaptive immunity. Seemingly counter-intuitive, lymphocyte concentrations frequently decrease in association with BRD (Burciaga-Robles et al., 2010; Hanzlicek et al., 2010; Hanedan et al., 2015). This reduction in circulating lymphocytes is not due to decreased overall cell count, but due to lymphocyte sequestration in lymphoid tissues for antigen presentation, and subsequent binding to epithelium (Abbas et al., 2007).

Reduction in hematocrit is also frequently observed in cattle with BRD (Burciaga-Robles et al., 2010; Hanzlicek et al., 2010; Kayser et al., 2019c). Hematocrit is merely the relative proportion of erythrocytes to total blood volume, therefore a reduction in hematocrit is due to the relative proportion of erythrocytes decreasing with increasing circulating leukocyte numbers.

Another blood biomarker altered in association with BRD is the organic molecule lactate. Typically seen as a non-specific response to hypoxemia, lactate is expected to increase as anaerobic glycolysis produces lactate (Andersen et al., 2013). Lactate concentrations increase with severity of clinical symptoms BRD and percentage of lung lesions (Coghe et al., 2000; Camkerten et al. 2010; Ellis et al., 2013; Šoltésová et al., 2015; Oosthuysen et al., 2017). Therefore, lactate is less likely to provide much utility in early disease detection but more so in prognostics.

Acute Phase Proteins

Acute phase proteins are generated by the liver in times of injury or illness. Though there are many acute phase proteins, those most commonly measured in cattle include fibrinogen and haptoglobin (HPT). Fibrinogen is involved in the blood clotting

cascade, and due to relative ease of measurement, was one of the first acute phase proteins used with regularity in veterinary medicine (Nikunen et al., 2007). Increased fibrinogen has been reported in calves with natural BRD (Nikunen et al., 2007; Youssef et al., 2015). Elevated fibrinogen has also been reported in experimental *Mannheimia haemolytica* (MH) challenges (Ganheim et al., 2003; Hanzlicek et al., 2010). Measurement of HPT and fibrinogen together appears to yield useful prognostic information regarding disease severity. Humblet et al. (2004) reported substantially increased fibrinogen and HPT in calves diagnosed with natural BRD that later required anti-inflammatories in addition to antimicrobials for recovery, compared to calves that recovered with antimicrobials alone. Haptoglobin is an acute phase protein that fights infection by competitively binding to iron to prevent bacterial proliferation, and increases in most instances of inflammation (Petersen et al., 2004). Increased HPT is commonly observed in BRD-diagnosed cattle, and its response is typically quite transient, regardless of peak magnitude (Nikunen et al., 2007; Burciaga-Robles et al., 2009; Holland et al., 2011; El-Deeb et al., 2020). El-Deeb et al. (2020) measured acute phase proteins and proinflammatory cytokines in BRD-affected feedlot calves to investigate use as diagnostic markers. They concluded that HPT and other acute phase proteins had strong utility for use as a BRD diagnostic and prognostic marker, as HPT was elevated in morbid calves and returned rapidly to baseline following antimicrobial therapy. Burciaga-Robles et al. (2009) reported decreased HPT concentration upon feedlot arrival in calves that remained healthy, intermediate in calves that were treated once, and greatest in calves that were treated multiple times for BRD. Holland et al.

(2011) similarly reported that cattle arriving to a feedlot with elevated HPT had reduced ADG and DMI during the 2 wk following arrival. Additionally, calves that arrived with high HPT experienced greater total morbidity rate, and an increased proportion of calves requiring three treatments. These studies indicate that degree of HPT concentration may indicate degree of immunocompetence and disease resilience.

Metabolites

Metabolomics provides an opportunity to identify biomarkers that are predictive of individual animals that may be immunocompromised and susceptible to BRD, in addition to calves in the early stages of BRD. Recent metabolomics studies in disease-challenged animals have shown utility in differentiating morbid and healthy cattle, which indicates it may support future development of chute-side diagnostics. Metabolomics analysis has successfully distinguished healthy dairy cows from those at risk for periparturient disease (Klein et al., 2012; Hailemariam et al., 2014) and healthy calves from those infected with *Mycobacterium avium* (De Buck et al., 2014). Additionally, auction-sourced versus single-source calves were observed to exhibit differing metabolites, suggestive that metabolomics could be of use to identify calves at greater risk for BRD (Buhler et al., 2019). Though few studies have examined metabolomics in regards to BRD in beef cattle, Aich et al. (2007) reported distinct metabolic profiles in preconditioned versus recently-weaned beef calves both prior to and following a bovine herpes virus-1 (BHV-1) challenge, suggesting stress may alter physiologic response to immune challenge. Basoglu et al. (2014) also reported metabolic distinctions in discovered dairy calves with BRD compared to healthy controls. More

recently, Blakebrough-Hall et al. (2020b) conducted a ^{1}H NMR metabolomic analysis of plasma metabolites in cattle diagnosed with natural BRD in a feedlot, and observed metabolic profile differences between BRD-diagnosed (n = 149) and healthy cohorts (n = 148) that were able to accurately (85%) differentiate BRD vs healthy cases. These results suggest selective metabolite profiles may be useful for development of chute-side sensor technologies to more effectively diagnose BRD status of calves at feedlot arrival to support more effective targeted metaphylactic treatment strategies (Blakebrough-Hall 2020b).

Physiological Changes

Febrile Response

Febrile response in a mammal is a non-specific response to infection mounted by the innate immune system in order to make the host less hospitable to pathogens. Temperature is by far the most widely used metric of disease diagnosis, as it is one of the few objective measurements widely available for relatively low cost. Increased temperature is the most common method of BRD diagnosis in commercial operations, as well as in experimental challenges (Hanzlicek et al., 2010; Theurer et al., 2013; Timset et al., 2016; Kayser et al., 2019b). Duration of the febrile response appears to be related to the severity of disease. Method of temperature measurement, such as rectal versus rumen, may alter antimicrobial intervention decisions. Rumen temperature is measured via thermoboluses placed in the reticulo-rumen and rectal temperature measured via rectal temperature probes. Rectal temperature is more common, as rumen thermoboluses are cost prohibitive outside of the research setting. Agreement between rectal and rumen

temperature within animal is important, as antimicrobial treatment protocols typically involve a temperature threshold. Rumen temperature has been reported to be slightly higher (0.6° C) than rectal temperature (Timset et al., 2011), therefore treatment protocols determined off rumen temperature should use a slightly higher threshold in determining BRD cases than what would typically be used as a rectal temperature threshold.

Rumination

Rumination behavior has been investigated as a potential disease indicator trait, as it is less expensive to measure than feed intake, but theoretically reflects the same physiology. Rumination is typically measured via collars with an accelerometer on the left side of the neck, using the movement caused by peristaltic muscle contractions to estimate rumination time. Marchesini et al. (2018) reported significant reductions in rumination as much as 6 d prior to clinical signs and treatment for BRD. In contrast, Kayser et al. (2020) employed statistical process control (SPC) algorithms to investigate utility of rumination as a predictor of BRD onset, and found it to have low sensitivity (12.5%). Age of cattle, diet provided, and disease severity likely alter the efficacy of utilizing rumination for disease detection.

Blood Gas

As pulse oximetry is a valuable tool in evaluating prognosis of human and small animal pneumonia patients, there has been research investigating whether blood oxygen saturation shifts in the early stages of BRD in cattle. Oxygen saturation can be measured by arterial blood gas analysis to measure oxyhemoglobin saturation (SO_2) or partial

pressure of oxygen (PO_2), or by pulse oximeters to non-invasively measure peripheral oxygen saturation (SpO_2). Of those parameters, arterial PO_2 is the most sensitive due to the sigmoidal shape of the saturation curve between PO_2 and SO_2 (Coghe et al., 1999). Many studies have reported reduction in PO_2, SO_2, or both traits, in association with clinical BRD. Ciszewski et al. (1991) collected arterial blood in Holstein calves challenged with bovine respiratory syncytial virus (BRSV) and healthy controls. By day 6 post-inoculation, the challenged calves presented an average arterial PO_2 of 76 mm Hg, while control calves maintained PO_2 of 94 mm Hg. Collie et al. (1992) similarly reported declining PO_2 with increasing severity of clinical symptoms in calves with chronic BRD. Woolums et al. (1999) conducted arterial blood gas analysis in calves 7 d following a BRSV challenge. Challenged calves became grossly morbid, and had significantly lower PO_2 than control calves (58.1 mm Hg vs 77.7 mm Hg). Ellis et al. (2013) also reported strong correlation between reduced PO_2 and lung lesion severity in calves challenged with BRSV. Šoltésová et al. (2015) collected a single arterial blood sample from 156 dairy calves arriving at a veterinary school for treatment of BRD and recorded subsequent health outcomes. Arrival SO_2 and PO_2 declined and PCO_2 increased with increasing disease severity. Ozkanlar et al. (2012) analyzed venous blood gas, presumably due to difficulty in arterial collection, in calves with natural BRD and compared to healthy controls. They reported significant reductions in PO_2, but did not observe a difference in SO_2. Oosthuysen et al. (2017) also investigated venous blood gas shifts following lipopolysaccharide (LPS) challenge, and were able to detect a brief decline in PO_2 at 2 hours following LPS administration, however no other changes were

observed at other time points. Hanzlicek et al. (2010) reported moderate to severe clinical symptoms of BRD and later lung lesions upon slaughter in calves challenged with MH, yet observed increasing arterial SO_2 and PO_2 in the days following inoculation. This indicates the first few collections were likely contaminated with venous blood, as arterial blood collection can be difficult.

Due to complexity of unadulterated arterial blood collection, the non-invasive SpO_2 is an attractive alternative. SpO_2 uses LED lights to estimate percentage of oxygen-bound hemoglobin based on optical reflectance. Surprisingly little research exists involving use of SpO_2 in BRD detection. Coghe et al. (1999) measured SpO_2, arterial SO2, and the agreement between the two in calves with moderate to severe BRD, and reported reductions in each parameter with increasing disease severity. The pulse oximeter had a negative bias at low arterial SO_2, but they concluded it was of little consequence as SpO_2 readings < 80% have little clinical relevance. Baruch et al. (2019) conducted a combined BHV-1/MH challenge in calves and recorded alterations in SpO_2. All of the calves developed mild or moderate clinical BRD symptoms following BHV-1 inoculation, and severity increased drastically after MH inoculation. Despite mild morbidity symptoms and elevated rectal temperature, SpO_2 was unaffected during the BHV-1-only period. However, by the day following MH inoculation SpO_2 had rapidly declined from 97.6% to 95.4%, and was reduced to 91.2% two days post-MH. Subsets of calves were serially slaughtered for lung examination on the day of MH inoculation, and 1, 3, 5, and 7 d post-MH inoculation. Degree of lung consolidation was significantly correlated with SpO_2; SpO_2 levels between 96-100% were associated with 11.5% lung

consolidation, SpO_2 levels between 93-95% were associated with 21.7% lung consolidation, while SpO_2 levels 92% and below were associated with 35% lung consolidation. These studies by Coghe et al. (1999) and Baruch et al. (2019) indicate that SpO_2 is likely not a suitable metric for preclinical disease detection, as deviations were observed only after animals presented with clinical symptoms and after other, more-easily measurable indices such as temperature had deviated. However, SpO_2 could certainly aid in distinguishing BRD from other disease etiologies, and in prognosis of BRD which would aid in the more judicious administration of antimicrobial treatment.

Behavioral Changes

Effect of Temperament

Prior to discussion of the behavioral changes associated with onset of BRD, the effect of temperament on immunological, physiological, and behavioral responses should be considered. Temperament in cattle is commonly evaluated in several ways: chute exit velocity, pen reactivity, and chute reactivity (Curley et al., 2006). Consideration of cattle temperament is incredibly important, as fractious cattle are more difficult to identify as morbid. Temperamental bulls had lower sickness scores following an LPS challenge (Burdick et al., 2011). Temperament could be a responsible factor when considering reviews such as from Griffin et al. (2014) that reported ADG was decreased in cattle with lung lesions at slaughter, many of which had never been treated for BRD. Not only are fractious cattle more difficult to identify as morbid, but even healthy fractious cattle have decreased ADG compared to calm cattle (Bruno et al., 2018; Olson et al., 2019). For that reason, disease-detecting systems which rely on

behavior monitoring must have adequate time for model training while the animal is healthy, as fractious and calm animals by definition do not behave the same. Additionally, impact of stress hormones such as cortisol must be considered. As glucocorticoids are known to be anti-inflammatory (Dhabhar, 2008) animals with elevated basal cortisol tend to have some suppression of immune responses. This is only in regards to the moderately increased cortisol found in more fractious animals, not the prolonged substantially increased cortisol observed in chronically stressed calves which generally leads to immunosuppression (Roth, 1985; Aich et al., 2009). Burdick et al. (2011) reported temperamental bulls had lower peak temperature and epinephrine following an LPS challenge. Dong et al. (2018) reported that cortisol inhibited production of pro-inflammatory cytokines in LPS-stimulated macrophages. In some cases, the inflammation associated with an immune response is more damaging to the host than the pathogen the immune response is targeting, indicating modest concentrations of cortisol may help to moderate the immune response following a health challenge (Lawrence and Gilroy, 2007; Jose and Madan, 2016). The anti-inflammatory mode of action of cortisol is in part due to its effect of decreasing affinity of L-selectin on the neutrophil with its receptor on the endothelium, which decreases neutrophil rolling and in turn decreases translocation to tissue (Burton et al., 1995). These effects of temperament should be considered when analyzing hematological reports of cattle and when developing behavior algorithms. Olson et al. (2019) reported temperamental cattle had shorter and smaller meals than calm cattle and overall less DMI, which could confuse algorithms in sickness detection. More research is needed to determine whether

there are temperament differences attributed to basal cortisol in animals that are more disease resilient.

Feeding Behavior

Alteration in feeding behavior is perhaps second only to febrile response regarding consistent shifts associated with morbidity. The same pro-inflammatory cytokines which signal the hypothalamus to initiate fever and production of acute phase proteins also induce anorexia (McCarthy, 2000). Sowell et al. (1999) reported reduced bunk visit behavior the week following feedlot arrival in steers that subsequently were diagnosed with BRD, however unfortunately the authors did not examine the behavior timeline relative to BRD diagnosis. Quimby et al. (2001) developed SPC algorithms to detect alterations in feed bunk duration to identify BRD in feedlot steers, and reported 87% accuracy in detection 4 d prior to visual diagnosis. Holland et al. (2011) reported calves with increased HPT upon feedlot arrival exhibited decreased BW and DMI, and were more likely to be treated 3 times for BRD. Following MH inoculation, Theurer et al. (2013) reported challenged calves spent less time at the hay bunk the day of challenge, and less time at the grain bunk the day following challenge. Wolfger et al. (2015) examined meal behavior of auction-sourced calves deemed at high risk for development of BRD. They reported 76% morbidity rate, and found reduced meal intake and meal frequency were associated with an increased risk for BRD 7 d prior to visual diagnosis. Kayser et al. (2019a) developed SPC algorithms to detect alterations in feeding behavior (DMI, bunk visit behavior) of feedlot bulls, and reported that feeding behavior began to differ 10 d prior to visual diagnosis, and DMI was altered 1 d prior to

visual diagnosis. Multivariate SPC models signaled 2 d prior to visual diagnosis, with an average accuracy of 81%. In a challenge model using BHV-1 and MH, Kayser et al. (2019b) reported reduction in DMI the day of MH inoculation and 4 d following. In a MH-only challenge study, Kayser et al. (2019c) reported a slight reduction of bunk visit frequency in challenged steers the day of MH inoculation and reduced DMI for the 3-d following. Belaid et al. (2019b) also observed reduced bunk visit duration and frequency 10 d and 7 d, respectively, prior to visual BRD detection in feedlot bulls. Finally, Kayser et al. (2020) investigated use of SPC algorithms to detect experimentally-induced BRD in steers inoculated with MH. They reported DMI and bunk visit behavior deviated the day of inoculation, with an average accuracy of 68.5%. As discussed, feeding behavior deviations surrounding morbidity events is well-documented. Future research should place emphasis on development of behavioral algorithms to detect BRD at the individual-animal level in real time.

Physical Activity

Monitoring physical activity is another strategy that has been proven to alter with onset of both natural and experimental BRD in cattle. For 1 wk following MH challenges, Hanzlicek et al. (2010) reported decreased step count and Theurer et al. (2013) reported increased lying time in challenged calves. Eberhart et al. (2017) observed that duration of lying bouts was increased for 24 h following inoculation in MH-challenged calves. Using accelerometers to measure duration of lying, standing and walking, Bayne et al. (2016) evaluated changes in activity behaviors in cattle with subclinical disease induced by inoculation with bovine viral diarrhea virus (BVDV).

Minimal differences in lying, standing and walking time were found between challenged vs controls steers, suggesting that severity of disease affects the magnitude of change in these activity behaviors during disease progression. The same reduction in activity has been observed in spontaneous BRD cases. Pillen et al. (2016) analyzed four activity traits (step count, duration of lying time, lying bout frequency, and motion index) of 364 beef steers during a 56-d feeding trial. Steers were monitored daily by feedlot personnel, and morbidity events were recorded. There was a 51.5% morbidity rate, and those morbid steers were observed to decrease step count 4 d prior to diagnosis and decreased motion index 5 d prior to diagnosis. While there were no significant differences in lying behavior, morbid steers tended to have reduced lying bout frequency as much as 6 d prior to diagnosis. More recently, Belaid et al. (2019b) evaluated use of a multifactorial model including feeding behavior (bunk visit duration and frequency) and physical activity (step count, lying bout frequency and duration) traits for detection of BRD in young bulls. Using multivariate logistic regression models with backward stepwise procedure, all combinations of these behavioral traits were evaluated for individual days up to 10 d prior to visual diagnosis of BRD. The best-performing model for prediction of BRD included BV duration, and frequency and duration of lying bouts, with an accuracy of 81% on day 9 prior to visual diagnosis. Interestingly, although repeated measures analysis revealed that step count was reduced in BRD bulls compared to healthy bulls up to 10 d prior to diagnosis, Belaid et al. (2019b) reported that step count was not retained in any of the multivariate logistic regression models for prediction of onset of BRD. Future research should investigate the week-to-week repeatability of activity traits, as

any behavior trait used for disease detection needs to be moderately high in repeatability in order for the model to be able to detect shifts associated with morbidity onset.

Summary

Bovine respiratory disease is a brutal, economically important disease that affects every feedlot. Development of sensor technology for preclinical detection, and disease prognosis, is of utmost importance. Commercial chute-side and remote sensors have been developed to identify cattle at greater risk for BRD-development or cattle in the early stages of disease etiology in an effort to tailor metaphylactic antimicrobial administration to only those individuals more likely to benefit from it. Development of these technologies relies on extensive knowledge of the shifts in biological systems associated with BRD. Knowledge of the relative timeline in which certain physiological systems deviate around a morbidity event in cattle would allow for more better prioritization of research objectives. Other behavior and physiological metrics such as DMI, fever, and activity deviate from baseline sooner, and are therefore more appropriate for early disease detection in cattle. Table 1 shows results from 7 natural BRD studies in which sensor data from days prior to visual BRD diagnosis were analyzed. There are several metrics that have been shown to deviate prior to presentation of clinical symptoms. Table 2 shows results from 7 studies in which BRD was experimentally-induced and sensor data was collected in the days following diagnosis. Feeding behavior appears to deviate prior to body temperature in natural BRD cases, but fever and feeding behavior deviation appears to occur concurrently in experimental BRD cases. Due to the obvious physiological importance of maintaining proper blood

oxygenation, it appears that oxygen saturation is one of the last biological mechanisms to deviate in cattle with BRD and may therefore have more prognostic than diagnostic value. As discussed above, activity metrics may prove useful in detection of BRD, but research first needs to confirm repeatability. Effect of temperament needs to be considered when monitoring behavioral changes with disease. Bovine respiratory disease is a multifactorial disease complex which demands a multifactorial solution. Sensor technology at the individual-animal level fitted with a multivariate algorithm could be developed to signal when feeding behavior, activity, and body temperature are determined to have shifted from baseline.

CHAPTER II

EFFICACY OF STATISTICAL PROCESS CONTROL PROCEDURES TO MONITOR DEVIATIONS IN PHYSICAL BEHAVIOR FOR PRECLINICAL DETECTION OF BOVINE RESPIRATORY DISEASE IN FEEDLOT CATTLE

Introduction

Bovine respiratory disease (BRD) is estimated to account for 70% of total feedlot morbidity and 50% of total feedlot mortality (Aich et al., 2009; Smith, 1998), therefore novel disease detection strategies are needed to help mitigate the impact of this disease. Cattle have evolved to mask clinical signs of disease as protection from predation, which limits the efficacy of visual observation by pen riders for preclinical detection of BRD (Timset et al., 2016). Remote sensor technologies may assist in earlier and more accurate detection of BRD to mitigate the impact of the disease, and promote more judicious use of antibiotics. Sensor technologies have been shown to effectively detect BRD in beef cattle by monitoring changes in feeding behavior and rumen temperature (Quimby et al., 2001; Timset et al., 2011; Kayser et al., 2019c). Accelerometer-based devices attached to ear tags, collars or hind legs have also been evaluated to monitor changes in feeding and physical activity behaviors relative to onset of BRD. Hanzlicek et al. (2010) observed that physical activity, measured as step count, was decreased for 7 d following an experimental MH challenge. In a MH challenge study with dairy steers, Eberhart et al. (2017) reported that MH-challenged calves spent more time lying down compared to control calves. Pillen et al. (2016) examined physical activity patterns of high-risk beef

calves in a commercial feedlot. A repeated measures analysis revealed that calves with clinically-confirmed BRD had significantly reduced motion index and step count up to 5 d prior to BRD diagnosis compared to healthy cohorts. Frequency of lying bouts and duration of standing were also reduced in BRD-diagnosed calves, but only during the day prior to BRD diagnosis.

Statistical process control (SPC) is a procedure that was originally designed to monitor the quality control of manufacturing processes (Shewhart, 1931), and has since been applied to monitor various processes in other industries including health care and agriculture (Berwick, 1991; De Vries and Reneau, 2010). Quimby et al. (2001) reported that SPC analysis of feeding behavior traits was able to detect morbid calves 4 d prior to pen rider diagnosis with an accuracy of 87%. More recently, Kayser et al. (2019a) reported that SPC analysis of individual feeding behavior traits was able to detect BRD prior to the onset of clinically-observed BRD with moderate to strong accuracy (range of 69 to 80%). Moreover, the accuracy of BRD detection was slightly improved (84%) when a multivariate SPC model that included 4 feeding behavior traits was evaluated. The objective of this study was to conduct a post-hoc analysis of data from Pillen et al. (2016) to evaluate the sensitivities and specificities of univariate and multivariate SPC models of physical activity data to detect the onset of BRD in high-risk steers.

Material and Methods

All animal care and use procedures were in accordance with the guidelines for use of Animals in Agricultural Teaching and Research as approved by the West Texas

A&M University Institutional Animal Care and Use Committee, (IACUC Protocol No. 02-10-13).

Experimental Animals and Design

This study was conducted at a commercial feedlot 19 km from Hereford, TX utilizing auction-sourced, high-risk male calves (n = 266, initial BW 180 ± 26 kg; Pillen et al., 2016). All calves were vaccinated the day following arrival with subcutaneous parenteral and intranasal modified-live respiratory vaccines (Titanium 3, Elanco Animal Health, Greenfield, IN; Inforce 3, Zoetis, Florham Park, NJ), an autogenous MH bacterin (American Animal Health, Grand Prairie, TX), and a Clostridial bacterin-toxoid (Covexin 8, Merck Animal Health, Madison, NJ). Additionally, calves were dewormed (Ivomec Plus, Merial Limited, Duluth, GA), administered a growth-promoting implant (Component ES, Elanco Animal Health), and provided metaphylactic antimicrobial treatment (Micotil, Elanco Animal Health). Calves were confirmed negative for persistent infection with BVDV, were hot-iron branded on the hip, and horned animals were horn-tipped. On arrival, 72.1% of the calves were intact bulls and were castrated during arrival processing. Finally, an accelerometer device was fixed to the right rear tarsus of each calf (IceQube, IceRobotics, Ltd., Midlothian, Scotland). Calves were blocked by castration status upon arrival, and randomly assigned to 1 of 2 pens. A complete description of animal management and diet composition used in the study has been previously reported (Pillen et al., 2016).

Data Collection

The accelerometer devices (IceQube) used in the study detect changes in electrical output voltage based on relative position, and apply proprietary algorithms to quantify daily frequency and duration of lying bouts, step count, and an index of activity motion. Accelerometer data were recorded at 15-min intervals, and later downloaded using the manufacturer's software (IceManager, IceRobotics, Ltd.). The calves were evaluated daily and clinical illness scores (CIS) and depression scores assigned by trained pen riders. The CIS (0-4) was based on respiration, apparent rumen fill, and nasal discharge, with the depression score (0-4) based on responsiveness to human approach. Both scoring systems were used to define BRD cases. For this study, BRD cases were calves that were assigned a score of 1 or higher for both scoring systems, or a score of ≥ 2 for either system. Rectal temperature was recorded at the time antimicrobial therapy was administered, but was not used to define BRD cases. The antimicrobial treatment regimen for BRD cases was Excede (Zoetis) on first treatment, Nuflor (Merck Animal Health) on second treatment, and Oxytet 100 (Norbrook Inc., Lenexa KS) on third treatment, with all antibiotics administered according to label instructions. Following antimicrobial treatment, calves were returned to their home pens. Calves that did not display clinical signs of BRD were used as controls. Health records were obtained from feedlot recordkeeping software (Animal Health International, Inc., Greeley, CO).

Statistical Analysis

Pillen et al. (2016) used a repeated measures analysis to compare frequency and duration of lying bouts, step count, and motion index between control and BRD-case

calves in the days prior to the onset of BRD diagnosis. The post-hoc analysis of data from this study used Shewhart (Proc Shewhart, SAS 9.4, Cary, NC) and cumulative summation (CUSUM) procedures (Proc CUSUM, SAS 9.4) to separately monitor deviations in frequency and duration of lying bouts, step count, and motion index on an individual-animal basis relative to the onset of BRD diagnosis. Although this was a retrospective analysis completed after the culmination of the study, the charts were constructed in a manner to mimic analysis in real time; the first 4 d of data were used for initial parameter estimation for each calf, and thereafter each day's observation was added to the model to recompute parameters as described by Kayser et al. (2020). This allows the accuracy of the chart to improve each day as data accumulates and the confidence in the parameter estimate increases.

In addition to evaluating univariate SPC models for frequency and duration of lying bouts, step count, and motion index using Shewhart and CUSUM procedures, principal components analyses (PCA; Proc Factor SAS 9.4) were utilized to construct multivariate SPC models that included various combinations of the 4 physical activity traits on an individual-animal basis. Based on preliminary analyses, 2 of these multivariate models were further evaluated including a resting model that incorporated frequency and duration of lying bouts, and a full model that used all 4 physical-activity traits. The accuracies of these multivariate models were then evaluated using Shewhart and CUSUM procedures in the same manner as described for the univariate models. Charting methods were conducted such that the signal for 'out of control' would occur for either the upper or lower thresholds for the univariate and multivariate models. The σ

(Shewhart) and H-value (CUSUM) threshold for each model were varied to determine effects of different control limits on signal timing and chart sensitivity and specificity, as described by Kayser et al. (2020). The sensitivity, specificity and accuracy values at various sigmas from the Shewhart chart analysis of step count is shown in Figure 1.

For each SPC model, when the chart signaled that the system was "out of control" the signal for that animal was assigned to 1 of 4 categories: false positives (FP) when the chart signaled out of control for a control animal, false negatives (FN) when the chart never signaled for a BRD-case animal, true positives (TP) when the chart signaled for a BRD-case animal, and true negatives (TN) when the chart did not signal a control animal. Out of control signaling was required to occur prior to the day of BRD diagnosis, as movement to the facility for health checks confounded activity data on the day of diagnosis. Chart performance was evaluated using Proc FREQ (SAS 9.4) based on sensitivity (TP / [TP + FN]), specificity (TN / [FP + TN]), and accuracy ([TP + TN] / [TP + FP + FN + TN]). In order to identify statistical difference between the variables, 95% confidence intervals (CI) were computed with a chi-square distribution using Proc FREQ (SAS 9.4). The signal day was the average number of days prior to visual BRD diagnosis that the chart signaled that a calf's metrics were deemed 'out of control'. The 95% CI for signal day were computed using Proc Univariate (SAS 9.4).

Results and Discussion

The morbidity and mortality rates of the calves in this study were 48 and 4.1%, respectively. The average first treatment for BRD occurred 16 d after feedlot arrival and ranged from 3 to 50 d post-arrival. Previously report results from a repeated-measures

analysis of the physical activity data found that step count and motion index were decreased in BRD case steers 4 and 5 d prior to visual diagnosis by pen riders, respectively. Other studies have also reported reductions in physical activity traits prior to the onset of BRD. Hanzlicek et al. (2010) reported decreased step count for one wk post experimental MH challenge in beef steers, and Eberhart et al. (2017) observed that duration of lying bouts was increased for 24 h in MH- compared to saline-challenged Holstein calves. Using leg-attached accelerometers to measure duration of lying, standing and walking, Bayne et al. (2016) evaluated changes in activity behaviors in cattle with subclinical disease induced by inoculation with low-virulent BVDV. Minimal differences in lying, standing and walking time were found between BVDV-challenged vs controls steers, suggesting that severity of disease affects the magnitude of change in these activity behaviors during disease progression. Belaid et al. (2019b) reported that physical activity (step count, frequency and duration of lying bouts) and frequency and duration of bunk visit (BV) events were decreased in bulls as much as 10 d prior to visual diagnosis of BRD. Reductions in feeding behavior patterns associated with onset of BRD have been well-documented. Daniels et al. (2000) and Buhman et al. (2000) reported reductions in frequency and duration of BV events in feedlot calves diagnosed with BRD. Using a 2-slope broken-line regression model, Jackson et al. (2016) reported that reductions in DMI and frequency and duration of BV events occurred 7 d prior to visual diagnosis of BRD in growing bulls. Collectively, these studies demonstrate that physical activity and feeding behavior traits have considerable merit for inclusion in

individual-animal prediction models, such as SPC analyses, for monitoring health status in animals.

In the current study, preliminary analyses revealed that the SPC models based on Shewhart procedures were more sensitive and specific than those based on CUSUM procedures (data not shown). Thus, only the results from SPC models based on Shewhart procedures will be discussed. Shewhart charts are designed to detect deviations from the mean with magnitudes greater than 3σ. Shewhart charts do not have "memory" unlike other SPC charts (e.g. CUSUM) meaning previous observations do not affect current observations (Nazir et al., 2013). Chart performance results for BRD detection utilizing the univariate and multivariate SPC models are shown in Table 3. The results presented for each SPC model were derived using the σ threshold that generated the highest diagnostic accuracy based on optimized chart sensitivity and specificity. The univariate models had relatively low sensitivities (19.4 to 40.3%) and moderate specificities (23.2 to 81.0%), such that overall accuracies ranged from 31.8 to 52.3%. Of the 4 univariate SPC models using Shewhart procedures, step count was the best performing, with overall diagnostic accuracy of 52.3% and average signal day of 1.8 d prior to clinical detection of BRD. However, the sensitivity of this SPC model based on step count was only 23.6%. The overall accuracy and average signal day of the complete multivariate SPC model were similar to the accuracies and average signal days of the univariate SPC models for motion index and step count. However, the complete multivariate SPC model was more sensitive and less specific in detecting BRD compared to the univariate SPC models for motion index and step count. The resting multivariate model based on

frequency and duration of lying bouts was also similar in overall accuracy to the respective univariate SPC models, although model performance based on signal day was slightly improved.

In a similar SPC analysis using Shewhart procedures, Kayser et al. (2020) analyzed changes in rest, standing, and over-activity data collected by collar-based accelerometers in steers that were experimentally challenged with MH. In that study, the univariate SPC models for these activity traits had moderate accuracies ranging from 47.8 to 68.8%, which were similar to results from the current study. In contrast, the univariate SPC models for DMI and feeding behavior traits were more accurate, ranging from 69.4 to 88.9% (Kayser et al. 2020). Indeed, SPC models utilizing feeding behavior traits appear to be relatively more sensitive and specific in detection of disease than physical activity traits. Quimby et al. (2001) and Kayser et al. (2019a) utilized CUSUM charts of feeding behavior traits to predict the onset of clinically-observed BRD, and reported accuracies ranging from 69 to 89%. Applying a hazard-analysis modeling approach, Wolfger et al. (2015) also found that monitoring deviations in feeding behavior was effective in predicting onset of BRD in high-risk feedlot steers.

Belaid et al. (2019b) evaluated the use of feeding behavior and physical activity traits for detection of illness in bulls at high risk for BRD. In that study, bulls were fitted with an accelerometer (Fedometer System, Rosh Pina, Israel) on the right front leg to measure step count and lying bout frequency and duration. Additionally, an antenna at the feed bunk was used to record animal proximity within 30 cm of the feed bunk to measure frequency and duration of BV events. Using multivariate logistic regression

models with backward stepwise procedure, all combinations of these behavioral traits were evaluated for individual days up to 10 d prior to visual diagnosis of BRD. The best-performing model for prediction of BRD included BV duration, and frequency and duration of lying bouts, with an accuracy of 81% on day 9 prior to visual diagnosis. The other multivariate logistic regression models were moderate to high in accuracy ranging from 66 to 81%, although the false positive rates were relatively high (range 42 to 66%). Interestingly, although repeated measures analysis revealed that step count was reduced in BRD vs healthy bulls for up to 10 d prior to diagnosis, Belaid et al. (2019b) reported that step count was not retained in any of the multivariate logistic regression models for prediction of onset of BRD.

While results between studies cannot be directly compared due to differences in methodologies used to develop prediction models, sensor technologies, and methods used to define BRD cases, results from the current study, Kayser et al. (2020) and Belaid et al. (2019b) suggest that physical activity traits are less predictive of onset of BRD than feeding behavior traits. In contrast, Knauer et al. (2018) found that SPC models based on milk consumption (L/d), drinking speed (mL/min) and unrewarded feeder events (non-consumption events) recorded by a computerized milk-feeding system had limited utility in predicting onset of disease in dairy calves. The calves in this study were diagnosed and treated for diarrhea, respiratory disease, or general “ill thrift”, with an overall treatment rate of 63%. The overall accuracies of univariate and various 2-trait SPC models using CUSUM procedures were moderate and ranged from 38.7 to 57.9%. The SPC model for drinking speed had the earliest signal day (-2.8) relative to visual

diagnosis, but the accuracy was moderate (53.8%). The 2-trait SPC model that included milk consumption and drinking speed had the highest accuracy (57.9%), but the majority of chart signals occurred after the calf had received treatment. Knauer et al. (2018) concluded that SPC analysis of milk consumption and behaviors offered no advantage compared to visual diagnosis. These conflicting results likely stem from this model including all reasons for treatment when only 30% of the treatments were for BRD, whereas the Quimby et al. (2001) and Kayser et al. (2019a) exclusively analyzed BRD case morbidity. Different results may have been reported by Knauer et al. (2018) if each diagnosis were investigated separately. Additionally, these calves were housed groups in pens with only an average of 17 calves, allowing potentially greater accuracy in disease detection by the animal managers.

Improved accuracy of feeding behavior models over strictly activity models, as observed in studies such as Kayser et al. (2020), may be due to physical activity being a less repeatable trait, as feeding behavior has been shown to be highly repeatable across time in beef and dairy cattle (De Vries and Reneau, 2003; Kelly et al., 2010). Indeed, the within-animal CV of step count in the present study was 40%, whereas we have observed CV of DMI to be 21% (Wottlin, unpublished data). An example control chart of step count for a calf that was diagnosed with BRD from the present study is shown in Fig. 2, and the day-to-day variance in this trait is clearly seen. Though we are unaware of a study that has compared repeatability of activity and feeding behavior in the same group of cattle, it is reasonable to deduce that feeding behavior is a more static behavior, less susceptible to environmental impacts than physical activity. Cattle activity has been

shown to be influenced by precipitation, ambient temperature, and temperature humidity index (Gonyou and Stricklin, 1984; Herbut and Angrecka, 2017; Hendriks et al. 2020).

Another potential reason for the relatively low accuracies of the SPC models observed in the present study is that average first treatment of BRD-diagnosed calves occurred on day 16 post feedlot arrival, which limited the data available for initial for SPC model training. Additionally, the extensive processing procedures (castration, dehorning, branding, modified-live vaccination, etc.) that were administered on the day following feedlot arrival likely induced considerable inflammatory responses that may have induced more sedentary behavior during for the first wk post arrival. Castration alone has been reported to induce substantial inflammation in young feedlot bulls (Roberts et al., 2018), and Richeson et al. (2008) found that calves administered modified-live vaccines upon feedlot arrival gained less than calves similarly-vaccinated 14 d later. These factors together would have resulted in limited SPC model training during the first wk post arrival.

An inherent obstacle to overcome in the development of SPC-type models deployed on sensor-derived continuously-recorded data for the prediction of onset of disease, is the accuracy of defined BRD cases. In the current study, BRD cases were based on 2 separate subjective measures (illness and depression scoring), which were not confirmed with elevated rectal temperature or other objective measure of illness (e.g., white blood cell count). Validation of these SPC models against the “gold standard” of human detection is inherently flawed, as the low sensitivity of BRD diagnosis based on visual signs of clinical illness has been reported (Timset et al., 2016). Sensors equipped

with a detection algorithm of moderately strong (~80%) accuracy may need to be put in place to test true performance of a sensor with treatment decisions, using validation based on lung lesions at slaughter or overall mortality rate to decrease reliance on the less-sensitive pen rider. Such sensor validation was employed by MacGregor et al. (2015); health intervention was administered to cattle in one group based solely on the recommendation of a feeding behavior system's algorithm, and compared to a second group of cattle that was routinely monitored and treated based on pen riders' discretion. The feeding behavior-based sensor system resulted in overall fewer pulls, lower mortality rate, lower medication charges per head, and greater treatment success rate. Low sensitivity of disease detection by feedlot personnel is primarily affected by the short duration that pen riders have to evaluate cattle for clinical signs of illness, and the instinctive fear that prey species possess resulting in masked symptoms. If emerging sensor systems must be validated against a rather tarnished "gold standard" of human detection, advancements in disease detection and management will be slow.

Conclusion

Sensor-based technology is being intensely researched for utility in disease detection of feedlot cattle. Statistical process control can effectively detect abnormal shifts in a system, and deployment on cattle feeding behavior has shown favorable results. However, this analysis resulted in low accuracy of BRD detection utilizing physical activity metrics, likely due to a high degree of within-animal day-to-day variation. Activity data may yet prove valuable in sensor-based disease detection research, however it appears that inclusion of feeding behavior metrics will yield the

most promising performance. Validation of sensors is based against human subjective identification of cattle disease, which is known to be of low sensitivity. Therefore, future research should focus on testing promising algorithms on objective measures (e.g. lung lesions at slaughter) rather than subjective human decisions. It is possible that a multivariate algorithm using activity and feeding behavior would assist in improving BRD detection and animal welfare.

CHAPTER III

DIFFERENTIAL HAPTOGLOBIN RESPONSIVENESS TO A *MANNHEIMIA HAEMOLYTICA* CHALLENGE ALTERED IMMUNOLOGIC, PHYSIOLOGIC, AND BEHAVIOR RESPONSES IN BEEF STEERS

Introduction

As bovine respiratory disease (BRD) remains the greatest threat to the health of beef cattle, research investigating mitigation strategies is of paramount importance. With ever-increasing public scrutiny on the use of antimicrobial technology in agriculture, identification of animals that are naturally more disease resilient would be valuable (König and May, 2018). Disease resilience is the ability to maintain performance even with infection pressure present, and to return rapidly to pre-infection state following exposure (Colditz and Hine, 2016; Putz et al., 2019). Resilient animals could be managed differently, requiring fewer health interventions. Certain physiological responses following immune challenges, such as haptoglobin (HPT) concentration, may represent degree of immunocompetence and disease resilience in cattle. These divergent responses may be related to other biomarkers (such as feeding behavior) which could be used to identify disease-resilient animals. A post-hoc analysis of data previously collected (Kayser et al., 2019a) revealed that HPT response was substantially different in steers challenged with *Mannheimia haemolytica* (MH), despite similar temperature and leukocyte responses. Therefore, the objectives of this study were to determine if differential HPT response to a MH challenge was a bioindicator for other immunologic,

physiologic and behavior response differences, and to determine if HPT responsive phenotype altered metabolite profiles.

Materials and Methods

All animal care and use procedures were in accordance with the guidelines for use of Animals in Agricultural Teaching and Research as approved by the Texas A&M University Institutional Animal Care and Use Committee and Institutional Biosafety Committee (IACUC # 2015-0379; IBC # 2015-068).

Experimental Animals and Design

This study utilized 36 beef steers (initial BW = 386 kg) originating from the Texas A&M University McGregor Research Center (McGregor, TX) and Beef Cattle Systems (College Station, TX) herds. Vaccines for viral respiratory pathogens were subcutaneously administered at 5 mo of age and 3 wk before weaning (Triangle 5; Boehringer Ingelheim, St. Joseph, MO). At 5 mo of age, steers were also vaccinated for clostridial disease (Covexin 8; Merck, Madison, NJ). Steers required to be seronegative for MH (whole-cell agglutination test; Texas Veterinary Medical Diagnostic Laboratory) to qualify for study enrollment, and the steers were confirmed not persistently infected with BVDV. Steers were stratified by source, exit velocity, initial BW and pre-trial ADG, and randomly assigned into treatments in a 2 × 2 factorial arrangement, with dietary live yeast (*S. cerevisiae boulardii* strain CNCM I-1079; Proternative Advantage; Lallemand Animal Nutrition) and MH inoculation (1.2 to 1.4 × 10^9 CFU/10-mL dose) being the two factors. Steers remained in the same 4 pens equipped with GrowSafe feed bunks (GrowSafe Systems Ltd., Calgary, Canada) for the duration of the study. Steers in

2 pens were fed diets containing live yeast, with steers in the other 2 pens fed a control diet. Steers were adapted to the diet, and thereafter DMI and feeding behavior were collected for 28 d prior to MH or PBS inoculation. Each pen housed equal numbers from each inoculation group. Live yeast supplementation did not affect any of the response variables, thus the effects of the LY treatment will not be addressed in this study.

Complete animal management and MH inoculation procedures are described in Kayser et al. (2019a). Briefly, an endoscope was passed through ventral meatus of the left nostril through to the right apical lung bronchus, where a 10-mL dose containing 1.2×10^9 CFU MH was administered followed by a 60-mL PBS flush. The CON steers were likewise inoculated with 70 mL of PBS. Steers were observed twice daily by 2 experienced evaluators for clinical signs of BRD, and clinical illness score (CIS) assigned that included signs of depression, inappetence, and respiratory distress (Scores of 1 to 4 [1 = mild, 2 = moderate, 3 = severe, and 4 = moribund] for each criterion). Steers with a CIS of 3 or greater were removed from the pen, rectal temperature measured, and antimicrobial therapy administered if temperature exceeded 40.5 °C. Steers were returned to home pen following the health evaluations.

Data Collection

Steers were fitted with rumen biothermal boluses (ThermoBolus, Medria, Châteauborg, France) that recorded rumen temperature at 5-min intervals, with a proprietary algorithm used to remove the effects of drinking events. During the study, steers were housed in pens equipped with electronic feed bunks (GrowSafe Systems Ltd.) that continuously recorded individual-animal feed intake and feeding behavior

traits that included frequency and duration of bunk visit (BV) events, non-feeding interval, head down (HD) duration, and time to bunk (TTB). Meal traits were extrapolated from BV traits using the Meal Criterion Calculation software v.1.8.7154.27227 (http://nutritionmodels.tamu.edu/mcc.html). Day-to-day variation in DMI, BV and meal traits was calculated as the root mean square error (RMSE) within animal by regressing each trait on trial day.

Blood samples (7-mL EDTA and 10-mL Vacutainers with no additive; Becton, Dickson and Company, Franklin Lakes, NJ) and BW were collected on days -4, 0 to 3, 5, 7, 10, and 14, relative to MH or PBS inoculation. The EDTA blood samples were analyzed for complete blood count analysis using an automated hemocytometer (ADVIA 120, Siemens Healthcare Diagnostics, Tarrytown, NY; Texas Veterinary Medical Diagnostic Laboratory). Haptoglobin analysis of serum samples were conducted using a commercial ELISA kit (Bovine Haptoglobin ELISA kit, Immunology Consultants Laboratory, Inc., Portland, OR) at the West Texas A&M University Animal Health Laboratory (Canyon, TX). The interassay and intraassay CVs of the haptoglobin assay were16.1 and 11.4%, respectively. Serum cortisol was measured using a solid phase radioimmunoassay (DSL-2100; Diagnostic Systems Labs, Webster, TX). The interassay CV of the cortisol assay was 8.8%. Plasma samples were stored at -80°C for subsequent metabolite analysis at the NMR Center at Montana State University (Bozeman, MT).

Preparation of Serum Samples for NMR Metabolomics Analysis

Prior to NMR analysis, plasma samples were thawed at 4°C and centrifuged at 12,000 x g for 5 min at 4°C to remove cells and other precipitated material. Samples

were diluted 1:1 with MeOH, incubated at -20°C for 30 min, and then centrifuged at 14,000 x g for 10 min. Thereafter, the supernatant was diluted 1:1 with chloroform, and then centrifuged at 10,000 x g for 8 min. The top polar layer was removed, placed in a 1.5-mL polypropylene tube in a vacuum concentrator overnight with no heat, and stored at -80°C until subsequent analysis. Dried extracts were re-suspended in 600 µL of NMR buffer consisting of 25 mM NaH2PO4/Na2HPO4, 0.4 mM imidazole, 0.25 mM 4,4-dimethyl-4-silapentane-1-sulfonic acid (DSS) in 90% H2O/10% D2O, pH 7.0. Following re-suspension, samples were centrifuged at 21,000 rpm for 1 min to pellet insoluble debris, and then transferred to 5 mm NMR tubes for NMR metabolomics analysis.

^{1}H NMR Experiments

NMR spectra of serum metabolite extracts were collected at 300 K using a Bruker 600 MHz AVANCE III solution NMR spectrometer equipped with a 5-mm triple resonance (1H, 13C, 15N) sensitivity enhanced CryoProbe, automatic sample loading system (SampleJet), and Topspin software (Bruker version 3.2). The 1D ^{1}H NMR spectra were recorded using the Bruker gradient-based water suppression "zgesgp" pulse sequence (Ramm Sander et al., 2013; Fuchs et al., 2019), with 256 scans, a ^{1}H spectral window of 7211.539Hz, 64K data points, and a dwell time interval of 69 µs, resulting in a data acquisition time of 4.5 s. Recovery delay times between acquisitions were set to 2.0 s with a pre-scan delay of 10.0 µs resulting in a total recycle delay of 5 s between scans. The 1D ^{1}H NMR spectra were phase- and baseline-corrected manually and pH and chemical shift calibrated using the Chenomx NMR Suite program (version 8.4;

Edmonton, Canada). The Chenomx^{TM} spectral reference library for 600 MHz (1H Larmor Frequency) magnetic field strength NMR was used to identify and quantify metabolites in resulting 1D ^{1}H NMR spectra of metabolite mixtures. Sodium trimethylsilylpropanesulfonate (DSS) was added to each NMR sample at a fixed concentration of 0.25 mM, and served as an internal chemical shift reference and internal calibration of relative ^{1}H signal intensities.

Statistical Analysis

The original study was a randomized complete block design with treatments arranged as a 2 × 2 factorial (LY and MH inoculation serving as the two factors), with individual animal as experimental unit. As the effect of LY supplementation was not significant for any of the response variables, the fixed effect of LY supplementation was not included in models for this study. Retrospectively, it was discovered 9 of the 18 steers that were inoculated with MH produced a minimal HPT response, whereas the other 9 MH-challenged steers produced a substantial HPT response. The classification of MH-challenged steers as HPT responsive or HPT non-responsive was based on a threshold of 20 mg/dL/d area under the curve values using Proc Expand (SAS 9.4, SAS Institute, Cary, NC). Thus, the steers were classified as HPT responsive (RES) or non-responsive (NON) following the MH challenge, and PBS-inoculated controls (CON). Preliminary analysis revealed that initial exit velocity was a significant covariate in the analysis of the feeding behavior data collected for 28 d prior to the MH challenge. Thus, initial exit velocity was included as a fixed effect. For the analyses of DMI and feeding

behavior data collected during the 28-d post MH challenge period, initial exit velocity was no longer a significant covariate and so was not included in the model.

Feeding behavior, rumen temperature, and blood data were analyzed in a repeated measures analysis using the mixed procedure of JMP (JMP, Version 14.0; SAS Institute, Cary, NC) with autoregressive covariance structure. The model included fixed effects of day, classification, and the interaction thereof. If that interaction was significant at $P < 0.05$, means were compared using Student's *t* test. Repeated measures analysis of rumen temperature was completed on an hourly basis for 36 h prior to and following MH inoculation due to the transient nature of the febrile response.

To remove diurnal effect, summary statistics of rumen temperature for the duration of the trial were computed for the third quarter of the day (12:00 to 18:00). Body weights of individual steers were regressed on day of trial using general regression platform (JMP, 14.0) and the regression coefficients used to calculate initial and final BW, and ADG for each period. Statistical significance was declared at $P \leq 0.05$ and tendencies at $P \leq 0.10$.

Metabolite concentrations data obtained from the NMR studies were analyzed using univariate and multivariate statistical analysis to assess whether distinct serum metabolite profiles could be used to discriminate between BRD infected and healthy animals. Metabolite concentrations normalized by sum were further log-transformed to ensure a Gaussian distribution of the data and auto-scaled (i.e. mean centered and divided by the standard deviation) prior to Principal Component Analysis (PCA) which was accomplished using the MetaboAnalyst software 4.0 (Chong et al., 2019; Ste. Anne

de Bellevue, Canada). Univariate analysis was conducted using metabolomic data from day 2 to 5, relative to MH inoculation, to elucidate maximal metabolic effects of experimental BRD. Statistical significance of metabolite level difference was assessed by unpaired parametric t-test with Mann–Whitney and Bonferroni correction.

Results and Discussion

The MH challenge model used in this study did not affect CIS between MH- versus PBS-inoculated steers. Hanzlicek et al. (2010) administered a substantially more potent MH challenge (4×10^{10} CFU/dose) and observed clinical signs of BRD in all MH-challenged calves. Theurer et al. (2013) and Amrine et al. (2014) observed slight to moderate signs of clinical illness in challenged calves that received a nearly 10-fold more concentrated MH dose (1×10^{10} CFU/dose). It is likely that the lack of clinical signs of BRD observed in the current study was due to a less-potent MH inoculum (1.4×10^{9} CFU/dose) administered. Effects of HPT responsive phenotype on hemogram, temperature, and feeding behavior are presented in table 4.

Hematology

The HPT responses of RES, NON and CON steers are presented in Fig. 3. The MH-challenged steers classified as RES exhibited greater ($P < 0.01$) HPT concentration on days 2 through 5 post-MH challenge compared to NON and CON steers. El-Deeb et al. (2020) evaluated acute phase proteins and proinflammatory cytokines in BRD-diagnosed feedlot calves, and concluded that HPT and other acute phase proteins had strong utility as diagnostic and prognostic biomarkers for BRD, as HPT was rapidly elevated in morbid calves, and quickly returned to baseline levels following

antimicrobial therapy. Humblet et al. (2004) observed that serum HPT concentrations were higher in BRD-diagnosed calves that required anti-inflammatory and antimicrobial therapy for recovery from disease compared to BRD-diagnosed calves that recovered with antimicrobial therapy alone. Burciaga-Robles et al. (2009) found that HPT concentration on feedlot arrival was lowest in healthy calves, intermediate for calves that subsequently were treated once, and highest in calves that were subsequently treated multiple times for BRD. Holland et al. (2011) organized feedlot cattle into pens based on a "low," "medium," or "high" arrival HPT concentration and compared performance and morbidity. They reported that increasing arrival HPT concentrations were associated with reduced ADG and DMI during the 2-wk post-arrival period, higher morbidity rates, and higher proportion of calves requiring 3 or more treatments to recovery from BRD. These studies indicate that degree of HPT concentration may be associated with immunocompetence and disease resilience. In contrast, Young et al. (1996) reported limited predictability of HPT; they measured serum HPT in 366 calves on day 0, 40, and 65 relative to feedlot arrival and recorded incidence of BRD 10 d following each blood collection. The HPT measured on day 0 and 40 was not predictive of subsequent disease in the next 10 d, but the HPT measured on day 65 was found to be associated with increased rate of subsequent BRD. Predictive value of HPT was calculated for each collection day and determined to be low, due to the inconsistency with which calves that had elevated HPT actually developed clinical BRD. Further, less than 60% of the calves with elevated HPT from at least one of the collection days had lung lesions at slaughter, which indicates the non-specific nature of the HPT response. Therefore, HPT response

may indicate immunocompetence or disease resilience, but results are inconsistent regarding its predictability of disease.

The MH-challenged steers exhibited greater ($P < 0.01$) neutrophil concentrations than CON steers on day 1 and 2 post-MH challenge, but the magnitude of increase was 35% greater ($P < 0.01$) in RES than NON steers (Fig. 4A). Neutrophils are phagocytic leukocytes, and are the first leukocyte to respond in immune insult or injury. The greater neutrophil concentrations observed in RES steers is logical as neutrophils release chemokines that then recruit acute-phase proteins such as HPT (Malech et al. 2014). The MH-challenged steers exhibited numerically decreased lymphocytes for 7 d following MH inoculation compared to the CON steers. However, the RES steers exhibited decreased ($P < 0.05$) lymphocytes compared to the CON steers on day 1 and 7, while NON were intermediate (Fig. 4B). Although the lymphocyte concentrations were depressed in the RES steers, the values were within standard reference limits (Jones and Allison, 2007), and were within the range reported by Hanzlicek et al. (2010) following an experimentally-induced MH challenge. Burciaga-Robles et al. (2010) found that neutrophil concentrations were greater and lymphocyte concentrations lower in calves exposed to BVDV prior to a MH challenge compared to calves that were MH challenged only, indicating that the severity of the disease insult alters leukogram profile.

Although the HPT classification × day interaction was not significant ($P = 0.11$) for cortisol, there was a classification effect with the RES steers expressing lower ($P < 0.05$) cortisol concentrations than NON and CON steers (Fig. 4C). This lower cortisol response in RES steers may have enabled the increased immunologic response following

the MH challenge as glucocorticoids are known to be anti-inflammatory (Dhabhar, 2008). Indeed, Dong et al. (2018) reported that cortisol inhibited production of pro-inflammatory cytokines in lipopolysaccharide-stimulated macrophages. In some cases, the inflammation associated with an immune response is more damaging to the host than the pathogen the immune response is targeting, indicating modest concentrations of cortisol may help to moderate the immune response following a health challenge (Lawrence and Gilroy, 2007; Jose and Madan, 2016).

There was a HPT classification × day interaction ($P < 0.01$) for platelets, with CON steers having the greatest concentration on day 5 compared to RES and NON steers. We cannot provide plausible explanation for the thrombocytosis that occurred on day 5 in CON steers as no outliers were observed. The RES steers had greater concentrations of platelets on days 7 to 14 than CON or NON steers (Fig. 4D). The thrombocytosis response in RES steers is expected, as RES steers also had greater neutrophil concentrations, which are known to release platelet activating factor (Whiteley et al., 1992). Although the role of platelets role in inflammation is not well-understood, Hanedan et al. (2015) reported numerically higher platelet concentrations in BRD-diagnosed calves, and elevated platelet concentrations have been associated with poor prognosis in human pneumonia patients (Prina et al., 2013).

Feeding Behavior

Feed intake was depressed ($P < 0.01$) in the MH-challenged steers following MH challenge compared to CON steers. However, the magnitude of the depression in DMI was greater in RES vs NON steers, with DMI remaining lower ($P < 0.05$) in RES steer

compared to NON and CON steers until day 8 post-MH challenge (Fig. 5A). The depression in feed intake prior to onset of clinical BRD has been well-documented (Quimby et al., 2001; Wolfger et al., 2015; Kayser et al., 2019a). Though cytokines were not directly measured in this study, it is likely RES steers had greater circulating concentrations of cytokines than NON steers, which have been found to be elevated in BRD-diagnosed calves (Ozkanlar et al., 2012). Pro-inflammatory cytokines are known to induce the anorexia as well as induce synthesis of HPT and other acute phase proteins (McCarthy, 2000; Dantzer, 2004). Higher cytokine concentrations in the RES steers would have contributed to the observed prolonged DMI depression compared to the NON steers. Similarly, Holland et al. (2011) reported that calves with higher concentrations of HPT on feedlot arrival had depressed DMI compared to calves that had lower HPT concentrations. Additionally, BV eating rate in RES steers was decreased ($P < 0.05$) from day 2 through 14 post-MH inoculation compared to CON and NON steers (Fig. 5B). The inappetence brought on by pro-inflammatory cytokines could have also contributed to the decreased BV eating rate in the RES steers in this trial, however BV frequency and duration were not affected by HPT classification for during this period (Fig. 5 C and D).

Febrile Response

The rumen temperature data from hours -8 through 28 relative to MH inoculation are presented in Fig. 6. The MH-challenged steers exhibited a mild, transient fever starting at hour 5 post-MH challenge, however, the RES steers had greater ($P < 0.01$) rumen temperature from 9 to 16 h post-MH challenge than the NON steers.

Temperature is a non-specific response to infection mounted by the innate immune system in order to make the host less hospitable to pathogens, and temperature responses remain the most common diagnostic method for defining BRD cases in commercial operations, as well as in experimental-challenge models (Hanzlicek et al., 2010; Theurer et al., 2013; Timset et al., 2016; Kayser et al., 2019b). Holland et al. (2011) reported that rectal temperature of calves with higher HPT concentrations at feedlot arrival tended to be higher during first treatment of BRD compared to calves with lower HPT concentrations on arrival. Additionally, Burdick et al. (2011) demonstrated that bulls with more excitable temperaments had greater basal cortisol concentrations and lower peak rectal temperature responses to an LPS challenge than bulls with calm temperaments. These findings would suggest that the lower-magnitude febrile response to the MH challenge in NON steers may have been associated with increased basal cortisol concentrations compared to RES steers.

Metabolomics

The ^{1}H NMR spectra of 364 plasma metabolites were evaluated for this study, resulting in the identification and quantification of 32 polar metabolites. Univariate analysis was conducted to examine the effect of HPT classification on relative metabolite concentrations between day 2 to 5 relative to MH challenge; these results are presented in Table 5. The RES steers had higher ($P < 0.05$) concentrations of D-lactic acid and phenylalanine compared to NON and CON steers. Additionally, RES steers had lower ($P < 0.05$) concentrations of allantoin, glucose, glutamine, and L-lactic acid, and

tended (P = 0.06) to have higher 3-hydroxybutyrate concentrations compared to NON and CON steers.

In contrast to the univariate analysis, multivariate analysis using unsupervised 2-dimensional principal component analysis (2D-PCA) was unable to differentiate RES from NON steers, nor combined MH-challenged steers from CON steers, based on distinct serum metabolite profiles. The 2D-PCA score plots of the metabolite analyses between RES and NON are presented for day of MH inoculation (day 0) and the day of peak HPT response (day 3) in Fig. 7. These results demonstrate a lack of separation of metabolite profiles due to HPT phenotype or due to the MH-challenge treatment. The lack of difference in metabolite profiles was likely associated with the fact the experimentally-induced MH challenge was moderate, which induced mild subclinical signs of BRD.

Blakebrough-Hall et al. (2020b) recently conducted a ^{1}H NMR metabolomic analysis of serum samples that were collected on day of diagnosis with BRD (n = 149) and randomly-selected healthy cohorts (n = 148). They identified 28 individual metabolites with different concentrations between BRD-diagnosed and healthy cattle. Five of the metabolites (3-hydroxybutyrate, glucose, glutamine, lactic acid, phenylalanine) identified by Blakebrough-Hall et al. (2020b) were similar those that were found to be different between MH- vs PBS-challenged steers in this study. In contrast to our findings, Blakebrough-Hall et al. (2020b) reported distinctive metabolite profiles differences based on PCA of metabolite profiles between BRD-diagnosed and healthy cohorts. Blakebrough-Hall et al. (2020b) concluded that hydroxybutyrate,

phenylalanine, lactate, tyrosine, citrate and leucine were metabolites of importance in identifying cattle with BRD. Cattle in the Blakebrough-Hall (2020b) study were visually diagnosed with natural BRD based on clinical symptoms, whereas the MH-challenge induced in the present study induced only mild subclinical responses. This could indicate that metabolic profiles of cattle do not measurably deviate with subclinical BRD.

However, metabolomics analysis has successfully distinguished healthy dairy cows from those that would develop postpartum diseases such as mastitis and metritis up to 4 wk prior to parturition with sensitivity of 85% (Hailemariam et al., 2014). Additionally, ^{1}H NMR metabolomic analysis of sera distinguished healthy calves from calves experimentally infected with *Mycobacterium avium* prior to onset of clinical symptoms (De Buck et al., 2014). Buhler et al. (2019) found that auction-sourced calves had differential metabolite profiles compared to calves that were sourced from a single ranch, suggesting that metabolomics could be of use to identify calves at greater risk for BRD. Further research is warranted to further identify metabolites that are responsive to a more severe experimentally-induced challenge to aid in the discovery of biomarkers that would be predictive of the onset of BRD.

Performance and Feeding Behavior of Pre- and Post-Challenge Periods

The effects of HPT classification on performance, DMI and feeding behavior patterns are presented in Tables 6 and 7 for the 28-d prior to and 28-d following the MH challenge, respectively. During the 28-d pre-challenge period, there were no differences in BW, ADG, DMI, or F: G between RES, NON and CON steers. However, during the 28-d post-challenge period, RES steers tended (P = 0.06) to have lower DMI and had 7%

lower ($P < 0.05$) final BW compared to NON and CON steers. This is due to the more substantial immune response mounted by the RES cattle following the MH-challenge. Holland et al. (2011) also observed decreased BW and DMI in calves that had detectable HPT concentrations on feedlot arrival compared to cattle that arrived with undetectable concentrations of HPT.

During the 28-d pre-challenge period, RES steers had 25% longer ($P \leq 0.05$) BV duration and 41% longer ($P \leq 0.01$) HD duration compared with NON steers, with CON steers being intermediate. Further, RES steers tended ($P = 0.07$) to have 21% longer meal duration than NON steers. As a consequence, RES steers had 22% slower ($P < 0.01$) BV eating rate and tended to have slower meal eating rate than NON or CON steers during this 28-d pre-challenge period. During the 28-d post-challenge period, RES steers still exhibited 26% slower ($P < 0.01$) BV eating rate and a tendency for slower meal eating rate compared to NON and CON steers. As BV and meal durations were not different between HPT classifications post-challenge, the slower eating rates in RES steers during this period were due to the tendency for lower DMI in association with the strong febrile and leukocyte responses discussed above. In BRD-diagnosed bulls, Jackson et al. (2016), reported faster BV eating rates 1 to 3 d prior to onset of clinical symptoms. As the RES steers had 22% lower ($P = 0.05$) serum cortisol concentrations than NON steers both prior to and following inoculation, we posit that the relatively slower eating rates exhibited both prior to and following the MH challenge suggest that the RES steers may have had more calm temperaments than NON steers. Cattle with calm temperaments have been reported to have lower basal cortisol concentrations than

cattle with excitable temperaments (Curley et al., 2006; Burdick et al., 2011), and have greater DMI, longer BV duration, and slower eating rate (Smith et al., 2017; Olson et al., 2019). Although RES steers had lower serum cortisol and BV eating rate prior to inoculation which suggests a calmer temperament, exit velocity was unexpectedly not affected by HPT classification in this study (data not shown).

In the 28-d pre-challenge period, RES steers exhibited 32% greater ($P < 0.04$) day-to-day variation in both BV frequency and HD duration compared to NON steers, with CON steers being intermediate. Likewise, during the 28-d post-challenge period, RES steers expressed 30% and 42% greater ($P < 0.01$) day-to-day variations in BV duration and HD duration, respectively, and tended ($P < 0.10$) to express greater day-to-day variations in DMI and meal duration compared to NON and CON steers. In aggregate, RES steers exhibited greater daily variance in feeding behavior patterns than NON steers both prior to and following the MH challenge.

Recent research has investigated the utility of day-to-day variation in feed intake and feeding duration as potential indicators of disease resiliency in pigs (Putz et al., 2019). In their natural-disease challenge model, infection pressure was maintained at the feeding facility by continually introducing diseased pigs from other facilities, resulting in 26% overall mortality rate. Daily variances in both DMI and feeding duration were found to be moderately heritable (0.21 – 0.26), and had a positive genetic correlation (0.37 to 0.62) to mortality and morbidity rates in pigs. Putz et al. (2019) concluded that decreased day-to-day variance may be indicative of resilience and presents a phenotype for selection of animals with improved disease resilience. Further, less day-to-day

variance in daily milk production has been associated with improved health and longevity in dairy cows (Elgersma et al., 2018). Dikmen and Mateescu (2019) determined decreased day-to-day variance in vaginal temperature was positively associated with heat tolerance, therefore more temperature-consistent heifers were deemed to be more heat resilient. Gijzel et al. (2017) concluded greater day-to-day variations in self-evaluated physical, mental, and social health metrics were associated with decreased disease resilience in geriatric human patients. Collectively, these results suggest the greater day-to-day variance in feeding behavior patterns of RES steers may be associated with decreased disease resiliency, and could in part explain the hyper immune reaction to a subclinical health challenge with no apparent benefit in reduction of disease severity or recovery when compared to NON seers.

Furthermore, basal cortisol concentration has been shown to be associated with disease resilience. Piglet survivability was shown to be positively correlated with size of adrenals and serum cortisol concentration (Leenhouwers et al., 2002) and rats expressing greater corticosterone concentrations were shown to be more tolerant of a heat stress challenge (Michel et al., 2007). Indeed, Richeson et al. (2016) reported decreased HPT response in animals that had been administered the glucocorticoid dexamethasone, frequently used to experimentally mimic natural cortisol. Mormede et al. (2011) propose that this improved disease resilience observed with higher basal cortisol concentrations is due to animals that have increased basal HPA axis activity adapt better to stressors and thus recover more quickly following a perturbation. This would suggest that the greater basal cortisol seen in NON steers could be a marker of resilience. More research is

needed to more fully explore traits for utility in identifying resilient animals, and to determine if our preliminary results indicating that animals exhibiting high levels of variance in behavior or production, and low basal cortisol, are in fact less resilient to disease.

Conclusion

Identification of disease resilient animals could result in decreased morbidity due to BRD in feedlots. Results of the current study indicate that although the MH-challenged steers exhibited immunological, physiological, and behavioral responses to the experimentally-induced challenge, these responses were more pronounced in the MH-challenged steers that mounted a greater HPT response. Steers that were HPT responsive displayed increased neutrophils, fever, and day-to-day variation in feeding behavior, and depressed cortisol, DMI, and eating rate. However, the disease challenge may not have been severe enough to illicit changes in metabolite profiles. We propose that these HPT-responsive steers were less disease resilient, as literature has shown association in low basal cortisol concentration and high day-to-day variation in feeding behavior as indicators of lower disease resilience. Therefore, HPT response and feeding behavior may be related, and may be indicator traits of disease resilience. Further research is needed to investigate how temperament, basal cortisol, and acute phase protein response relate to disease resilience.

CHAPTER IV

EFFECTS OF EXPERIMENTALLY-INDUCED HYPOXEMIA ON HEMODYNAMICS AND BLOOD GASES, AND THE PERFORMANCE OF PULSE OXIMETERS IN CATTLE

Introduction

New methods of detecting bovine respiratory disease (BRD) earlier enter the market regularly in an attempt to improve disease recognition in a species that has evolved to mask symptoms of injury and illness. Current detection relies on visual appraisal of cattle, and has been shown to be only moderately accurate (Timset et al., 2016). Since arterial oxyhemoglobin saturation (SO_2) decreases with respiratory disease in cattle (Ozkanlar et al., 2012; Šoltésová et al., 2015), it is possible that a related non-invasive alternative, peripheral oxygen saturation (SpO_2), could be a biomarker indicative of BRD onset. Invasive SO_2 (determined from a blood sample) is a direct measure of the oxyhemoglobin present, while non-invasive SpO_2 (measured by a pulse oximeter) is an estimation of oxyhemoglobin proportion based on different light reflectance patterns in peripheral tissues. If SpO_2 could serve as a BRD biomarker, it could provide utility as a diagnostic and prognostic tool for non-invasive measure of oxygen saturation status in animals suspected of BRD. Non-invasive knowledge of oxygen status would give feedlot health managers another option for confirmation of BRD positive cases, as well as administer antimicrobial interventions more judiciously. First, changes in hemodynamics such as heart rate (HR) and blood pressure (BP), as well

as accuracy of SpO_2, needs to be understood at sub-normal levels of SO_2. Therefore, our objective for this study was to induce hypoxia in a graded manner in anesthetized cattle to investigate both the hemodynamics associated with hypoxemia, and the performance of clinical SpO_2 monitors against a benchtop blood gas analyzer.

Materials and Methods

All animal care and use procedures were in accordance with the guidelines for use of Animals in Agricultural Teaching and Research as approved by the Texas A&M University Institutional Animal Care and Use Committee (IACUC # 2018-0108).

Experimental Animals

Eight Holstein steers (BW = 127 ± 10 kg) were observed to be in good health for 3 wk prior to experimental hypoxia. Calves were halter trained 2 wk prior to experimental hypoxia to aid in ease-of-handling and to decrease stress experienced by the animals. Calves were food-restricted for 24 h and water-restricted for 12 h prior to the procedure to minimize risk of aspiration pneumonia while under anesthesia. The experiment spanned 5 d: one calf was evaluated day 1, another calf on day 2, and two calves each on days 3 to 5.

Anesthesia Induction and Data Collection

On arrival at the Texas A&M Large Animal Hospital (College Station, TX), steers were weighed and an electrocardiogram (ECG) band (Polar; Bethpage, NY) was fixed around the girth just behind the elbow. Next, a jugular catheter (16g Mila, Mila International, Inc., Erlanger, KY) was placed for administration of medication and Lactated Ringers Solution (5 mL/kg BW/ h) before and during anesthesia. Calves were

premedicated with IV midazolam (0.1 – 0.2 mg/kg), then general anesthesia was induced using IV ketamine (4 mg/kg). Once anesthetized, calves were rolled on a cart from the processing area to the surgery suite, where they were placed on a padded table in lateral recumbency. Next, calves were intubated with a cuffed endotracheal tube and connected to a patient monitoring anesthesia machine (Datascope Passport 2, Mindray DS, Mahwah, NJ). The anesthesia machine was equipped with ECG, indirect BP, an esophageal temperature probe, and a gas analyzer with capacity to analyze fraction of inspiratory of isoflurane, inspiratory fraction of oxygen (FiO_2), expiratory concentration of isoflurane, and capnometry. Anesthesia was maintained with inhaled isoflurane to maintain the end tidal anesthetic between 1 – 1.5 minimum alveolar concentration and IV butorphanol (0.05 – 0.1 mg/kg). Body temperature was maintained with warming blankets (HotDog, Eden Prairie, MN). Steers were instrumented with two additional pulse oximeters (Animal Clip Oximeter Pod, AD Instruments, Colorado Springs, CO; Masimo SET, Masimo Corp., Irvine, CA), placed on the tongue. In order to avoid falsely-low rates due to pressure-induced ineffective perfusion, the clips were moved and the tongue massaged every 10 min. The Animal Clip Oximeter Pod (AD Instruments) was connected with an invasive BP monitor transducer to an additional bedside patient monitor (PowerLab, AD Instruments). A veterinary surgeon then clipped and sterilized the medial tibia area, then placed a catheter (18g Mila, Mila International, Inc.) in the saphenous artery for serial arterial collection. Arterial blood samples (1 mL) were collected using specialized blood gas syringes (25 g Micro Heparin, CareFusion,

San Diego, CA) and were analyzed by a co-oximeter (pHOx, Nova Biomedical, Waltham, MA) for lactate, pH, and SaO_2 within 15 minutes of collection.

Stepwise Desaturation

Several baseline measurements were collected from each calf at elevated O_2 administration (> 22% FiO_2). SpO_2 readings were recorded from the two devices placed on the tongue simultaneous to blood sample collection. Once the second baseline sample value was returned, oxygen saturation was manipulated by decreasing FiO_2 and increasing supplied nitrogen through an oxygen blender (Air-Oxygen Mixer, Sechrist, Anaheim, CA). Delicate precision was impossible in the manipulations, so changes were made slowly and SpO_2 monitored continuously to ensure an unsafe hypoxic level was never sustained. On the third calf, it was discovered that desaturation could be achieved in a much more stable manner if increasing concentration of bottled air was supplied, rather than pure nitrogen. Thus, bottled air was used to induce hypoxia on calves 3 – 8.

The first hypoxia grade was achieved by slowly reducing the FiO_2 to 19 – 21%. After 5 min at this level to allow time for tissues to desaturate, several blood collections were made per calf. If the calf remained stable, FiO_2 was further reduced to 17 – 18%, maintained for 5 min, and several more collections made. Number of collections at each grade depended on patient stability and total time under anesthesia, as calves were not permitted to be anesthetized longer than 2 h. If the calf remained stable, the final FiO_2 step of 15 – 16% was reached and maintained for just 2 min prior to blood collection. The calves were then returned to hyperoxic FiO_2 levels (≥ 22%) and maintained until full resaturation was indicated by the bedside SpO_2 monitors. If time permitted, an

additional round of FiO_2 reduction occurred for continued sampling. If at any point the anesthesiologist felt that the calf was critically hypoxemic, FiO_2 was immediately increased to 100% until SpO_2 monitors reported ≥ 98%.

For recovery, calves were maintained at hyperoxic FiO_2 levels for 5 – 10 min while isoflurane and butorphanol administration were ceased. The catheters and all instruments were removed, and extubation occurred when the calf was breathing independently and had total control of the airway and tongue. Following, calves were transported on a rolling cart to a quiet room for recovery. All calves tolerated the procedure well, and were alert and responsive within 45 min of extubation.

Results and Discussion

Blood Oxygen Saturation

As expected, SO_2 and SpO_2 declined ($P < 0.01$) with reductions in FiO_2 (Table 8). The SpO_2 monitors reflected changes in delivered oxygen concentration within 30 s, however, SO_2 appeared to have a 10 min latency in reflecting blood oxygenation changes. This latent response time is likely indicative of the time required for alveolar washout to fully occur. Alveolar washout is the process of gas in the small alveolae of the lung being fully replaced by whatever the animal is breathing, such the partial pressure of gas in the alveolae is the same as the partial pressure of the inspired gas (Tawhai and Hunter, 2001). Re-saturation with oxygen occurred much more quickly than desaturation, reflecting the biological reluctance for hemoglobin to release oxygen under hypoxic conditions, and perhaps some evolutionary adaptations to conserve oxygenation. One such adaptation is hypoxic pulmonary vasoconstriction, the

physiological mechanism by which small arteries in the lung constrict, diverting blood to the more oxygenated areas of the lung (Dunham-Snary et al., 2017).

Co-oximeter-derived SO_2 and SpO_2 from either pulse oximeter were not well correlated ($r^2 = 0.20 – 0.34$), though the means at each FiO_2 grade appear in agreement. While rate of change and accuracy of SpO_2 may have differed from SO_2, both parameters reflected changes in FiO_2. In lamb fetuses exposed to hypoxia by maternal artery occlusion, SO_2 declined to around 30% from the baseline of 69%, but rapidly recovered upon removal of the occlusion (Newman et al. 2000). Šoltésová et al. (2015) reported arterial SO_2 in calves measured upon clinic arrival was reduced with increasing severity of respiratory disease. SpO_2 declined to 84% in senior men after 7 min of exposure to hypoxia (Shatilo et al., 2008). Additionally, Povea et al. (2005) reported SpO_2 declined to a surprising 67% in men after 5 min of exercise in hypoxic conditions (11.5% FiO_2). The pulse oximeter in that study was placed on the ear lobe, so it is possible that the pressure generated by the clip was affecting perfusion, resulting in falsely-low readings after a period of exercise. Alternatively, pulse oximeters may also exhibit decreased accuracy at lower SO_2 levels. Such bias was reported by Coghe et al. (1999); their pulse oximeter had a negative bias at low SO_2, but they concluded it was of little importance as SpO_2 readings < 80% have little clinical relevance. Baruch et al. (2019) observed changes in SpO_2 following a combine viral-bacterial respiratory challenge in Holstein calves, during which a subset of calves was serially slaughtered. They reported SpO_2 was significantly correlated with degree of lung consolidation, and SpO_2 levels between 96 – 100% were associated with 12% lung lesions. SpO_2 levels

between 93 – 95% were associated with 22% lung lesions. This indicates that SpO_2 probably does not change early in the disease process to have utility in preclinical disease detection, but can certainly aid in diagnosis from other disease etiologies, and in prognosis of respiratory disease.

Hemodynamics

Increases in HR and BP are expected under hypoxic conditions, as the body attempts to compensate for decreased oxygenated hemoglobin by moving more blood at a faster rate to the tissues. In this study, HR was increased ($P < 0.01$) at FiO_2 levels < 19%, and BP increased ($P < 0.01$) at FiO_2 concentrations < 22% (Table 8). The two pulse oximeters in the present study, which measure HR via optical reflectance, agreed well ($r^2 = 0.91 – 0.96$) with the ECG band (Table 9). In human athletes subjected to hypoxic conditions at 11.5% FiO_2, HR significantly increased from normoxia conditions (Povea et al., 2005). Senior men experienced increasing HR and BP after just one min in hypoxic conditions, and those metrics continued to increase throughout the full 7-min experiment (Shatilo et al., 2008). Engelen et al. (1996) also reported a rapid, linear rise in HR, followed by a brief plateau and a second rapid rise in HR of human athletes exercising with 12% and 15% FiO_2. Contrastingly, Salman et al. (2005) reported declining HR in anesthetized rabbits undergoing experimental graded hypoxia at FiO_2 < 10%, and no changes in BP.

Blood Chemistry

In the present study, lactate was unaffected by graded hypoxia (Table 8). Typically with hypoxemic mammals, lactate is expected to increase as anaerobic

conditions result in lactate as an end-product of glycolysis (Andersen et al., 2013). Lactate increased 6-fold in lamb fetuses exposed to hypoxia (Newman et al. 2000). Ellis et al. (2013) reported substantial increases in arterial blood lactate with increasing percentage of lung consolidation in calves suffering from BRSV. Many others have also reported increasing lactate concentrations with increasing BRD severity in calves (Coghe et al., 2000; Šoltésová et al., 2015; Oosthuysen et al., 2017). Though pH was also unchanged in the present study with graded hypoxia (Table 8), pH was reduced in hypoxic fetal lambs (Newman et al., 2000), and in calves diagnosed with BRD (Gunes and Atalan, 2006; Ozkanlar et al., 2012). Reduction in pH with respiratory disease is caused by an increase in H+ ions to counteract the accumulating CO_2 in the blood and lungs; this phenomenon is known as respiratory acidosis, and is associated with poor prognosis (Ucgun et al., 2006). Calves in the current study may not have been exposed to hypoxia for a long enough duration to induce respiratory acidosis.

Conclusion

Results from this study revealed that SpO_2 sensors were able to detect reductions in SO_2 induced by experimentally-induced hypoxia in cattle, indicating that SpO_2 sensors may have utility in BRD diagnosis and/or prognosis. Additionally, other metrics (e.g., HR, BP, lactate) evaluated in this study may also be suitable biomarkers for the diagnosis and/or prognosis of BRD in beef cattle. The use of pulse oximeter sensors would provide a valuable tool to help differentiate BRD from other diseases or to evaluate the severity of BRD cases to facilitate more judicious administration of antimicrobial usage.

CHAPTER V

PHYSIOLOGICAL, BEHAVIORAL, AND IMMUNOLOGICAL RESPONSES TO AN EXPERIMENTALLY-INDUCED RESPIRATORY DISEASE CHALLENGE IN GROWING STEERS

Introduction

As bovine respiratory disease (BRD) remains the foremost illness affecting beef cattle, different methods of detection are clearly required. Due to prey instincts, cattle can effectively mask symptoms of disease and injury, resulting in low sensitivity of detection by feedlot personnel (Timset et al., 2016). Remote sensors enable continuous allow for monitoring of a behavior or physiological trait without interference of human presence, thereby improving detection of deviations from an animal's baseline. Some remote sensor technologies that have been shown to detect BRD prior to visual diagnosis include feeding behavior (Quimby et al., 2001), step count (Pillen et al., 2016), and temperature (Timset et al., 2011). Studies in natural BRD cases (Gunes and Atalan, 2006; Šoltésová et al., 2015) and experimental BRD cases (Woolums et al., 1999; Ellis et al., 2013) have shown arterial blood gas metrics such as oxygen saturation (SO_2) and partial pressure of oxygen (PO_2) decline in clinically morbid cattle, indicating measurement of blood oxygen saturation may have potential for sensor development. Pulse oximetry provides a remote and non-invasive alternative to arterial blood gas testing by measurement of peripheral oxygen saturation (SpO_2). To our knowledge, there have been no studies investigating the progression of respiratory disease in feedlot cattle

using a continuously-recording pulse oximeter, and only limited research in beef cattle involving serial arterial blood collection following a respiratory disease challenge. Therefore, in order to aid in the discovery of biomarkers which can be used to identify cattle with BRD and to better understand the physiological changes associated with the early respiratory disease processes, our objective was to evaluate changes in physiological (blood oxygen saturation, rumen temperature), behavioral (feeding behavior, rumination) and immunological (hemogram, plasma protein) responses to an experimentally-induced respiratory disease challenge in growing beef steers.

Material and Methods

All animal care and use procedures were in accordance with the guidelines for use of Animals in Agricultural Teaching and Research as approved by the Texas A&M University Institutional Animal Care and Use Committee, (IACUC # 2018-0108) as well as the Texas A&M University Institutional Biosafety Committee (IBC # 2018-153).

Experimental Animals and Design

Twenty-four *Bos taurus* steers (initial BW = 264 kg) that were sourced from the Texas A&M University McGregor Research Center herd were used in this study. The steers were vaccinated subcutaneously for clostridial diseases (Covexin 8; Merck, Madison, NJ) at 2 and 5 mo of age. At 5 mo of age, steers were administered a killed vaccine subcutaneously with protection against respiratory viral pathogens (Triangle; Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO). At weaning, steers received a modified live vaccination subcutaneously with protection against respiratory viral pathogens (Titanium 5; Elanco Animal Health; Greenfield, IN). Blood samples

collected at 5 mo of age and 28 d post weaning were analyzed to determine serum titers for bovine herpes virus-1 (BHV-1). To qualify for study enrollment, heifers had to be seropositive (> 4 titer level) for BHV-1 as determined by a neutralization test (Texas A&M Veterinary Medical Diagnostic Laboratory, Amarillo, TX). The average of BHV-1 serum titers was 3 at 5 mo of age, and 27.3 at 1 mo post-weaning, indicating immunologic response following vaccination. All steers were observed as healthy and were acclimated to the research facility for 28 d prior to the health challenge.

Steers were stratified by initial BW, exit velocity, and BHV-1 titer dilution, and randomly assigned to 1 of 2 treatments that included inoculation with phosphate buffered saline (PBS; control steers) or with BHV-1 on day -3 and *Mannheimia haemolytica* (MH; VB-challenged steers) on day 0. Steers were housed in 4 pens equipped with electronic feed bunks (GrowSafe Systems Ltd., Calgary, Canada), with two pens of challenge steers (n = 8 per pen) and two pens of PBS control steers (n = 4 per pen). Throughout the study, steers were fed ad libitum a grower diet consisting of 36.5% dry-rolled corn, 26% dried distiller's grains, 30% chopped alfalfa, 5% molasses, and 2.5% mineral-vitamin premix on a DM basis.

Inoculum Preparation and Procedure

Viral inoculum was prepared the day prior to administration, and originated from the Cooper strain of BHV-1 (USDA Center for Veterinary Biologics; Ames, IA). Briefly, the virus was inoculated onto roller bottles with bovine kidney cells grown in Eagles minimum essential medium with Earles basic salt and 10% fetal bovine serum. The inoculum was grown in a 5% CO2 incubator until there was approximately 90%

cytopathic effect observed. Following, the virus was harvested by freeze/thaw, centrifuged at 500 × g for 20 min, harvested and stored at −70 °C. Finally, the viral inoculum was suspended in PBS to achieve the desired concentration of 1×10^8 plaque forming units (PFU) per mL. Pre-loaded syringes with the proper dose per naris per animal were placed in an iced cooler for transport to the inoculation site. On day -3 of the trial, 1 mL of either BHV-1 or PBS was aerosolized into each naris with a 3-mL syringe fitted with an atomization device (MAD Nasal; Teleflex, Morrisville, NC). The PBS control steers were processed first to avoid cross-contamination. Following viral inoculation of the VB-challenged steers, the entire processing facility was disinfected with sprayed dilute bleach solution.

The MH inoculum was prepared as described by Mosier et al. (1995) early the morning of administration (day 0). The MH was grown on trypticase soy agar containing 5% sheep blood in a CO_2 incubator for 18 h. Individual colonies were then suspended in brain-heart infusion broth and incubated at 37 °C with aeration for 18 h. Following, the bacteria were pelleted by centrifugation at 3,000 × g for 15 min at 4 °C and washed with PBS twice. After the second wash, bacteria were suspended in PBS. The anticipated final concentration of 1×10^9 CFU per mL was estimated based on optical density. Cultured plate counts of the inoculum later confirmed final concentration at 2.15×10^9 CFU per mL. The inoculum was aliquoted into syringes in 10 mL doses and were placed in a Styrofoam cooler on ice for transport to the inoculation site (18 km). Syringes containing 10 mL of PBS for control steers were prepared at the same time. Syringes containing 60 mL of PBS from the same lot number were prepared to flush the inoculum

through the lavage tube. On day 0 of the trial, steers were restrained in a hydraulic squeeze chute with a head sweep then endoscopically administered 70 mL of PBS, or 10 mL of MH inoculum followed by 60 mL of PBS flush. Again, PBS control steers were processed first to prevent cross-contamination. A 1 m endoscope was passed though the nares into the trachea to the right cranial lung lobe where the solutions were injected through a sterile bronchoalveolar lavage tube into the tracheal bifurcation. Inoculation procedures were not observed to have any adverse side-effects, and the steers tolerated the procedure well.

Data Collection and Body-Mounted Sensors

Steers were fitted with biothermal boluses (SmaXtec, Graz, Austria) and rumination collars (SCR, Madison, WI) 14 d prior to VB or PBS inoculation to allow for acclimation and baseline data collection. Biothermal boluses were inserted into the reticulorumen of steers using a lubricated balling gun passed over the tongue to the entrance of the esophagus. The bolus device continuously-recorded temperature and an activity score at 15-min intervals, which were transmitted wirelessly via a base station to a data acquisition computer. The activity score, based on a 3D accelerometer, provided a metric of general movement (www.smaxtec.com). The rumination collars were placed around the neck of the calves with the accelerometer-based sensor on the left dorsal side of the neck, with data transmitted wirelessly via a base station to the data acquisition computer at 2-h intervals.

On the day prior to inoculation with BHV-1, a pulse oximeter (Maxim Integrated, San Jose, CA) was placed on each ear of all steers to collect SpO_2 and HR

data. The sensor was equipped with a red and an infrared (IR) LED and a photodiode to measure light reflectance from oxygenated hemoglobin in arterial blood. These data were used to calculate R ([RedAC / RedDC] / [IRAC / IR DC]), which was then processed to determine SpO_2 and HR data. The sensor was equipped with an internal clock, and was programmed to record for 40 s at 40-min intervals from 17:00 to 07:00, resulting in 15 40-s data collection periods. A built-in accelerometer also collected data during this period to provide quality audits of the data. Algorithms were developed to discard data when the accelerometer readings indicated the animal was active, as reflectance data are susceptible to motion artifact. The rostral aspect of the ear pinna was chosen as the attachment site, instead of the ventral aspect of the tail head as in some monitors, in order for future products to fit seamlessly into existing feedlot processing workflow. After many rounds of development, the design of this monitor placed the SpO_2 sensor placed between two 1 mm diameter stainless steel posts in the ear. The sensor was connected by a 10 cm wire extension to a main board and battery housing, which was mounted to a traditional plastic ear tag pierced through the ear approximately 6 cm away from the SpO_2 sensor attachment site. Data was stored on a local SD card until sensor removal on day 12 relative to MH inoculation.

Blood was collected and BW was recorded on d -3, -1, 0, 2, 3, 5, 7, 10, 14, 21, and 36 relative to MH or PBS inoculation. This study focused on the immediate post-challenge effects based on the data collected from day -3 through 14. Arterial blood was collected serially by direct arteriopuncture of the caudal auricular artery using a specialized syringe (25-gauge Micro Heparin, CareFusion, San Diego, CA) for blood gas

analysis as validation of the SpO_2 data. A benchtop blood gas analyzer (Prime+, Nova Biomedical, Waltham, MA) and a handheld blood gas analyzer (CG4+ iStat, Abbott, Princeton, NJ) were used to determine blood oxygen saturation. Both devices report partial pressure of carbon dioxide (PCO_2), PO_2, SO_2, pH, and lactate. The time between arterial collection and sample analysis was < 10 min. Venous blood was collected serially by direct venipuncture of the jugular vein using an 18-gauge needle into two vacutainers, one without additive for serum haptoglobin analysis and one with EDTA for blood gas and complete blood count (CBC) analysis. Non-clotted samples were promptly transported to the Texas A&M Veterinary Medical Diagnostic Laboratory for CBC analysis using an automated hemocytometer (ADVIA 120, Siemens Healthcare Diagnostics, Tarrytown, NY). Whole blood was spun at 3000 rpm for 10 min promptly after collection, then serum was stored at -20°C until analyzed for haptoglobin using a commercial ELISA plate (Bovine Haptoglobin ELISA kit, Immunology Consultants Laboratory, Inc., Portland, OR). The interassay and intraassay CV were 1.38% and 3.66%, respectively.

Feeding behavior and intake data were captured by the GrowSafe system, beginning 28 d prior to challenge to allow for acclimation and baseline data collection. The GrowSafe 6000 software processes individual animal feed intake data nightly, and daily data for a pen is discarded if the assigned feed disappearance is less than 95%. No days for this study were deleted, as data was of good quality and the system did not experience malfunction. Daily data begins at midnight and ends at 2359 h. A bunk visit (BV) begins when an animals' electronic identification tag is detected by the antenna

that surrounds the top perimeter of a bunk, and ends when an animal departs the bunk. Neck bars prevent more than one animal visiting the bunk at a time.

Clinical Illness Scoring

Steers were evaluated once daily by an experienced evaluator for symptoms of BRD. The clinical illness score (CIS) included depression, reactivity, dyspnea, inappetence, and nasal discharge, with evaluators assigning scores of 1 to 4 (1 = mild, 2 = moderate, 3 = severe, and 4 = moribund) for each criterion. Observer was not blinded to treatments or timeline, but CIS was used for treatment administration decisions under veterinarian guidance. Steers with a CIS of 2 or greater were removed from the pen, rectal temperature measured, and antimicrobial therapy administered if temperature exceeded 40.5 °C. Steers were returned to home pen following the health evaluation. For first treatment, steers received tulathromycin (Draxxin, Zoetis, Parsippany, NJ) at 2.5 mg/kg BW. Following a 7-d post-treatment interval, steers received ceftiofur hydrochloride (Excenel, Zoetis, Parsippany, NJ) administered at 2.2 mg/kg BW daily for 3 days if a second treatment was needed. If the rumen temperature bolus reported a temperature exceeding 42.5 °C, steers were administered transdermal flunixin (Banamine, Merck Animal Health, Madison, NJ) along the dorsal midline at 3.3 mg/kg BW according to veterinarian guidance.

Statistical Analysis

For this study, animal served as experimental unit. Rumen temperature data were divided into quarters of the day (e.g. 0000 – 0600 h = Quarter 1, 0601-1200 h = Quarter 2, etc.) in order to remove the effect of diurnal patterns, as described by Kayser et al.

(2020). Data within daily quarter was then averaged and analyzed across day. Rumen temperature, rumination, activity, DMI, feeding behavior, haptoglobin, and hemogram data were analyzed in a repeated measures model using the MIXED procedure of SAS with autoregressive covariance structure (version 9.4, SAS Institute Inc., Cary, NC). The model included fixed effects of day, inoculation, the interaction thereof, and calf within treatment as a random effect. When a significant treatment × day interaction was detected for a variable, the SLICE output option was used to identify within-day treatment differences.

The performance, rumen temperature, rumination, activity, DMI, and feeding behavior data from study day 21 to 77 were analyzed to evaluate residual effects of VB challenge, with treatment as fixed effect and calf within treatment as a random effect. Body weights of individual steers were regressed on day of trial using general regression platform (JMP, 14.0) and the regression coefficients used to calculate initial and final BW, and ADG for the post-challenge period. Statistical significance was declared at $P \leq 0.05$ and tendencies at $P \leq 0.10$.

Results and Discussion

All steers were clinically normal (CIS = 0) for the 3-d post BHV-1 inoculation. Following the MH challenge, all VB-challenged steers exhibited mild to moderately severe clinical signs within 8 h, which persisted through the 14 d thereafter. Clinical illness responses observed in this study were consistent with the literature. Theurer et al. (2013) administered a MH challenge at half the concentration (1×10^{10} CFU/dose) of the present study and reported mild to moderate clinical illness, while Hanzlicek et al.

(2010) administered a MH challenge at twice the concentration (4×10^{10} CFU/dose) of the present study and observed gross clinical BRD in all calves. Quiescent behavior, including lack of feed ingestion, in times of sickness allows for conservation of energy for immunological responses (Dantzer, 2004). Compounding the effects of the health challenge, a weather event occurred the day following MH challenge that included rain, wind, and fluctuating ambient temperatures (Fig. 8). Statistical analysis was not completed for CIS as the evaluator was not blinded to treatments or timeline. One VB-challenged steer died day 7 relative to MH inoculation; his CIS the day prior had only been 1.5 but he had been administered antimicrobial treatment 5 d prior, on day 2 post-MH. Additionally, 2 VB-challenged steers had CIS of 3.5 on days 9 and 16 post-MH inoculation and were humanely euthanized according to protocol.

Feeding Behavior, Body Weight, and Rumination

The effects of treatment on DMI, BV frequency, BW, and rumination from days -3 to 14 are presented in Table 10. There was a treatment × day interaction for DMI, with VB-challenged steers having reduced ($P < 0.01$) DMI from the day of MH inoculation through day 14 (Fig. 9A). Further, VB-challenged steers visited the feed bunk fewer ($P < 0.04$) times post MH inoculation compared to PBS steers (Fig. 9B). This reduction in feeding behavior, in conjunction with energetic cost of febrile response, resulted in reduced ($P < 0.02$) BW in the VB-challenged steers from day 2 through 14 following MH inoculation (Fig. 9C). The reduction in DMI in association with morbidity is well-documented (Quimby et al., 2001; Wolfger et al., 2015; Kayser et al., 2019a) and is associated with an increase in proinflammatory cytokine concentration.

Though cytokines were not directly measured in this study, cytokines have been shown to be elevated in BRD-diagnosed calves and are known to induce anorexia and desire to rest (McCarthy, 2000; Dantzer, 2004; Ozkanlar et al., 2012). Likewise, Belaid et al. (2019a) observed reduced BV frequency the day prior to visual BRD detection in veal calves. In a combined BHV-1/MH challenge study, Kayser et al. (2019a) reported a reduction in DMI the day of MH inoculation and the 4-d following MH challenge. In a MH-only challenge study, Kayser et al. (2019b) reported a slight reduction of BV frequency in challenged steers the day of MH inoculation and reduced DMI for the 3 days following. The concentration of MH used in the current study (2.15×10^{10} CFU/dose) was higher than that administered in the two aforementioned studies, and thus explains the more persistent reduction in intake and feeding behavior.

Rumination rate was reduced ($P < 0.04$) in VB-challenged steers on days 1 and 7 post MH inoculation, and tended to be reduced ($P = 0.07$) in PBS steers on day 3 (data not shown). Biological relevance of the rumination rate results was not apparent and the trends of the responses did not follow the observed changes in DMI. In contrast, Marchesini et al. (2018) reported reductions in duration of rumination up to 6 d prior to observed clinical diagnosis and treatment for BRD in feedlot cattle using the same collar-attached accelerometers (SCR) used in the present study. It is unclear why similar deviations in rumination were not observed in the current study. Kayser et al. (2020) investigated utility of monitoring rumination measured from the Medria collar (Châteauborg, France) to detect clinical BRD following inoculation with MH, and found that it had low sensitivity (12.5%).

Temperature and Activity

The effects of treatment on rumen and rectal temperature, and activity from days -3 to 14 relative to MH or PBS inoculation are presented in Table 10. Rectal temperature of VB-challenged steers was elevated days 2 through 14 compared to PBS steers (Fig 10A). Preliminary analysis revealed that rumen temperature during the third quarter (1200 to 1800 h) was most responsive to the VB challenge, thus the results reflect that interval. Rumen temperature rapidly increased ($P < 0.01$) in VB-challenged steers within several hours following the MH inoculation, and remained elevated ($P < 0.01$) until day 6 post MH inoculation (Fig 10B). Rumen temperature of PBS steers was slightly elevated from their baseline on days 1 and 2 post PBS challenge, which likely reflects the stress associated with collecting serial blood samples. Additionally, rumen temperature was briefly elevated in PBS steers on day 8, which likely was associated with an increase in ambient temperature (see Fig 8.). Interestingly, mean rumen temperatures across all trial days were on average 0.4°C higher than the corresponding rectal temperature. This is important to consider for proper evaluation of herd health, particularly for antimicrobial treatment decisions using a rumen or rectal temperature threshold in the protocol. The same relationship of rumen temperature being slightly higher (0.6° C) than rectal temperature was reported by Timset et al. (2011). Additionally, Timset et al. (2011) reported rumen hyperthermia events occurred 24 to 48 h prior to visual clinical symptoms such as nasal discharge and cough. Temperature is by far the most widely used metric of disease diagnosis, as it is one of the few objective measurements widely available for low cost. Febrile response following experimental

BRD challenges has been reported extensively (Hanzlicek et al., 2010; Theurer et al., 2013; Timset et al., 2016; Kayser et al., 2019a), the duration of which appears to be related to the severity of challenge. Kayser et al. (2019a) reported rectal temperature was only elevated in VB-challenged heifers until day 3 post MH, whereas it was elevated until day 14 in the present study. Again, this is due to the more concentrated MH dose the VB-challenged steers in the present study received.

As expected, daily average activity was reduced in VB-challenged steers days 0 through 9 relative to MH inoculation, compared to the remarkably steady activity of PBS steers across trial day (Fig. 10C). Decreased step count and increased lying time have been reported in MH-challenged calves for one wk post inoculation (Hanzlicek et al., 2010; Theurer et al., 2013). The same reduction in activity has also been observed in natural BRD cases; Pillen et al. (2016) and Belaid et al. (2019b) reported decreased step count in feedlot cattle 5 to 10 d prior to visual disease detection by pen riders. Reduction in activity is an evolutionary adaptive sickness behavior, with the purpose of energy conservation and redirection to immunologic processes (Dantzer, 2004). With the instinct of cattle to mask clinical symptoms, remote sensors such as temperature boluses and activity monitors show great utility in disease detection.

Complete Blood Count and Blood Proteins

Main effects of treatment on CBC and blood proteins from days -3 to 14 are presented in Table 10, and Fig. 11 shows results of CBC analysis with significant treatment × day interactions. Neutrophil concentrations were elevated ($P < 0.04$) in the VB-challenged steers on days 2 and 3, and remained numerically elevated through day

14 compared to PBS control steers. Neutrophils are the first leukocyte to respond to immune insult or injury, and increased concentrations are expected with any morbidity event. Increased neutrophils have been reported in many experimental respiratory health challenges (Burciaga-Robles et al., 2010; Hanzlicek et al., 2010; Kayser et al., 2019a; Kayser et al., 2019b). There was a main effect of VB challenge on lymphocytes, with VB-challenged steers expressing lower ($P = 0.03$) concentrations compared to PBS steers, but a treatment × day interaction was not detected. Similarly, Burciaga-Robles et al. (2010), Hanzlicek et al. (2010), and Kayser et al. (2019a) observed reduction in lymphocytes in MH-inoculated calves, and Hanedan et al. (2015) observed reduced lymphocytes in natural BRD cases. Reduced circulating lymphocyte concentration during immune activation is due to sequestration in lymphoid tissues. Hematocrit was depressed ($P < 0.05$) in VB-challenged steers on days 3, 5, and 10 compared to PBS steers. Hematocrit reduction following disease challenge is common (Burciaga-Robles et al., 2010; Hanzlicek et al., 2010; Kayser et al., 2019a) and expected, as the relative proportion of red blood cells decreases with increasing circulating leukocyte numbers. Platelets were elevated ($P < 0.01$) in VB-challenged steers on days 7 to 14 compared to PBS steers. This is anticipated, as neutrophils release platelet activating factor to stimulate platelets which then assist in the inflammatory process, though the detailed mechanism for thrombocytosis associated with respiratory disease is not fully understood (Whiteley et al., 1992). It appears that excessive numbers of activated platelets in pulmonary tissue as a result of respiratory disease results in endothelial cell apoptosis as the platelets degranulate (Kuckleburg et al., 2005). This vasculitis and tissue

consolidation could contribute to lung lesions seen at slaughter in BRD-diagnosed cattle. Numeric increase in platelets was reported by Hanedan et al. (2015) in feedlot cattle with natural BRD, and increased platelet number has been reported to indicate poor prognosis in human pneumonia patients (Prina et al., 2013).

The fibrinogen and HPT responses in the VB-challenged and PBS steers are presented in Fig 12. Fibrinogen was elevated ($P < 0.01$) in VB-challenged steers compared to PBS steers on days 2 to 10, with the nadir on day 5. Hanzlicek et al. (2010) also reported elevated fibrinogen in MH-challenged animals. Interestingly, human patients with thrombocytosis had increased fibrinogen in a prospective analysis conducted by Prina et al. (2013), suggesting that platelets and fibrinogen may have some interrelationship of mutual activation in severe respiratory disease cases. The VB-challenged steers experienced a severe HPT response compared to the PBS steers, with greater concentrations on days 2 to 7 and a nadir on day 3. Haptoglobin is an acute phase protein that combats infection by competitively binding iron to prevent bacterial proliferation, and increases in most instances of inflammation (Petersen et al., 2004). Increased HPT is commonly observed in BRD-diagnosed cattle, and its response is typically quite transient regardless of peak magnitude (Burciaga-Robles et al., 2009; Holland et al., 2011; El-Deeb et al., 2020). The HPT response VB-challenged steers exhibited in this study was of much greater magnitude than in the VB challenged animals studied by Kayser et al. (2019a). This is expected, as Humblet et al. (2004) reported that increasing HPT concentration is associated with increasing disease severity.

Blood Gas and Chemistry

A benchtop analyzer (Prime+ Vet, Nova Biomedical) was used for blood gas analysis on day -3, but due to intermittent technical malfunction, a handheld analyzer (iStat, Abbot) was used for the remaining blood collection days. Whenever possible, blood samples were analyzed on both instruments to develop a paired data set for generation of predictive equations to investigate agreement between devices. Correlation equations were developed (Table 11) based on 28 paired arterial samples and 42 paired venous samples, and were used to transform the benchtop analyzer blood gas results from day -3 into estimates of the handheld analyzer. Correlations between the iStat CG4+ cartridge and Nova Prime+ for pH, PCO_2, PO_2, and lactate were moderate to strong (r^2 = 0.66 – 0.98). Correlation for arterial SO_2 was found to be low (r^2 = 0.07) and correlation for venous SO_2 was moderate (r^2 = 0.67), which is reasonable due to the small reference range for that parameter. Regardless, re-analysis using the transformed day -3 blood gas data did not alter any statistical significance or conclusions from analysis of the original, untransformed data. Indrasari et al. (2018) conducted a correlation analysis between the iStat handheld analyzer using CG4+ cartridges compared to the Nova pHOx (the generation preceding the Prime+ used in the present study). They reported similarly strong correlations (r = 0.84 – 0.99) for pH, PCO_2, PO_2, and lactate, however they did not report values for SO_2 comparison.

The main effects of treatment on arterial and venous blood gas and chemistry metrics from days -3 to 14 relative to MH inoculation are presented in Table 12. During the 17-d experimental period, the VB-challenged steers had lower (P < 0.02) arterial SO_2

and PO_2 compared to PBS steers. However, the treatment × day interaction was not significant for either variable, indicating the arterial oxygenation parameters were not acutely responsive to the MH challenge. Arterial SO_2 and PO_2 responses are shown in Fig. 13 A and B for illustrative purposes. There was no effect of VB challenge on venous SO_2. The VB challenge tended to cause decreased venous PO_2, but a significant treatment × day interaction was not detected. The VB challenge did not affect arterial or venous PCO_2 and although there were significant treatment × day interactions, the biological relevance is not apparent (data not shown).

The VB challenge did not affect arterial pH or lactate in a biologically relevant manner. However, a treatment × day interaction was detected such that venous pH of VB steers was elevated ($P < 0.04$) on days 0 and 10 relative to MH inoculation (Fig 13C). Interestingly, though venous lactate had a treatment × day interaction ($P = 0.02$), none of the within-day treatment differences were significant upon execution of the SLICE procedure, presumably due to the large standard error (Fig 13D).

Literature yields conflicting results regarding arterial blood oxygen shifts with BRD diagnosis and progression, and an important factor to consider is the difficulty associated with serially collecting arterial blood in cattle. Muylle et al. (1996) cautioned about ease of thrombosis when collecting arterial blood and stressed the importance of complete animal restraint. Due to the hydraulic chute orientation for the present study, we elected to collect from the intermediate branch of the auricular artery. Inter-animal variation existed in the artery's location and diameter, thereby altering success rate of collection between steers. Additionally, more fractious animals were more prone to

thrombosis as they tended to have more sporadic movement during collection, despite use of a hydraulic head sweep. Thrombosis could be mediated by applying firm pressure to the collection site for 2 min immediately following needle withdrawal; this technique became part of the collection protocol for both calm and fractious animals with favorable results.

Hanzlicek et al. (2010) reported that MH-challenged cattle, with moderate to severe clinical signs of BRD and post-slaughter confirmed lung lesions upon slaughter, had increased arterial SO_2 and PO_2 immediately following the MH challenge. As collection of arterial blood samples is relatively difficult, these results would suggest that the initial arterial blood samples may have been contaminated with venous blood. In contrast, other studies have reported appropriate reductions in blood oxygenation in BRD cases. Ciszewski et al. (1991) collected blood from the carotid artery in Holstein calves challenged with BRSV. By day 6 post-inoculation, the challenged calves presented an average arterial PO_2 of 76 mm Hg, while control calves maintained PO_2 of 94 mm Hg. Collie et al. (1992) also reported declining PO_2 with increasing CIS in calves suffering from chronic BRD. Woolums et al. (1999) challenged 17 Holstein calves with BRSV, and conducted arterial blood gas analysis on day 7 post-inoculation. Challenged calves became grossly morbid, and had significantly lower PO_2 than control calves (58.1 mm Hg vs 77.7 mm Hg). Ellis et al. (2013) also reported reduced PO_2 in calves challenged with BRSV, and concluded that parameter was strongly correlated with severity of lung lesions. Šoltésová et al. (2015) reported arrival SO_2 and PO_2 declined and PCO_2 increased with increasing disease severity in dairy calves diagnosed with

BRD. Ozkanlar et al. (2012) analyzed venous blood gas, presumably due to difficulty in arterial collection, in calves with natural BRD and compared it to healthy controls. They reported significant reductions in PO_2, but did not observe a difference in SO_2. Oosthuysen et al. (2017) also investigated venous blood gas shifts following LPS challenge, and were able to detect a brief decline in PO_2 at 2 hours following LPS administration. Ciszewski et al. (1991) reported HR increased on day 3, temperature increased day 4, respiration rate increased day 5, and finally PO_2 was reduced day 6 following BRSV challenge in calves. Due to the obvious physiological importance of maintaining proper blood oxygenation, it appears that oxygen saturation is one of the last biological mechanisms to deviate in cattle with BRD and may therefore have more prognostic than diagnostic value. We are unaware of any evidence suggesting blood oxygen saturation deviates prior to observable clinical symptoms, thereby making it more useful as a prognostic tool, not suitable for preclinical disease detection.

Elevated lactate is commonly seen in a variety of disease etiologies, but most commonly is associated with decreased tissue oxygenation as glycolysis produces lactate under anaerobic conditions (Andersen et al., 2013). Lactate response is non-specific, and concentrations are seen to increase with increasing severity of BRD (Coghe et al., 2000; Camkerten et al. 2010; Šoltésová et al., 2015; Oosthuysen et al., 2017). Venous pH was elevated in VB-challenged steers in the present study, a phenomenon which was also observed by Oosthuysen et al. (2017) in cattle following LPS challenge and by Hanzlicek et al. (2010) following MH challenge. This is contrary to what is expected, as pH typically declines in association with respiratory disease as accumulating CO_2 in the

blood and lung causes an influx of H+ ions (Ucgun et al., 2006). Such respiratory acidosis is associated with poor prognosis, and was observed by Gunes and Atalan (2006) and Ozkanlar et al. (2012) in young calves diagnosed with BRD.

Pulse Oximetry

Of the 48 SpO_2 sensor tags (2 per steer) originally placed on animals the day prior to BHV-1 inoculation, only 11 tags remained mechanically intact with successful data collection until day 4 post MH inoculation. This included data from 2 PBS steers and 9 VB-challenged steers. This experience illustrates the supreme difficulty in maintaining mechanical integrity of delicate sensors on confined cattle, particularly one as sensitive to motion artifact as SpO_2 is. The tag had 3 attachment points, which did result in fair inflammation and necrosis to the ear pinna, but this design was found to be least susceptible to motion artifact. However, the ear may be too prone to inflammation, thereby confounding SpO_2 readings as the vessels are all quite small, and cattle frequently self-groom by head scratching which contributed to tissue damage. The sensor tag recorded the metric R (which is inversely related to SpO_2) and HR; these metrics are shown in Fig 14. There were not significant treatment × day interactions for either metric, mean data are merely shown by day for illustrative purposes. One should consider the very low sample size available when interpreting this data. The metric R appears to trend upward over time, which would theoretically indicate declining SpO_2. However, other variables may have also contributed to increasing R. Over time, the sensor could have loosened on the ear slightly which would have resulted in less light passing through the tissue and causing a falsely elevated R. Further, inflammation

progression of the cartilage tissue from the tag application would have resulted in apparent decreased peripheral perfusion. Surprisingly little research can be found using SpO_2 monitors to detect BRD outside of the clinical setting, probably due to difficulty in obtaining accurate and repeatable measurements. Coghe et al. (1999) listed some difficulties of accurate SpO_2 measurements as: poor blood perfusion, poor placement, skin pigmentation, ambient light interference, motion artifact, among others.

Coghe et al. (1999) investigated agreement between arterial blood gas analysis and pulse oximetry in 149 calves diagnosed with moderate to severe BRD. Upon arterial blood gas analysis, 125 calves had SO_2 values > 90%, 15 were between 80 – 90%, and the remaining 9 were < 80%. All calves with < 80% SO_2 values subsequently died. In the precision and bias analysis, it was discovered the SpO_2 monitor slightly overestimated oxygen saturation at high SO_2 levels, and slightly overestimated it at low levels. The authors stated that due to the sigmoidal shape of the saturation curve, a subtle decrease in SO_2 represents a substantial decrease in PO_2. In a more recent study, Baruch et al. (2019) serially measured SpO_2 in Holstein calves using an intranasal probe following a combined BHV-1/MH challenge. Inoculation with MH occurred 6 d following BHV-1, then calves were serially slaughtered on day of MH inoculation, and 1, 3, 5, and 7 days after. All of the calves developed mild or moderate BRD symptoms following BHV-1 inoculation, and disease severity increased drastically after MH inoculation. Despite mild morbidity symptoms and elevated rectal temperature, SpO_2 was unaffected during the BHV-1-only period. However, SpO_2 rapidly declined from 97.6% to 95.4% the day following MH and was 91.2% two days post-MH. Oxygen

saturation was inversely related with lung consolidation; SpO_2 levels between 96-100% were associated with 11.5% lung consolidation, SpO_2 levels between 93-95% were associated with 21.7% lung consolidation, and SpO_2 levels 92% and below were associated with 35% lung consolidation. These studies by Coghe et al. (1999) and Baruch et al. (2019) indicate that SpO_2 is likely not a good metric for preclinical disease detection, as they found deviations in SpO_2 only once animals were moderately morbid and after other, more-easily measurable indices such as temperature had deviated. Indeed as discussed above, research (Woolums et al. 1999; Ozkanlar et al., 2012; Ellis et al., 2013) has shown that even the more sensitive metric PO_2 does not decrease until moderate BRD. Other behavior and physiological metrics such as DMI, fever, and activity deviate from baseline sooner, and are therefore more appropriate for early disease detection in cattle.

Post-Challenge Results

Feeding behavior, rumen bolus, and rumination data were analyzed from day 21 – 77 post MH challenge to examine long-term effects of moderate to severe BRD (Table 13). Several of the VB steers sporadically presented with clinical symptoms up to 36 d post-MH inoculation, and received antimicrobial intervention per protocol. These morbidities may have been due to other causative pathogens, and the cattle merely were still in a weakened immune state with impaired lung function, and vulnerable to infection. Beyond day 36, no calves presented with clinical symptoms of BRD. There were no differences ($P > 0.30$) in rumen temperature, activity score, or rumination between VB-challenged and PBS steers in this post challenge period, as those

parameters had transient, acute responses immediately following the challenge. As expected, initial and final BW were lighter ($P < 0.02$) in the VB-challenged steers, due to the 14-d following inoculation during which DMI was so severely impacted. However, during this 56-d post challenge period, there was only a tendency ($P = 0.06$) for DMI to be reduced in the VB-challenged steers and no difference when DMI was calculated as a percentage of BW. Further, ADG and G: F were not different between VB-challenged and PBS steers. Other studies (Stovall et al., 2000; Step et al., 2008; Reinhardt et al., 2012; Blakebrough-Hall et al., 2020) have also shown no or minimal performance differences between natural BRD cases following treatment and healthy cohorts, suggesting animals can fully recover and be productive after a sickness event. Stovall et al. (2000) recorded BRD events in heifers in the feedlot receiving period, then compared performance in the finishing period and carcass qualities. There were no differences in ADG or final BW between BRD and control cattle, however fewer BRD cattle graded choice upon slaughter. Step et al. (2008) reported no performance differences during the receiving period in market-sourced versus preconditioned calves, despite significant differences in morbidity rates. Reinhardt et al. (2012) and Blakebrough-Hall et al. (2020) reported differences in final BW and ADG only in BRD cattle that had been treated 2 or more times. In contrast, Griffin (2014) stated in his review that ADG was decreased in cattle with lung lesions at slaughter, many of which had never been treated for BRD. However, cattle temperament may be a confounding variable in determining effect of a natural BRD event on feedlot performance, as

fractious cattle are more difficult to identify as morbid, and even healthy fractious cattle have decreased ADG compared to calm cattle (Bruno et al., 2018; Olson et al., 2019).

In the present study, there were no differences ($P > 0.14$) in any feeding behavior traits other than time to bunk in the post challenge period. The VB-challenged steers tended to take longer ($P = 0.08$) to come to the bunk after feed delivery, and had a much greater ($P = 0.03$) day-to-day variance in their time to bunk. This is probably due to longer time to bunk in the earlier phase of this 56-d interval and shorter towards the end as they fully recovered.

Conclusion

The experimentally-induced VB challenge model used in this study successfully induced severe DMI, feeding behavior, body weight gain, and febrile responses compared to PBS control steers. The hemogram responses of the VB-challenged steers further supports that substantial immune responses were induced by the experimental VB challenge in this study. However, the experimental VB challenge did not induce acute changes to blood oxygen saturation prior to onset of observed clinical signs of BRD in this study. Based on blood gas results in this study, as well as lack of supporting evidence from previous research, these results indicate that SpO_2 is likely not a viable indicator for preclinical detection of BRD. However, SpO_2 has been shown to be well correlated with arterial SO_2, and therefore provides a non-invasive alternative for prognosis of clinical BRD. Further research is warranted to further develop an optimal SpO_2 sensor capable od real-time monitoring of blood gases in beef cattle.

CHAPTER VI

CONCLUSIONS

Advances in sensor and computational technologies have prompted considerable interest in the development of real-time animal-health monitoring systems. These systems would enable producers to more accurately detect onset of disease that would support more effective intervention strategies to mitigate disease outbreaks, as well as provide opportunities to more objectively monitor animal welfare status. Effective animal health monitoring systems are dependent upon: (1) robust sensors capable of accurately monitoring behavioral or physiologically-based response variables on an individual-animal basis, (2) statistical-based quality control procedures that can differentiate abnormal vs normal variation of a monitored trait to accurately signal that an animal is 'out of control', and (3) effective database management systems to support decision-support analytics. This research focused on addressing 1 and 2 above, by validating accelerometer, temperature, and pulse oximeter sensors in cattle diagnosed with BRD, or in cattle experimentally-challenged with BRD pathogens.

In the first study, physical activity data collected from leg-attached accelerometers from BRD-diagnosed and healthy calves were analyzed using Shewhart statistical process control (SPC) procedures to determine if the activity sensors could detect onset of BRD prior to feedlot personnel. Univariate and multivariate models had moderate sensitivity (40 to 57%) and specificity (23 to 81%), and signaled up to 2 d prior to visual diagnosis. The moderate diagnostic accuracies of the SPC models

reported in this study may be due to the relatively high within-animal daily variation in physical activity, and minimal time for early model training. Activity data may yet prove valuable in sensor-based disease detection research, however it appears that inclusion of feeding behavior metrics will yield the most promising performance. Validation of sensors is often based on subjective observation of clinical signs to diagnose BRD cases, which is known to have low to moderate sensitivities. Therefore, future research should focus on validating potential sensors using more objective BRD case definitions (e.g. lung lesions at slaughter). It is likely that animal health monitoring systems that utilize multiple sensor technologies and multivariate SPC-type procedures to signal deviations in physiology and/or behavior responses would be more sensitive and specific in detecting onset of BRD.

The second study investigated potential markers of BRD resilient animals using behavioral, physiological, and immune alterations of steers that mounted substantial or minimal haptoglobin (HPT) responses following an experimental challenge with *Mannheimia haemolytica* (MH) were compared with PBS-challenged controls. The HPT-responsive steers had greater post-challenge neutrophil, lymphocyte, rumen temperature, and DMI responses than HPT-nonresponsive steers, but differences in metabolite profiles were not detected between HPT-responsive phenotypes. Lower basal cortisol concentration in HPT-responsive steers may have enabled these greater immunologic and feeding behavior responses compared to HPT-nonresponsive steers. Further, HPT responsive steers exhibited increased day-to-day variation in feeding behavior prior to and following the MH challenge. Therefore, HPT response and feeding

behavior may be indicator traits of disease resilience, as research has shown greater variation in production and behavior to be associated with decreased resilience. Further research is needed to investigate how temperament, basal cortisol, feeding behavior, and acute phase protein response relate to disease resilience.

The objective of the third study was to investigate performance of pulse oximeters and cattle hemodynamics during experimental hypoxia. Results from this study revealed that SpO_2 monitors could detect reductions in SO_2 imposed by the experimentally-induced hypoxia in cattle, indicating that SpO_2 monitors may have utility in BRD diagnosis and prognosis. Further, heart rate and blood pressure may also be biomarkers for BRD diagnosis and prognosis, as those metrics were elevated in hypoxemic steers. Pulse oximeters would provide a valuable tool at feedlots, as SpO_2 would help differentiate BRD from other diseases, and its utility as a prognostic tool would aid in the more judicious administration of antimicrobial treatment.

The fourth and final study was conducted to investigate immunologic (leukocytes, acute phase proteins), physiologic (temperature, blood gas), and behavior (activity, rumination, feeding behavior) responses following a combined viral (bovine herpes virus-1; BHV-1) and bacterial (MH) challenge (VB) in steers to further explore the use of pulse oximetry for preclinical detection of BRD. The experimentally-induced VB challenge model used in this study successfully induced severe DMI, feeding behavior, body weight gain, and febrile responses compared to PBS-challenged control steers. However, VB challenge did not result in acute changes to blood oxygen saturation prior to onset of observable clinical signs of BRD. Based on blood gas results

in this study, as well as lack of supporting evidence from previous research, these results indicate that SpO_2 is likely not a viable indicator for preclinical detection of BRD. However, SpO_2 has been shown to be well correlated with arterial SO_2, and therefore provides a non-invasive alternative for clinical prognosis of BRD. Further research is warranted to further develop an optimal SpO_2 sensor capable of real-time monitoring of blood gases in beef cattle.

Based on evidence from the literature and the studies discussed above, future research should focus on identification of more disease resilient animals based on genotypic and phenotypic markers, and development of sensor technologies which monitor both temperature and feeding behavior as these metrics have consistently shown to deviate early in the disease process.

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FIGURES

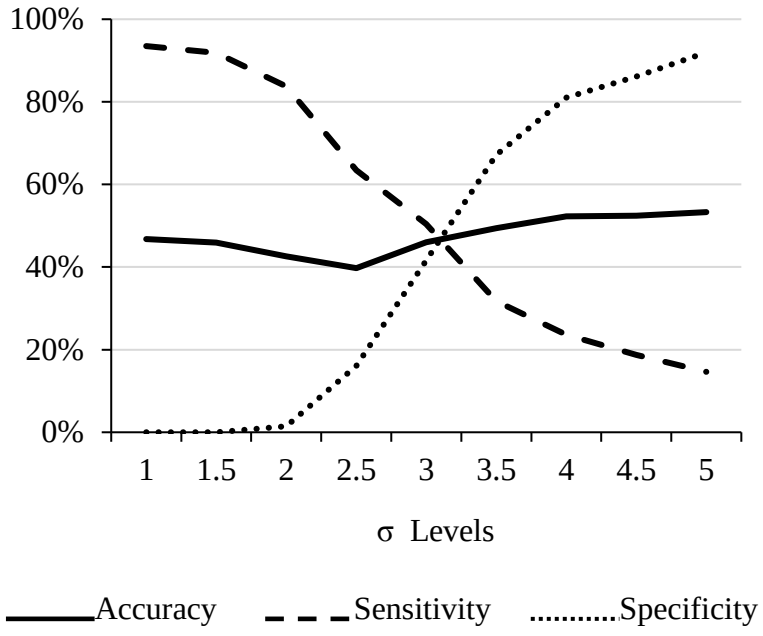

Figure 1. Sensitivity, specificity and accuracy values at various sigmas from the Shewhart chart analysis of step count.

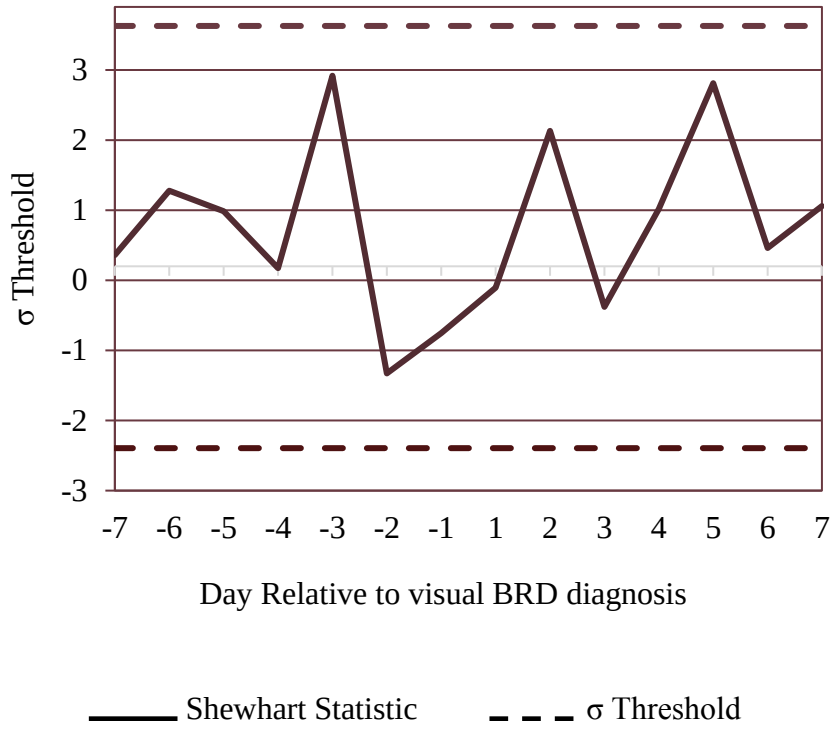

Figure 2. Shewhart control chart of step count, obtained from a leg-based accelerometer, from a calf who was diagnosed with bovine respiratory disease by pen riders on day 35 post feedlot arrival.
Note: Day 0 is omitted, as morbid cattle were walked to the hospital pen for treatment with antimicrobials.

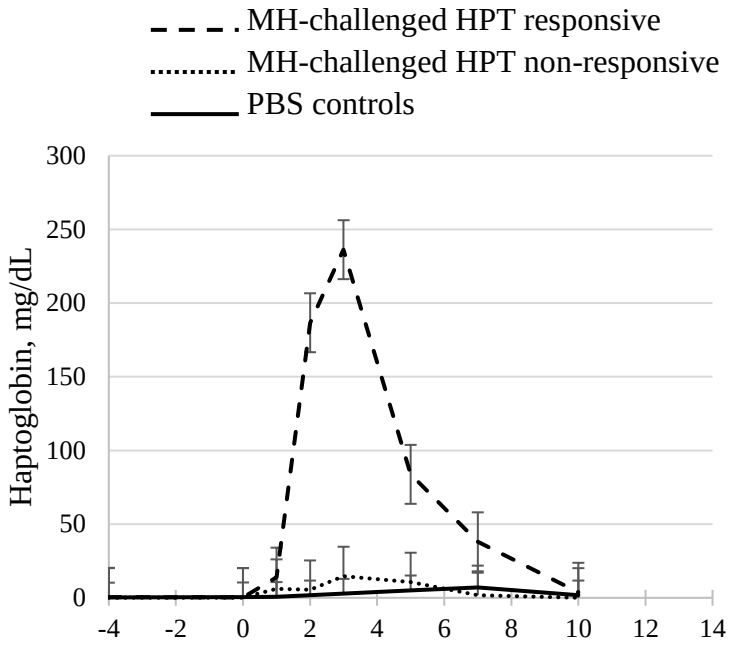

Figure 3. Least squares means of haptoglobin (HPT) by day relative to challenge with Mannheimia haemolytica (MH) or phosphate-buffered saline (PBS; control). Differential HPT responsiveness to MH challenge was used to classify cattle into responsive and non-responsive groups.
Within day values with separated error bars are different ($P < 0.05$).

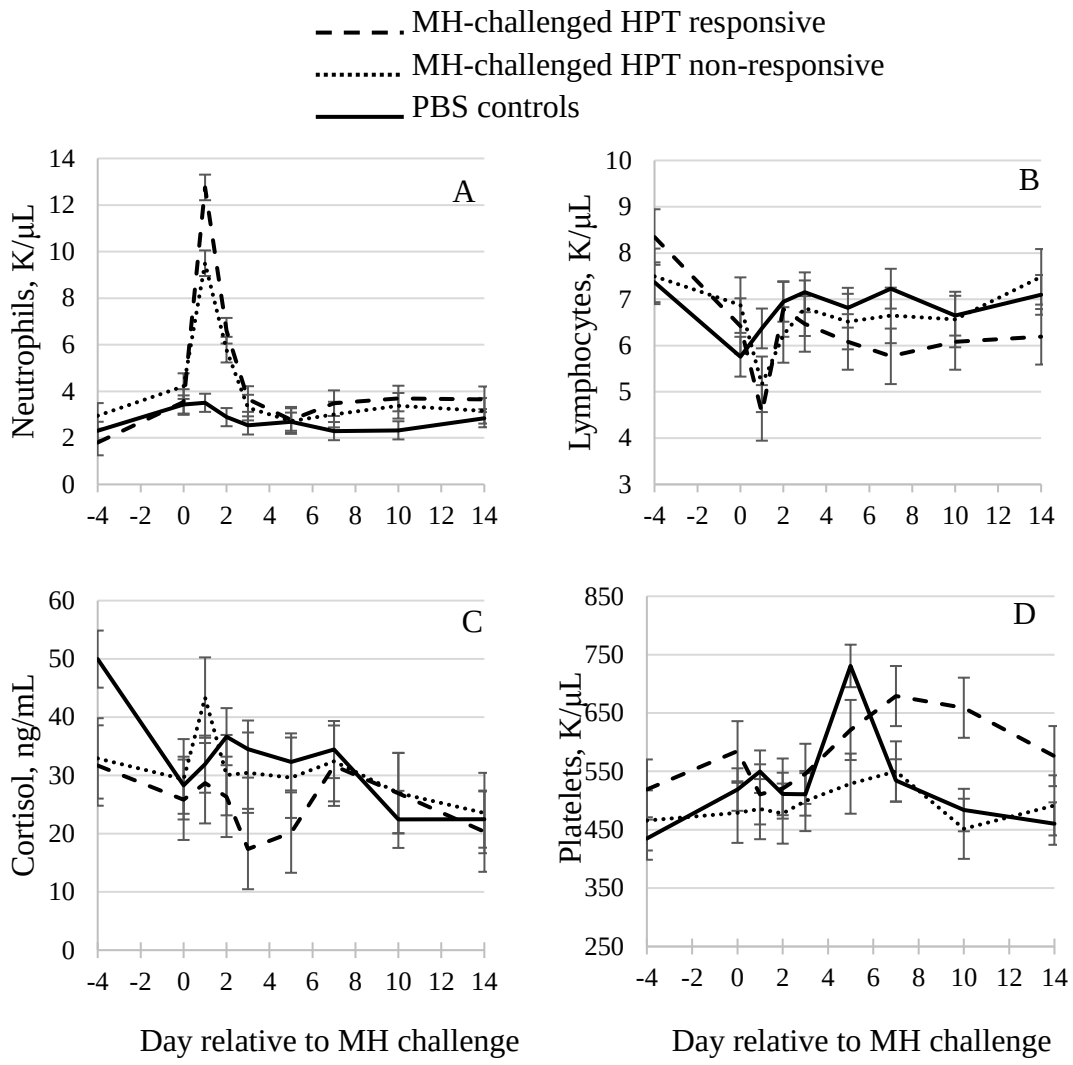

Figure 4. Least squares means of (A) neutrophils, (B) lymphocytes, (C) cortisol, and (D) platelets by day relative to challenge with *Mannheimia haemolytica* (MH) or phosphate-buffered saline (PBS; control).
Differential haptoglobin (HPT) responsiveness to MH challenge was used to classify cattle into responsive and non-responsive groups. Within day values with separated error bars are different ($P < 0.05$).

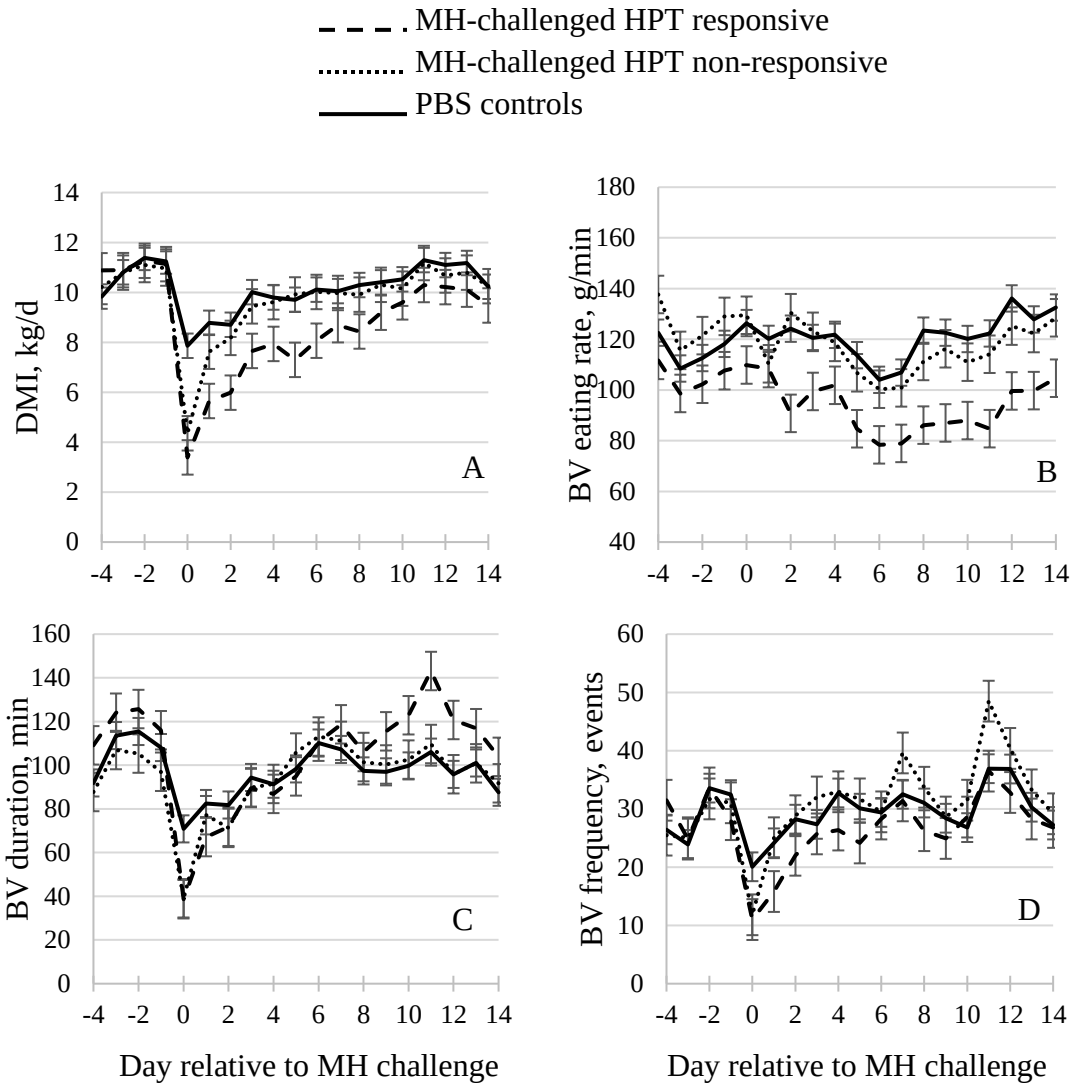

Figure 5. Least squares means of (A) DMI, (B) bunk visit eating rate, (C) bunk visit duration, and (D) bunk visit frequency by day relative to challenge with *Mannheimia haemolytica* (MH) or phosphate-buffered saline (PBS; control).
Differential haptoglobin (HPT) responsiveness to MH challenge was used to classify cattle into responsive and non-responsive groups. Within day values with separated error bars are different ($P < 0.05$).

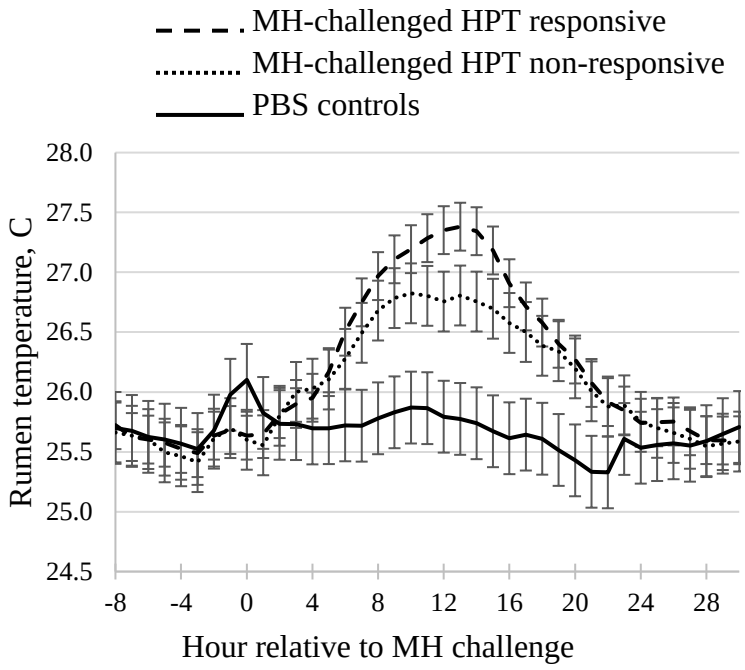

Figure 6. Least squares mean hourly rumen temperature by hour relative to challenge with *Mannheimia haemolytica* (MH) or phosphate-buffered saline (PBS; control). Differential haptoglobin (HPT) responsiveness to MH challenge was used to classify cattle into responsive and non-responsive. Within day values with separated error bars are different ($P < 0.05$).

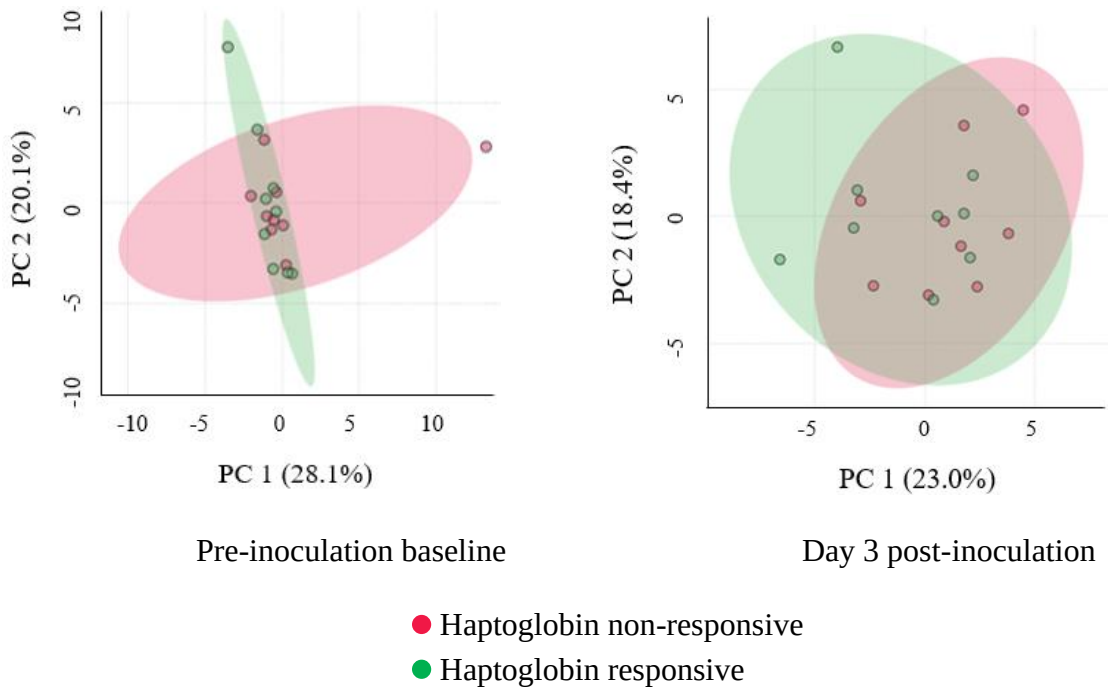

Figure 7. 2D principal component (PC) analysis scores plots (2D-PCA) scores plots resulting from the analysis of plasma metabolic profiles of haptoglobin-responsive (green circles) versus non-responsive (red circles) cattle, following inoculation with *Mannheimia haemolytica*, with shaded regions illustrating respective 95% confidence intervals.

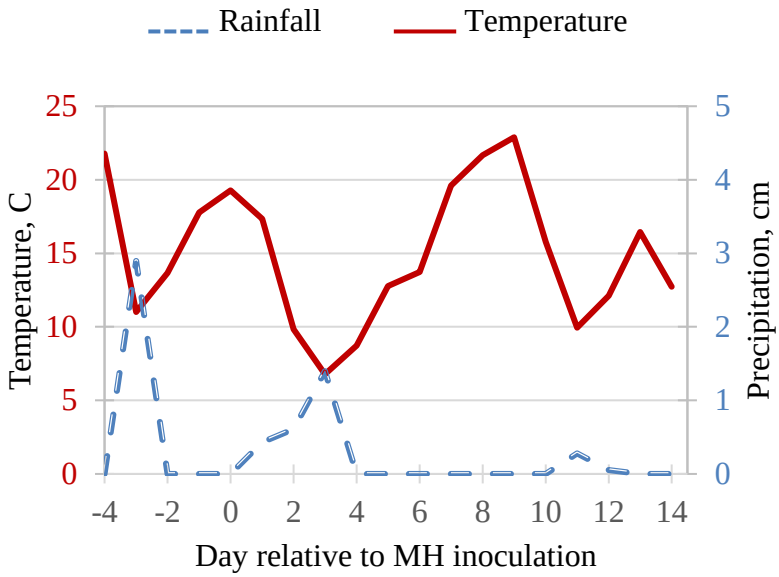

Figure 8. Rainfall and ambient temperature to which cattle were exposed to following a combined bovine herpes virus (day -3) and *Mannheimia haemolytica* (MH; day 0) respiratory health challenge.

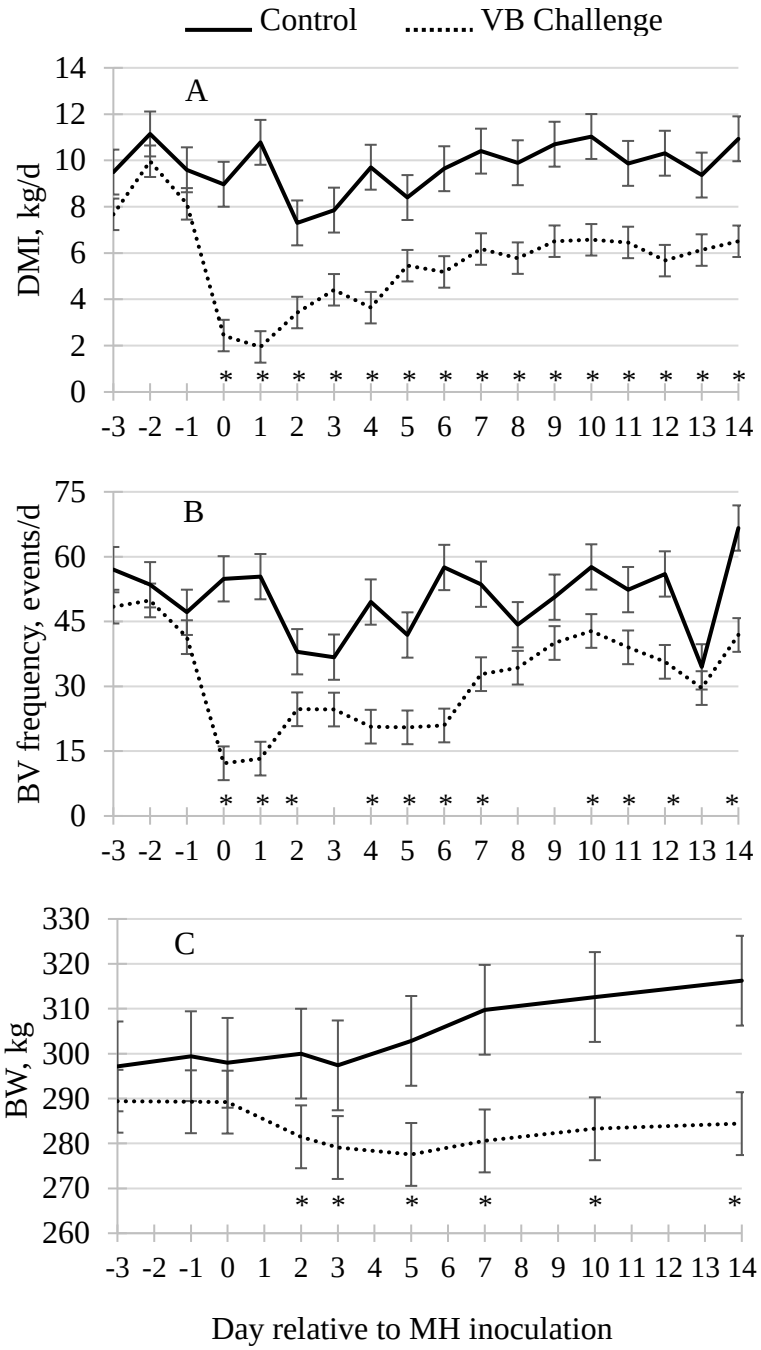

Figure 9. Least squares means (± SEM) for (A) dry matter intake, (B) bunk visit (BV) frequency, and (C) body weight in beef steers relative to experimental inoculation with viral-bacterial challenge (VB; bovine herpesvirus-1 on day -3 followed by *Mannheimia haemolytica* on day 0) or phosphate-buffered saline (PBS; control). Model included effects for trial day and repeated measures on individual steers.
*Significant difference ($P < 0.05$) between treatment group within trial day.

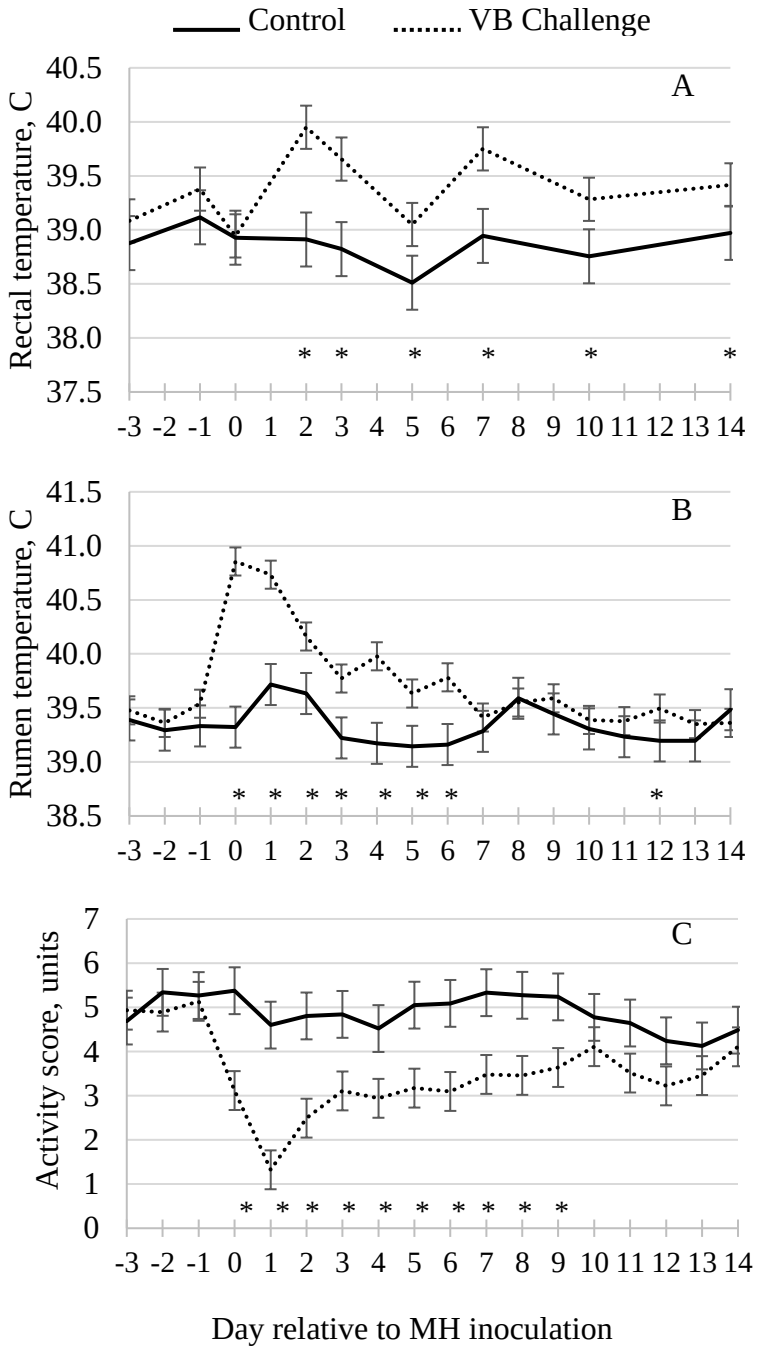

Figure 10. Least squares mean (± SEM) for (A) rectal temperature, (B) third quarter (1200 to 1800 h) rumen temperature and (C) activity score in beef steers by day relative to experimental inoculation with viral-bacterial challenge (VB; bovine herpesvirus-1 on day -3 followed by *Mannheimia haemolytica* [MH] on day 0) or phosphate-buffered saline (PBS; control).
Model included effects for trial day and repeated measures on individual steers.
*Significant difference ($P < 0.04$) between treatment group within trial day.

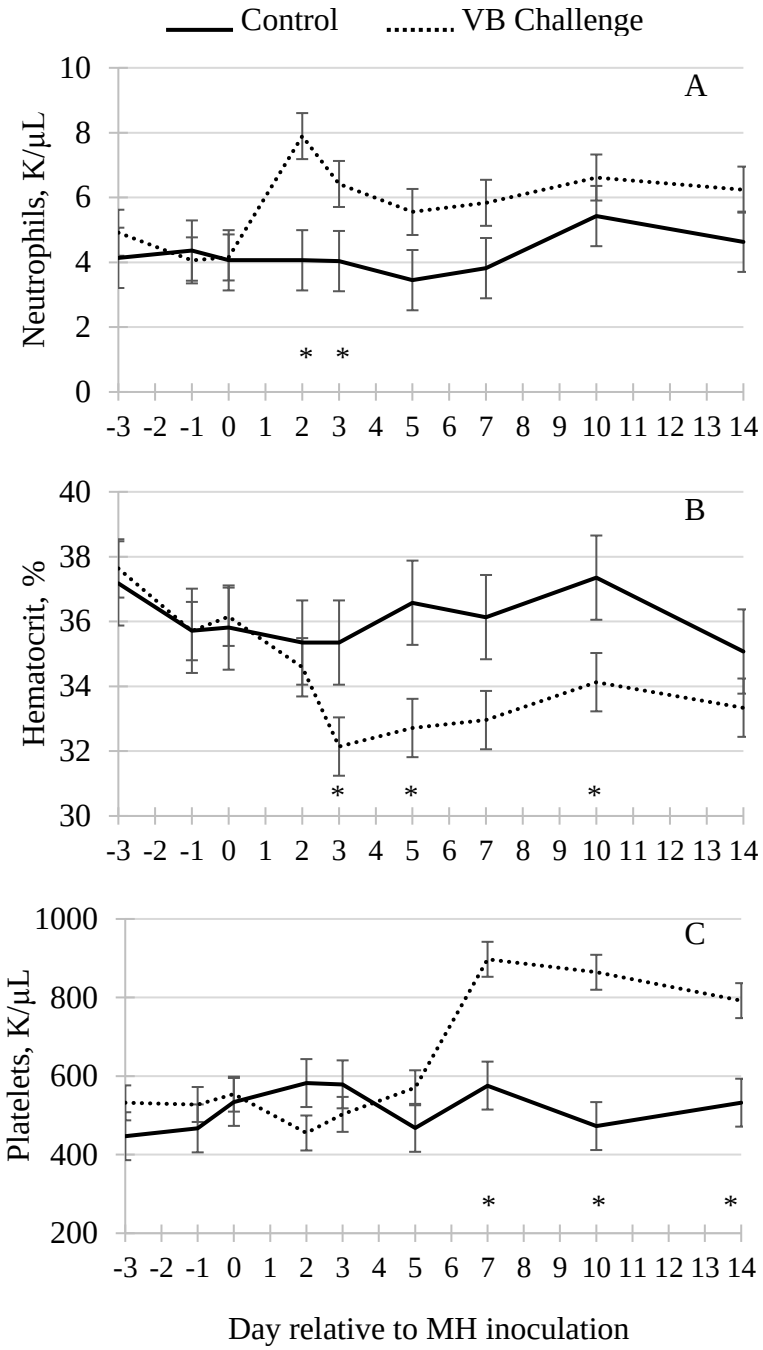

Figure 11. Least squares mean (± SEM) for (A) neutrophils, (B) hematocrit, and (C) platelets in beef steers by day relative to experimental inoculation with viral-bacterial challenge (VB; bovine herpesvirus-1 on day -3 followed by *Mannheimia haemolytica* [MH] on day 0) or phosphate-buffered saline (PBS; control).
Model included effects for trial day and repeated measures on individual steers.
*Significant difference ($P < 0.04$) between treatment group within trial day.

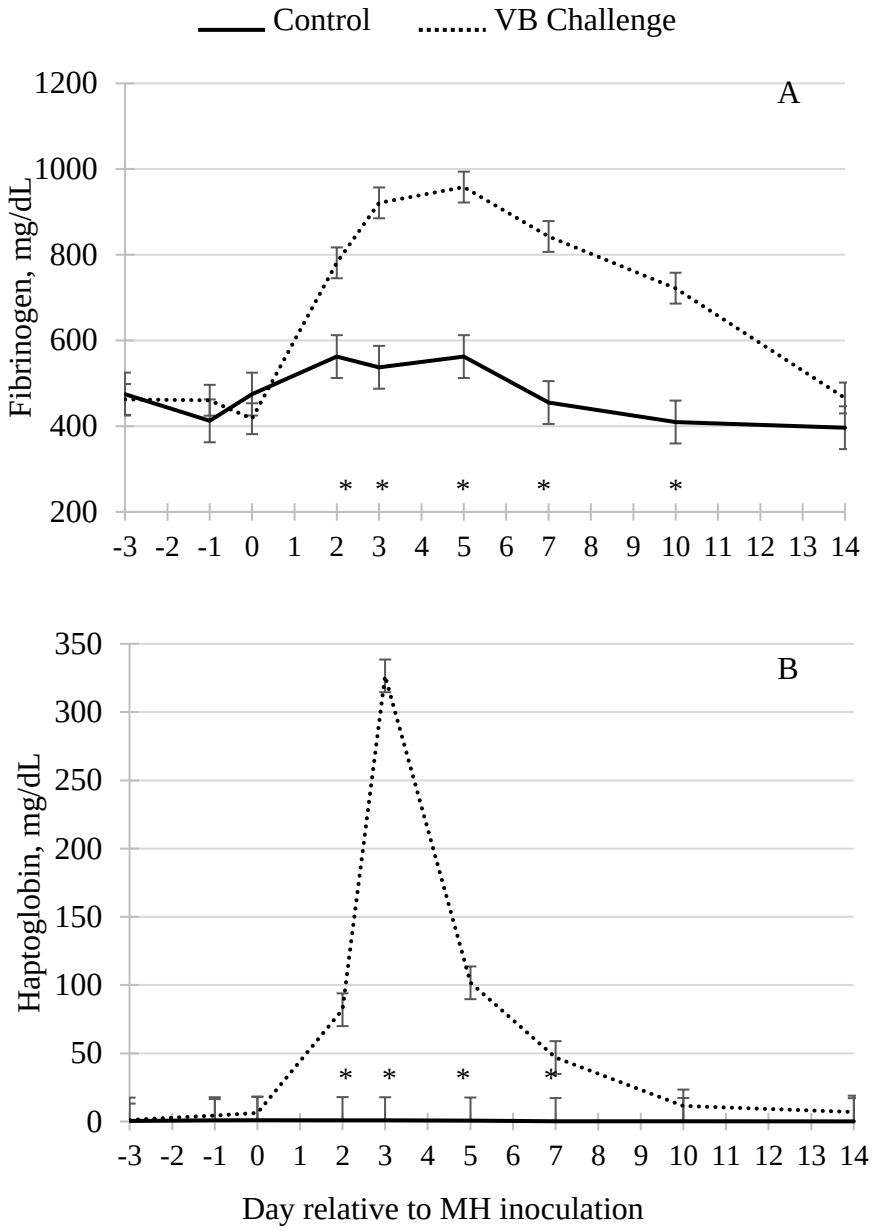

Figure 12 Least squares mean (± SEM) for (A) fibrinogen and (B) haptoglobin in beef steers by day relative to experimental inoculation with viral-bacterial challenge (VB; bovine herpesvirus-1 on day -3 followed by *Mannheimia haemolytica* [MH] on day 0) or phosphate-buffered saline (PBS; control).
Model included effects for trial day and repeated measures on individual steers.
*Significant difference ($P < 0.01$) between treatment group within trial day.

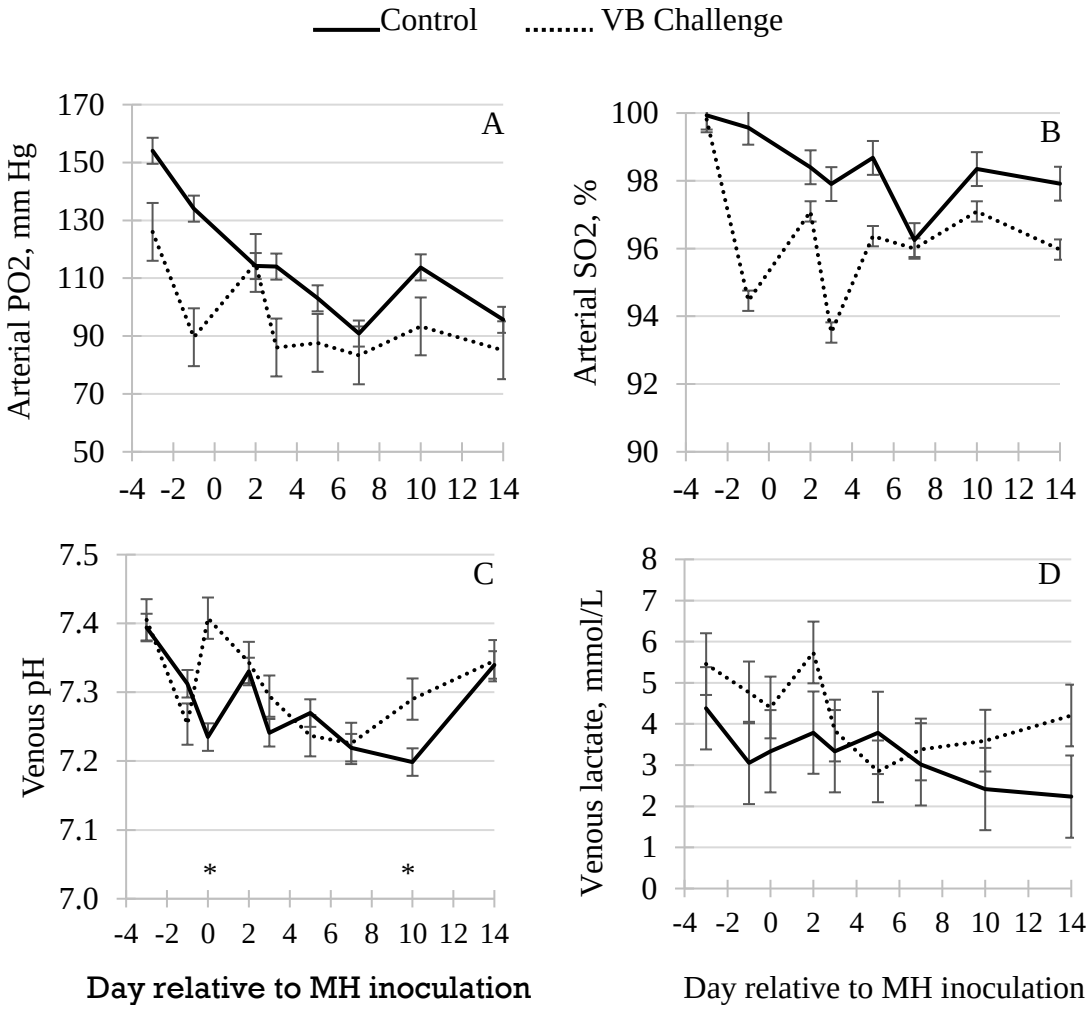

Figure 13 Least squares mean (± SEM) for (A) arterial PO2, (B) arterial SO2 (C) venous pH, and (D) venous lactate in beef steers by day relative to experimental inoculation with viral-bacterial challenge (VB; bovine herpesvirus-1 on day -3 followed by *Mannheimia haemolytica* [MH] on day 0) or phosphate-buffered saline (PBS; control).
Model included effects for trial day and repeated measures on individual steers.
*Significant difference ($P < 0.02$) between treatment group within trial day.
Panels with no significant differences are included for illustrative purposes.

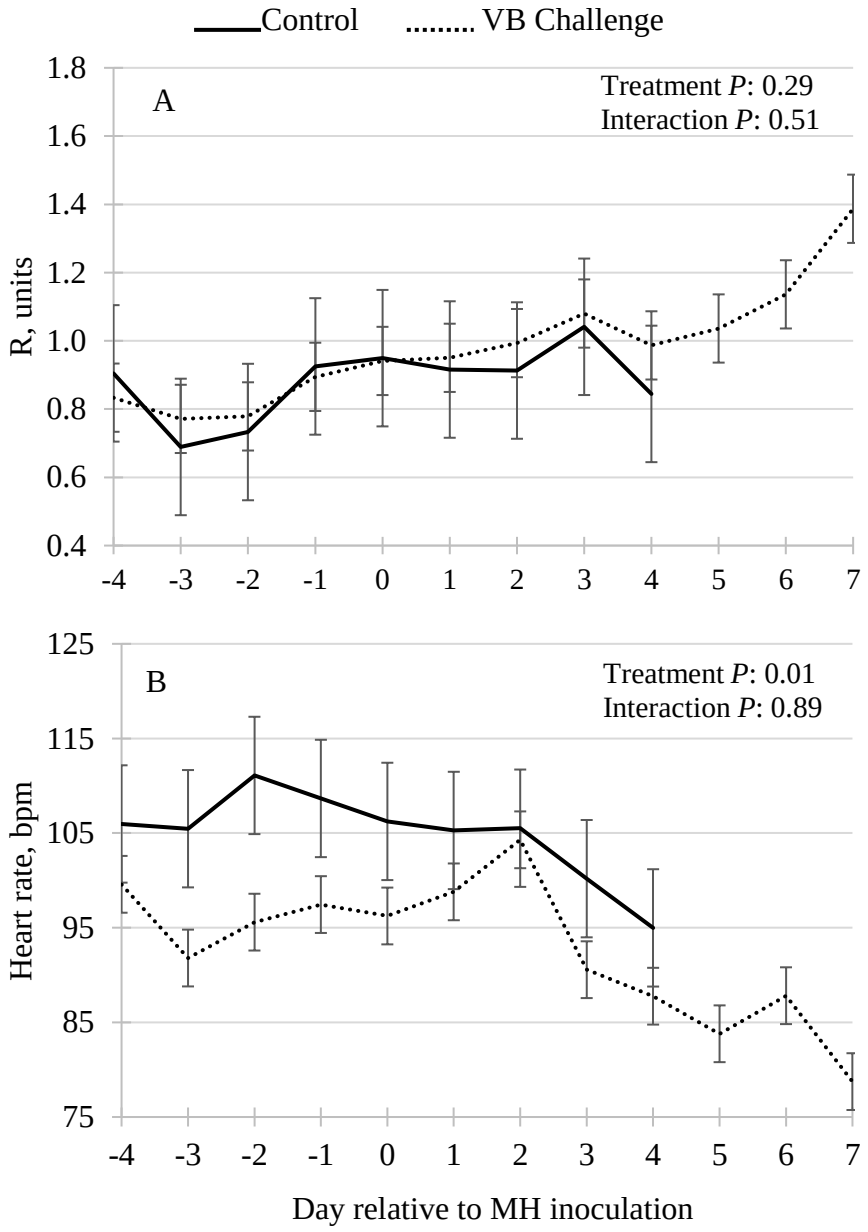

Figure 14 Least squares mean (± SEM) for (A) R and (B) heart rate in beef steers by day relative to experimental inoculation with viral-bacterial challenge (VB; bovine herpesvirus-1 on day -3 followed by *Mannheimia haemolytica* [MH] on day 0) or phosphate-buffered saline (PBS; control).
"R" is a metric that is inversely related to SpO_2. Model included effects for trial day and repeated measures on individual steers. There were no significant treatment × day interactions; data are presented for illustrative purposes.
Data from only 2 control steers and 9 VB steers were successfully recorded.

TABLES

Table 1. Timeline of symptom presentation in feedlot cattle diagnosed with spontaneous bovine respiratory disease (BRD).

				Day relative to visual BRD diagnosis					
Study	N	BW (kg) or age (d)	Morbidity rate, %	≤-6	-5	-4	-3	-2	-1
Belaid, 2019b	770	127 d	9.2	Step count, lying frequency, BVF, BVD					
Kayser, 2019a	231	391 kg	12.9	Eating rate			BVF, BVD		DMI, time to bunk
Marchesini, 2018	214	441 kg	8.9		Rumination		Activity		
Pillen, 2016	364	176 kg	51.2		Motion index	Step count			Standing time
Quimby, 2001	125	191 kg	66.0			BVD			
Timset, 2011	24	269 kg	87.5					Fever	Fever
Wolfger, 2015	213	294 kg	76.0	Meal intake, BVF, BVD					

Key: BVF = bunk visit frequency; BVD = bunk visit duration

Table 2. Timeline of physiological deviations in cattle with experimentally-induced bovine respiratory disease (BRD).

					Day relative to Mannheimia haemolytica (MH) or bovine respiratory syncytial virus (BRSV) inoculation					
Study	Pathogen	N	BW (kg) or age (d)	Morbidity rate, %	0	1	2	3	4	≥5
Baruch, 2019	BHV1/MH	30	211 kg	100%	Fever, SpO_2	Lung consolidation				
Ciszewski, 1991	BRSV	24	30 d	100%				HR	Fever	RR, PO_2
Eberhart, 2017	MH	12	135 d	83%	Fever, lying time, RR					
Hanzlicek, 2010	MH	14	199 kg	100%	Fever, step count	Neutrophils, hematocrit, pH	Lactate, standing time			RR, HR, PO_2
Kayser, 2019b	BHV1/MH	38	230 kg	0%	Fever, DMI	Neutrophils, lymphocytes	HPT	Hematocrit		
Kayser, 2019c	MH	36	352 kg	5%	Fever, DMI, BVF, BVD				Eating rate	
Theurer, 2013	MH	18	240 kg	100%	Fever, hay BVD, lying time, HPT	Grain BVD				

Key: BHV1 = bovine herpes virus 1; SpO_2 = peripheral O_2 saturation; HR = heart rate; RR = respiration rate; PO_2 = partial pressure of O_2; HPT = haptoglobin; BVF = bunk visit frequency; BVD = bunk visit duration

Table 3. Performance of frequency and duration of lying bouts, step count, and motion index for the detection of bovine respiratory disease (BRD) in beef steers using Shewhart control charting during the 56-d study.

Trait	Sensitivity	Specificity	Accuracy	Signal Day	Sigma
Univariate models					
Frequency of lying bouts	23.4%	67.9%	45.6%	0.62	4.0
Duration of lying bouts	40.3%	23.2%	31.8%	-1.00	3.5
Motion index	19.4%	71.5%	45.4%	-2.00	4.0
Step count	23.6%	81.0%	52.3%	-1.79	4.0
Multivariate models[1]					
Complete	57.4%	29.2%	43.3%	-1.86	4.0
Resting	43.9%	37.2%	40.6%	-1.81	4.0

[1]Complete multivariate model included all 4 activity traits, the resting multivariate model included frequency and duration of lying bouts.

Table 4. Effect of haptoglobin (HPT) responsive phenotype on physiological responses and feeding behavior from days -4 to 14 relative to inoculation with Mannheimia haemolytica (MH) or phosphate-buffered saline (control) in cattle.

	MH-challenged				*P*-value		
Item[1]	HPT Responsive	HPT Non-responsive	Control	SE	Group	Day	Group × Day
Feeding behavior traits							
DMI, kg/d	8.76	9.80	10.2	0.49	0.08	<0.01	0.01
BV eating rate, g/min	95.8[b]	118[a]	120[a]	4.6	<0.01	<0.01	0.03
BV duration, min/d	104	95.3	97.1	6.5	0.57	<0.01	0.45
BV frequency, events	26.7	31.2	29.3	2.6	0.23	<0.01	0.47
Rumen temperature traits							
Temperature, °C	39.6	39.5	39.5	0.1	0.27	<0.01	0.01
Temperature SD, °C	0.574[a]	0.544[ab]	0.482[b]	0.031	0.03	<0.01	0.01
Hematologic variables							
Haptoglobin, mg/dL	62.9	4.37	2.21	6.17	<0.01	<0.01	<0.01
Cortisol, ng/mL	25.4[b]	31.0[a]	32.6[a]	2.3	0.05	0.07	0.11
Neutrophils, K/µL	4.68[a]	4.24[a]	2.76[b]	0.31	<0.01	<0.01	<0.01
Monocytes, K/µL	0.412	0.563	0.432	0.061	0.17	0.31	0.70
Eosinophils, K/µL	0.294	0.335	0.354	0.061	0.71	0.47	0.85
Lymphocytes, K/µL	6.30	6.62	6.82	0.51	0.70	<0.01	<0.01
Erythrocytes, K/µL	7.95	8.09	8.10	0.22	0.84	<0.01	0.24
Hematocrit, %	33.1	34.5	34.2	0.9	0.47	<0.01	0.66
Hemoglobin, g/dL	12.3	12.7	12.8	0.4	0.51	<0.01	0.12
MCH, pg	15.5	15.9	15.8	0.3	0.62	0.07	0.88
MCV, fL	41.8	42.7	42.3	0.8	0.74	<0.01	0.57
Platelets, K/µL	579	492	526	35	0.22	<0.01	<0.01

[1] BV = bunk visit; MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin.

Table 5. Effect of haptoglobin (HPT) responsive phenotype on metabolite concentrations from day 2 to 5 relative to inoculation with Mannheimia haemolytica (MH) or phosphate-buffered saline (control) in cattle.

	MH-Challenged				*P*- values		
Metabolite	HPT Responsive	HPT Non-responsive	Control	SEM	Group	Day	Group × Day
3-Hydroxybutyrate	0.26	0.21	0.21	0.02	0.04	0.01	0.25
3-Hydroxyisobutyrate	0.032	0.024	0.025	0.003	0.11	0.02	0.52
3-Hydroxyisovalerate	0.027	0.026	0.027	0.003	0.53	<0.01	0.06
Acetate	0.37	0.40	0.36	0.03	0.39	<0.01	0.77
Alanine	0.19	0.19	0.18	0.01	0.62	<0.01	0.78
Allantoin	0.24^{b}	0.31^{a}	0.28^{ab}	0.02	0.02	<0.01	0.62
Betaine	0.18	0.17	0.16	0.01	0.45	0.09	0.49
Creatine	0.11	0.10	0.11	0.01	0.66	0.19	0.08
Creatinine	0.071	0.071	0.071	0.003	0.99	0.05	0.93
Dimethyl sulfone	0.87	0.88	0.83	0.03	0.34	<0.01	0.95
Formate	0.083	0.089	0.083	0.01	0.44	<0.01	0.32
Fructose	0.37	0.32	0.33	0.03	0.27	<0.01	0.73
Glucose	4.78^{ab}	5.10^{a}	4.43^{b}	0.20	0.03	<0.01	0.72
Glutamine	0.21^{b}	0.25^{a}	0.23^{ab}	0.01	0.01	<0.01	0.82
Glycine	0.17	0.17	0.16	0.01	0.38	<0.01	0.43
Hippurate	0.021	0.024	0.025	0.002	0.22	<0.01	0.93
Histidine	0.029	0.035	0.031	0.002	0.11	<0.01	0.23
Isoleucine	0.14	0.14	0.14	0.01	0.56	<0.01	0.97
Leucine	0.21	0.22	0.21	0.01	0.40	<0.01	0.98
D-Lactic acid	0.021^{a}	0.019^{ab}	0.017^{b}	0.001	0.03	0.13	0.91
L-Lactic acid	2.05^{b}	2.82^{a}	2.05^{b}	0.22	0.04	0.49	0.91
Malonate	3.37	3.38	3.21	0.13	0.33	<0.01	0.95
Methanol	0.48	0.47	0.53	0.17	0.96	<0.01	0.99
Methionine	0.026	0.026	0.028	0.001	0.46	<0.01	0.99
Phenylalanine	0.088^{a}	0.081^{ab}	0.074^{b}	0.003	<0.01	<0.01	0.59
Pyruvate	0.069	0.071	0.059	0.01	0.07	<0.01	0.82
Succinate	0.005	0.006	0.005	0.001	0.59	<0.01	0.66
Threonine	0.15	0.12	0.14	0.01	0.11	<0.01	0.70
Tryptophan	0.043	0.043	0.044	0.002	0.92	<0.01	0.99
Tyrosine	0.078	0.090	0.085	0.004	0.09	<0.01	0.57
Urea	13.5	12.6	13.8	1.04	0.69	<0.01	0.70
Valine	0.29	0.32	0.29	0.01	0.14	<0.01	0.99

Table 6. Differences of performance and feeding behavior between cattle who were haptoglobin (HPT) responsive or not following inoculation with *Mannheimia haemolytica* (MH) and saline-inoculated controls in the 28 d prior to the immune challenge.

	MH-challenged				
Item[1]	HPT Responsive	HPT Non-Responsive	Control	SE	*P*-value
No. animals	9	9	18		
Performance and growth traits					
Initial BW, kg	344	358	355	7	0.37
Final BW, kg	392	402	401	8	0.63
ADG, kg	1.71	1.56	1.62	0.08	0.46
DMI, kg	11.3	10.6	11.3	0.3	0.20
F: G	6.61	6.83	7.11	0.28	0.32
*Bunk visit (**BV**) traits*					
BV frequency, events	57.9	48.5	51.8	3.9	0.23
BV duration, min	140[a]	112[b]	121[ab]	7	0.03
BV eating rate, g/min	84.5[c]	103[a]	97.9[b]	4.7	0.03
Meal traits					
Meal criterion, min	5.38	5.76	5.68	1.08	0.97
Meal frequency, events	14.3	14.0	15.2	1.5	0.77
Meal duration, min	193	160	167	10	0.07
Meal eating rate, g/min	62.7	71.3	71.8	3.5	0.11
Intensity traits					
Head down duration, min/d	78.2[a]	46.4[c]	65.0[b]	7.8	0.02
Time to bunk, min	25.1	30.6	30.6	8.0	0.84
Day-to-day variation, RMSE[1]					
DMI, kg/d	1.42	1.34	1.50	0.09	0.37
BV frequency, events	17.1[a]	11.6[b]	15.0[ab]	1.2	0.01
BV duration, min	26.9	23.7	23.7	1.5	0.19
Meal frequency, events	3.13	3.25	3.24	0.32	0.96
Meal duration, min	32.5	27.9	30.2	2.7	0.48
Head down duration, min	17.9[a]	12.2[b]	15.3[ab]	1.4	0.03
Time to bunk, min	30.5	34.6	35.0	8.2	0.90

[1]Day-to-day variance was calculated as the root mean square error (RMSE)

Table 7. Differences of performance and feeding behavior between *Mannheimia haemolytica* (MH) -challenged cattle who were haptoglobin (HPT) responsive or not and saline-inoculated controls in the 28 d post-challenge.

	MH-challenged				
Item	HPT Responsive	HPT Non-Responsive	Control	SE	*P*-value
No. animals	9	9	18		
Performance and growth traits					
Initial BW, kg	378	393	394	8	0.27
Final BW, kg	405[b]	434[a]	434[a]	9	0.04
ADG, kg/d	1.29	1.48	1.47	0.07	0.20
DMI, kg/d	9.31	10.3	10.6	0.44	0.06
F:G	7.85	7.14	7.47	0.53	0.60
*Bunk visit (**BV**) traits*					
BV frequency, events	30.0	34.5	32.1	2.5	0.46
BV duration, min	108	95.7	98.1	6.2	0.31
BV eating rate, g/min	86.8[b]	109[a]	111[a]	4.5	0.01
Meal traits					
Meal criterion, min	6.22	6.69	5.58	0.71	0.43
Meal frequency, events	11.4	10.8	12.3	0.8	0.34
Meal duration, min	135	132	126	7	0.59
Meal eating rate, g/min	70.1[b]	79.3[ab]	85.7[a]	3.5	0.01
Intensity traits					
Head down duration, min	64.8	45.7	56.6	5.9	0.09
Time to bunk, min	56.5	41.4	38.1	8.1	0.19
Day-to-day variation, RMSE[1]					
DMI, kg	1.77[a]	1.47[ab]	1.21[b]	0.12	0.01
BV frequency, events	8.89	9.76	7.44	0.82	0.07
BV duration, min	27.7[a]	19.3[b]	15.3[c]	1.6	0.01
Meal frequency, events	2.62	2.22	2.11	0.21	0.15
Meal duration, min	34.5[a]	28.6[a]	19.3[b]	2.2	0.01
Head down duration, min	17.5[a]	10.1[b]	10.5[b]	1.2	0.01
Time to bunk, min	71.6	63.2	51.1	7.8	0.10

[1]Day-to-day variance was calculated as the root mean square error (RMSE)

Table 8. Least squared means (± SEM) of changes in hemodynamics, oxygen saturation, and temperature at varying levels of inspired oxygen (FiO_2) in anesthetized calves.

	FiO2 level				
Item[1]	≤ 16%	17 - 18%	19 - 21%	≥ 22%	*P* values
No. measurements	12	52	32	38	
pHOx Ultra					
SO_2, %	76.5 ± 2.9[c]	87.8 ± 1.5[b]	94.1 ± 2.2[a]	98.2 ± 2.6[a]	<0.01
Lactate, mmol/L	4.57 ± 0.3	4.73 ± 0.1	4.84 ± 0.2	5.22 ± 0.2	0.29
pH	7.39 ± 0.01	7.38 ± 0.01	7.38 ± 0.01	7.39 ± 0.01	0.48
Polar ECG					
Heart rate, bpm	108 ± 4.2[a]	104. ± 1.7[a]	91.0 ± 2.2[b]	85.8 ± 2.3[b]	<0.01
Datascope Passport 2					
SpO_2, %	79.2 ± 2.1[c]	83.4 ± 1.0[c]	87.4 ± 1.3[b]	93.9 ± 1.2[a]	<0.01
Heart rate, bpm	104 ± 4.4[a]	103. ± 2.1[a]	91.1 ± 2.7[b]	90.1 ± 2.5[b]	<0.01
Diastolic BP, mmHg	102 ± 6.1[a]	110. ± 2.9[a]	106. ± 3.7[a]	84.4 ± 3.7[b]	<0.01
Systolic BP, mmHg	146 ± 5.7[a]	151 ± 2.7[a]	147 ± 3.5[a]	129 ± 3.5[b]	<0.01
PowerLab					
SpO_2, %	77.9 ± 2.0[d]	82.7 ± 0.8[c]	86.8 ± 1.1[b]	91.7 ± 1.2[a]	<0.01
Heart rate, bpm	101 ± 3.5[a]	103. ± 1.7[a]	89.3 ± 2.3[b]	82.0 ± 2.4[c]	<0.01
Diastolic BP, mmHg	103 ± 5.7[a]	110. ± 2.7[a]	105. ± 3.7[a]	87.5 ± 3.9[b]	<0.01
Systolic BP, mmHg	150 ± 5.2[ab]	154. ± 2.5[a]	153. ± 3.4[a]	138. ± 3.6[b]	<0.01

Different superscripts within row represent differences at $P < 0.001$.

[1] pHOx Ultra, Nova Biomedical; Waltham, MA; Polar electro-cardiogram heart rate monitor, Bethpage, NY; Datascope Passport 2, Mindray, Mahwah, NJ; BP = Blood pressure; PowerLab, AD Instruments; Colorado Springs, CO.

Table 9. Coefficients of determination between gold-standard instruments and patient-side instruments.

Instruments[1] of comparison	r^2	RMSE	*P* values
Heart rate monitors			
Polar vs Datascope heart rate	0.96	3.08	<0.01
Polar vs PowerLab heart rate	0.91	4.68	<0.01
Oxygen saturation monitors			
pHOx SaO_2 vs Datascope SpO_2	0.25	8.39	<0.01
pHOx SaO_2 vs PowerLab SpO_2	0.20	6.65	<0.01
Datascope SpO_2 vs PowerLab SpO_2	0.34	6.19	<0.01

[1] pHOx Ultra, Nova Biomedical; Waltham, MA; Polar electro-cardiogram heart rate monitor, Bethpage, NY; Datascope Passport 2, Mindray, Mahwah, NJ; PowerLab, AD Instruments; Colorado Springs, CO.

Table 10. Physiological and behavioral responses in steers experimentally challenged with bovine herpes virus-1 and *Mannheimia haemolytica* (MH; VB Challenge) or phosphate-buffered saline (PBS Control) between day -3 and 14 relative to MH inoculation.

	Treatment			*P*-values		
Parameter[1]	VB Challenge	PBS Control	SEM	Treatment	Day	Interaction
No. of steers	16	8				
Performance metrics						
Body weight, kg	284	304	4	0.01	0.01	0.01
DMI, kg/d	5.68	9.73	0.44	0.01	0.01	0.01
BV frequency, count	31.8	50.4	5.2	0.01	0.01	0.01
Rumination, min/d	200	233	41	0.49	0.01	0.01
Temperature and activity						
Rumen temperature, C	39.7	39.3	0.1	0.01	0.01	0.01
Rectal temperature, C	39.4	38.9	0.3	0.01	0.01	0.01
Activity score, units	3.51	4.87	0.39	0.06	0.01	0.01
Complete blood count						
Leukocytes, K/µL	11.9	11.3	0.8	0.63	0.11	0.05
Neutrophils, K/µL	5.74	4.21	0.33	0.06	0.02	0.05
Monocytes, K/µL	0.611	0.492	0.15	0.27	0.70	0.93
Eosinophils, K/µL	0.292	0.354	0.12	0.65	0.94	0.68
Lymphocytes, K/µL	5.20	6.25	0.34	0.03	0.01	0.12
Erythrocytes, M/µL	9.58	9.50	0.47	0.86	0.84	0.77
Hemoglobin, g/dL	12.4	13.1	0.4	0.20	0.02	0.90
Hematocrit, %	34.4	36.1	0.9	0.22	0.01	0.01
MCV, fL	41.1	37.5	4.8	0.55	0.83	0.82
MCH, pg	13.3	13.8	0.4	0.36	0.01	0.99
Platelet, K/µL	633	529	43	0.02	0.01	0.01
Blood proteins						
Fibrinogen, mg/dL	670	476	27	0.01	0.01	0.01
Haptoglobin, mg/dL	64.5	0.592	12.2	0.01	0.01	0.01
Plasma protein, g/dL	18.8	12.3	8.5	0.54	0.06	0.95

The *P*-value threshold for significance is 0.05.

[1]BV = bunk visit; Activity score = activity metric measured by a rumen bolus; MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin.

Table 11. Agreement between the iStat (Abbott; Priceton, NJ) and Prime+ (Nova Biomedical; Waltham, MA) blood gas analyzers developed from 28 arterial and 42 venous blood samples.

Item[1]	r^2	Correlation Equation
Arterial		
SO_2, %	0.07	iStat = 36.0 + 0.62 × Nova
PO_2, mm Hg	0.86	iStat = -20.6 + 1.15 × Nova
PCO_2, mm Hg	0.66	iStat = 9.86 + 0.81 × Nova
pH	0.92	iStat = 0.69 + 0.91 × Nova
Lactate, mmol/L	0.96	iStat = -0.22 + 0.92 × Nova
Venous		
SO_2, %	0.67	iStat = 21.7 + 0.69 × Nova
PO_2, mm Hg	0.77	iStat = 1.25 + 0.84 × Nova
PCO_2, mm Hg	0.81	iStat = 17.0 + 0.74 × Nova
pH	0.96	iStat = 1.37 + 0.81 × Nova
Lactate, mmol/L	0.98	iStat = -0.09 + 0.88 × Nova

[1]SO_2 = percentage of O_2 saturated hemoglobin; PO_2 = partial pressure of O_2; PCO_2 = partial pressure of CO_2.

Table 12. Blood gas and biochemical analyses in steers experimentally challenged with bovine herpesvirus-1 and *Mannheimia haemolytica* (MH; VB Challenge) or phosphate-buffered saline (PBS Control) between day -3 and 14 relative to MH inoculation.

Parameter[1]	Treatment		SEM	*P*-values		
	VB Challenge	PBS Control		Treatment	Day	Interaction
Number of steers	16	8				
Arterial						
SO_2, %	96.3	98.4	1.3	0.02	0.11	0.60
PO_2, mm Hg	96.5	121	10.7	0.01	0.01	0.40
PCO_2, mm Hg	36.4	38.7	1.8	0.21	0.05	0.01
pH	7.49	7.48	0.02	0.47	0.30	0.01
Lactate, mmol/L	4.16	3.12	0.81	0.38	0.01	0.06
Venous						
SO_2, %	65.6	68.4	2.8	0.22	0.30	0.64
PO_2, mm Hg	41.8	45.0	2.1	0.06	0.01	0.91
PCO_2, mm Hg	58.6	63.2	3.4	0.10	0.01	0.01
pH	7.31	7.28	0.02	0.06	0.01	0.01
Lactate, mmol/L	4.25	3.26	0.76	0.41	0.01	0.02

The *P*-value threshold for significance is 0.05.

[1]SO_2 = percentage of O_2-saturated hemoglobin; PO_2 = partial pressure of O_2; PCO_2 = partial pressure of CO_2

Table 13. Performance, feed efficiency, rumen temperature, activity, and feeding behavior traits in steers experimentally challenged with bovine herpesvirus-1 on day -3 and *Mannheimia haemolytica* (MH) on day 0 (VB Challenge) or phosphate-buffered saline (PBS Control) between days 21 and 77 relative to MH inoculation.

	Inoculation			
	VB Challenge	PBS Control	SEM	*P*- value
No. animals	13	8	--	--
Performance				
Initial BW, kg	303	328	6	0.01
Final BW, kg	380	409	8	0.02
ADG, kg/d	1.37	1.45	0.07	0.47
DMI, kg/d	8.68	9.84	0.40	0.06
DMI, % BW	2.53	2.67	0.10	0.28
G: F	0.163	0.152	0.014	0.20
Physiological Traits				
Rumen temperature, C	39.6	39.7	0.1	0.30
Activity, units	4.49	4.62	0.26	0.73
Rumination, min/d	265	269	20	0.88
Eating Traits				
Bunk visit (**BV**) frequency, count	43.4	46.2	2.6	0.46
BV duration, min	93.6	93.6	7.4	0.99
Head down (**HD**) duration, min	44.5	40.2	8.1	0.72
BV eating rate, g/min	98.8	107	7.3	0.46
Time to bunk, min	46.7	20.8	9.8	0.08
Meal Traits				
Meal criterion, min	8.44	7.11	0.88	0.30
Meal duration, min	144	152	6	0.40
Meal frequency, min	9.51	10.2	0.63	0.45
Meal eating rate, g/min	61.2	65.5	3.4	0.38
Day-to-day variance, RMSE[1]				
DMI, kg	1.66	1.70	0.15	0.84
BV frequency, count	13.7	15.5	0.9	0.20
BV duration, min	21.5	20.4	1.9	0.69
HD duration, min	12.0	10.4	2.0	0.58
Meal duration, min	31.7	27.1	2.1	0.14
Meal frequency, count	2.25	2.17	0.18	0.74
Time to bunk, min	75.9	38.2	11.1	0.03

[1]RMSE = root mean square error