Utility of meloxicam therapy in cattle prior to long distance transportation

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Utility of meloxicam therapy in cattle prior to long distance transportation

by

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DEDICATION

I wish to dedicate this thesis to my parents, my brother and close family members who have supported me with their time, knowledge, love and understanding. You have helped me become the person who I am today. To both of my parents; thank you for deciding two young boys needed to be involved in raising cattle. To my mother; thank you for always lending a helping hand and doing everything in your power to make my life easier during times of academic stress and pressure. Your love for me is never questioned and because of that I am eternally grateful. To my father; your purposeful or inadvertent (to this day I am not sure which) exposure to the real world of veterinary medicine started me on this path. Many long nights watching you spay a dog or run calls after school are fond memories that will be with me forever. Without your insight during times of difficulty and doubt, I would not have completed this degree. To my brother; I hope that I have served as an exemplary role model in school and life. To my Aunt; thank you for exposing me in the scientific process at a young age. That spark ignited and still burns bright to this day. Above all else, I want to thank God for every gift and privilege that was bestowed upon me. Not a day will go by in my life where I won’t be truly thankful.
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<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ACD</td>
<td>Acid Citrate Dextrose Solution</td>
</tr>
<tr>
<td>ACTH</td>
<td>Adrenocorticotropic hormone</td>
</tr>
<tr>
<td>ADG</td>
<td>Average Daily Gain</td>
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<tr>
<td>AMDUCA</td>
<td>American Medicinal Drug Use Clarification Act</td>
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<td>ANCOVA</td>
<td>Analysis of Covariance type</td>
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<tr>
<td>AMEL</td>
<td>Arrival Meloxicam</td>
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<td>ANOVA</td>
<td>Analysis of Variance</td>
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<tr>
<td>BRD</td>
<td>Bovine Respiratory Disease</td>
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<td>BVDV</td>
<td>Bovine Viral Diarrhea Virus</td>
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<tr>
<td>BW</td>
<td>Body Weight</td>
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<td>CBC</td>
<td>Complete Blood Count</td>
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<td>CD</td>
<td>Cluster Differentiation</td>
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<td>CMI</td>
<td>Cell mediated immunity</td>
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<tr>
<td>CNT</td>
<td>Lactose Placebo; Non-Transport</td>
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<tr>
<td>CONT</td>
<td>Placebo</td>
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<tr>
<td>COX</td>
<td>Cyclo-oxygenase</td>
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<tr>
<td>CTR</td>
<td>Lactose Placebo; Transport</td>
</tr>
<tr>
<td>DART</td>
<td>Depression, Appetite loss, Respiratory character change, Temperature elevation</td>
</tr>
<tr>
<td>DMI</td>
<td>Dry Matter Intake</td>
</tr>
<tr>
<td>ELDU</td>
<td>Extra Label Drug Use</td>
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<tr>
<td>ELISA</td>
<td>Enzyme-Linked Immunosorbent Assay</td>
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<tr>
<td>FDA</td>
<td>Food &amp; Drug Administration</td>
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<tr>
<td>G:F</td>
<td>Gain to Feed ratio</td>
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<tr>
<td>HCW</td>
<td>Hot Carcass Weight</td>
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<tr>
<td>HP</td>
<td>Haptogoblin</td>
</tr>
<tr>
<td>IACUC</td>
<td>Institutional Animal Care and Use Committee</td>
</tr>
<tr>
<td>IFN</td>
<td>Interferon</td>
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<tr>
<td>IL</td>
<td>Interleukin</td>
</tr>
<tr>
<td>HPLC-MS</td>
<td>High Pressure Liquid Chromatography- Mass Spectrometry</td>
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<tr>
<td>MCH</td>
<td>Mean Corpuscular Hemoglobin</td>
</tr>
<tr>
<td>MCHC</td>
<td>Mean Corpuscle Hemoglobin Concentration</td>
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<tr>
<td>MCV</td>
<td>Mean Corpuscular Volume</td>
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<tr>
<td>MEL</td>
<td>Meloxicam</td>
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<td>MFI</td>
<td>Mean Fluorescent Intensity</td>
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<td>MLV</td>
<td>Modified Live Virus</td>
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<td>MMP-9</td>
<td>Matrix Metalloproteinase 9</td>
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<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<tr>
<td>MNT</td>
<td>Meloxicam; Non-Transport</td>
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<tr>
<td>MP-FCM</td>
<td>Multiparameter Flow Cytometry</td>
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<tr>
<td>MTR</td>
<td>Meloxicam; Transport</td>
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<tr>
<td>NSAID</td>
<td>Non-Steroidal Anti-Inflammatory Drug</td>
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<td>NT</td>
<td>Non-transport</td>
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<tr>
<td>PBMC</td>
<td>Peripheral Blood Mononuclear Cell</td>
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<tr>
<td>PGE2</td>
<td>Prostaglandin E2</td>
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<tr>
<td>PMEL</td>
<td>Pre-transportation Meloxicam</td>
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<tr>
<td>PO</td>
<td>Per os</td>
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<tr>
<td>RDW</td>
<td>Red Blood Cell Distribution Width</td>
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<tr>
<td>RIA</td>
<td>Radio Immunoassay</td>
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<tr>
<td>SP</td>
<td>Substance P</td>
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<tr>
<td>TCO2</td>
<td>Total serum carbon dioxide</td>
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<tr>
<td>TNF</td>
<td>Tumor Necrosis Factor</td>
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<tr>
<td>TR</td>
<td>Transport</td>
</tr>
<tr>
<td>USDA</td>
<td>United States Department of Agriculture</td>
</tr>
<tr>
<td>VN</td>
<td>Viral Neutralization</td>
</tr>
<tr>
<td>WC</td>
<td>Work Shop Cluster</td>
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<tr>
<td>γδ</td>
<td>gamma delta</td>
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ABSTRACT

Transportation is one of the most common production practices for cattle throughout the United States and the world. During this event, cattle handling, a new environment, and other compiled life events cause stress. Transport and stress influence inflammation, immune function and subsequently can lead to bovine respiratory disease. This disease complex is one of the largest production losses that the beef industry deals with on a daily basis. In this dissertation, we tested the efficacy of a non-conventional therapeutic, meloxicam, to mitigate the negative response of transportation. The encompassed chapters hypothesized meloxicam given orally prior to transportation would reduce stress, reduce acute phase protein inflammation, reduce immune system inhibition, improve production performance benefits and improve clinical outcomes of disease. In the first trial, meloxicam was administered per os prior to a long-distance transportation at 1mg/kg. The study demonstrated a reduction in the stress leukogram and an inverse relationship of meloxicam to circulating cortisol in beef steers. In the second study, we compared the adaptive immune function invitro through multiparameter flow cytometry. Subjects were grouped in non-transport or transportation groups. Nested in these groups the subjects were treated with meloxicam or placebo. Meloxicam and transportation had an effect on adaptive immune function. Meloxicam administered prior to transport can be interpreted as inhibitory or homeostatic when compared to the singular effect of transportation on the immune system. Finally, the last phase of research was a clinical field trial to elucidate clinical outcomes of bovine respiratory disease. Three treatment groups of pre-transportation meloxicam, on arrival meloxicam and
placebo were compared. Meloxicam had no effect on clinical outcomes of BRD. In addition, there was no change in the process of disease severity or rectal temperature at BRD identification. In similarity, there was no change in average daily gain, feed conversion or body weight performance measured at 42 days. The lack of significant differences over the course of the initial receiving culminated in no observable differences of harvest parameters. The dissertation research confirms that meloxicam is beneficial when administered prior to transportation for reduction of the stress and potential normalization of immune function but lacks clinical efficacy in mitigation of disease outcomes that translate to beneficial performance and harvest outcomes.
CHAPTER 1.
GENERAL INTRODUCTION

Dissertation Organization

The present dissertation is organized into 6 chapters focusing on cattle transportation and a novel therapeutic intervention; meloxicam. This first chapter is an outline of the dissertation that will be presented in the remaining chapters. Things of relevance in this chapter are the justification for investigation and the guiding hypothesis of each chapter. The second chapter is a literature review of cattle transportation designed for publication. The review is focused on the circulating biomarkers in cattle that have been monitored prior to and after transportation. In addition, the review highlights other non-antimicrobial therapeutics that have been used before or after transport in cattle. Circulating biomarkers of interest are related to behavior, stress, immune function, and acute phase proteins. The third chapter of the doctoral thesis is a research publication focusing on the effects of meloxicam (PO) on circulating physiologic biomarkers in beef steers after long distance transportation. The fourth chapter is the second research publication of my doctoral project. This project utilized flow cytometry to investigate the effects of transport and meloxicam (PO) on adaptive immunity. The fifth chapter of my doctoral program is a clinical trial formatted for publication and focused on feed yard clinical outcomes. The trial outcomes of interest consisted of; monitoring for signs of BRD using the DART system and examining live performance weight and carcass characteristics at harvest. The comparison of this study focuses on oral administration of meloxicam prior to transportation, meloxicam on arrival and inactive excipient placebo groups. The final; sixth chapter of the thesis dissertation presents the summary and
conclusions that can be drawn from the research conducted in this dissertation as well as the guidance for future research.

The coauthors listed on the submitted manuscripts had contributions to research as described below: Johann Coetzee was my major professor and was involved on all fronts of research such as study design, manuscript feedback, data collection, and analysis. Matthew Stock and Locke Karriker were instrumental in the aid of data collection and manuscript revisions. Procurement of cattle for research would not have been possible without the guidance and support of Terry Engelken, Rhonda Vann, Caleb Lockard, Clint Krehbiel and Blake Wilson. Darrel Busby managed cattle over the feeding period. Larry Wulf was instrumental in teaching the chemistry involved for drug extraction and quantification. Walter Hsu was a mentor through the methods and process of RIA. Jeff Lakritz was critical in quantifying Hp-MMP9 through ELISA. Andrew Carpenter and Barry Bradford where critical at quantifying TNF alpha in their specialized in-house ELISA. Chong Wang and Natalia Cernicchiaro contributed additional insight and validation of my own statistical findings. James Roth and Ratree Platt were integral in the development of the flow cytometry method, and execution of the flow cytometry. Erin Kalkwarf performed the BVDV antibody titer VN assay in the Iowa State Diagnostic Lab. Data for additional biomarker quantification through annexin A1 quantified by Jeff Caswell.
Relevance of NSAIDS as a Justified Therapeutic Administered Prior to Transportation for the Reduction of Pain and Inflammation in an Effort to Mitigate Bovine Respiratory Disease.

The nonintegrated nature of the beef system as well as the spatially fixed elements of infrastructure in specific economic, geographic, and environmental conditions requires almost all beef cattle to encounter a transportation event at some point in their lifetime (Gorsich et al. 2016). Economic loss associated with transportation is closely associated with disease outcomes. Commonly “shipping fever” or fibrinous pneumonia due to *Mannheimia haemolytica* as well as other respiratory pathogens have been suggested to cost the feedlot industry an average of 7% of total production costs (Griffin 1997). BRD associated inflammation increases the overall production costs and adds to the upfront antimicrobial cost (Gifford et al. 2012). More recent surveys from the national health monitoring system has seen a two fold increase in price to treat respiratory disease since 1999 without a decline in BRD incidence (NAHMS 2013). Summaries for cattle harvested in 2015 year, according to the USDA Livestock Slaughter, reached upwards of 30 million head and accounting for total losses near 1 billion dollars annually.

Transportation effects on cattle have been evaluated extensively in multiple settings and modalities across the world. Livestock are subject to transport by road, railcar and ship (Phillips and Santurtun 2013, Ashby et al. 1979, Schwartzkopf-Genswein and Grandin 2014). Each transport experience has been suggested to have associated detrimental aspects, however they vary in severity. Transportation in the United States involves animal handling, comingling, weight loss and stress (Broom 2007). Cattle
appear to recover uneventfully from the transportation event within 24-48 hours of rest (Earley, Drennan and O'Riordan 2013). However, calves are at a higher risk to develop respiratory disease. Disease pathogenesis is a multifactorial process that ultimately results from impairment of the innate and adaptive immune responses (Caswell 2014). The impairment can be detected in circulating physiologic parameters associated with metabolism, the endocrine system, and the innate and adaptive immune system (Marques et al. 2012, Fike and Spire 2006, Bernardini et al. 2012). Variations in responses have been characterized with differences in sex, breed, age and length of the transportation events. These have been used to understand the underlying process that contributes to the stressful nature of transport predisposing cattle to disease responses with limited success.

Current practices for controlling BRD in cattle involve the use of antimicrobial metaphylaxis to combat the anticipated morbidity over the feeding period (Ives and Richeson 2015, Vandonkergoed 1992). The advent of niche marketing and consumer demand have opened marketing channels for cattle never treated with antimicrobial agents (Fox et al. 2008). With this rising consumer demand and a potential regulatory future eliminating antimicrobials as seen in the European union, other therapeutics for disease reduction or performance enhancement warrant investigation (Lean 2013). This potential shift leaves a void in the veterinary medical profession for research into novel prophylactic treatments for transportation detriments. Previous work has shown benefit with the use of non-steroidal anti-inflammatory drugs (NSAIDs) after transportation in the face of veterinary practices known to increase BRD, such as arrival castration (Coetzee et al. 2012a). The inflammatory and stress response associated with transportation is a primary source of problems for incoming calves. In addition NSAIDs
have been studied as a means for analgesic relief resulting in mitigation of the stress response in cattle (Coetzee et al. 2012b) and have been shown to reduce lung lesions (Lockwood, Johnson and Katz 2003).

There is no approved therapeutic that is labeled for pain or stress relief in cattle undergoing transportation. Veterinary approval for the use of meloxicam falls under the animal medicinal drug use clarification act (AMDUCA) of 1994 as extra label drug use (ELDU). Guidance for ELDU requires that drug use be under the guidance of a veterinarian, with approved animal and human drugs, when the health of the animal is threatened, and not for production purposes. In addition, these drugs may not be used in feed and a violative residue is prohibited. Meloxicam is a NSAID that is a member of the oxicam class and selectively but not preferentially prefers COX II inhibition (Plumb 2015). Kinetically meloxicam has been investigated to have a longer half-life than the only approved NSAID for treatment of pyrexia in cattle, flunixin meglumine (Coetzee et al. 2015). Currently meloxicam is approved for use in the European Union and Canada as follows: as an adjunct therapy for BRD, diarrhea, mastitis and the pain alleviation for calf disbudding (EMA 2016). Uses in cattle during veterinary procedures, such as castration have reduced disease outcomes in treated animals (Coetzee et al. 2012a). Due to the aforementioned information, we develop a hypothesis for candidate drug investigation as a therapeutic to impact beef steers undergoing long distance transportation.

**Guiding Hypothesis for the Dissertation**

The hypothesis of the first research project in the dissertation was the investigation of meloxicam (15 mg / tablet) delivered orally at 1 mg / kg prior to long distance transportation would reduce the inflammation and stress associated with transportation.
Previous literature implemented an efficacious dose at 1mg/kg in cattle (Coetzee et al. 2012a). We analyzed hematology and plasma parameters to confirm these outcomes. Hematology consisted of a CBC prior to transport, on arrival and six days later. Cortisol, cytokines and acute phase proteins were also analyzed at these timepoints. Meloxicam treatment reduced the stress leukogram. Drug concentration was inversely proportional to cortisol, neutrophils, and basophils. Our results allowed us to accept this hypothesis and further target investigation of meloxicam usage during long distance transportation. As a result of chapter three’s findings, we attributed the treatment effects of meloxicam required investigation of four specific aims for this dissertation: (1) stress and inflammation, (2) changes in immune function, (3) reduction in respiratory disease, (4) decreased disease translating to increased animal performance.

The fourth chapter study design evaluates the effects of meloxicam and transportation on the adaptive immune system through humoral and cell mediated responses. The null hypothesis was applied. Meloxicam administration prior to transportation would have no effect on immune system function compared to control groups. The second alternative hypothesis was directed at transport effects, suggesting that stress would be deleterious to immune function decreasing the CMI. Immune function was tested with a booster vaccination on arrival to evaluate cellular responses seven days after arrival. The cell mediated immune response of PBMC’s stimulated with BVDV antigen was quantified in vitro through flow cytometry (Platt et al. 2009). The humoral response was evaluated through antibody quantification in VN assay conducted at the Iowa State University Veterinary Diagnostic Lab. The study findings allowed rejection of the null hypothesis. This project demonstrated the drug treatment of
meloxicam was either homeostatic or inhibitory on the adaptive immune system day 7 post arrival vaccination. In addition, transportation was non-inhibitory on adaptive immune function at arrival and seven days after the on-arrival booster vaccination. This data allowed us to reject the alternative hypothesis. Though the third and fourth chapters gave valuable insight into the goals of the first two specific aims, there was still a necessary research culmination required to complete the third and fourth aims of the dissertation.

As a result of in vitro evaluation for inflammation and immune response after transportation, the fifth chapter is focused on the alternative hypothesis that meloxicam has stress reducing, homeostatic immune modulating benefits to reduce disease with in the first forty-two days of the feeding period. Due to the anti-inflammatory and analgesic properties of the NSAID, a secondary alternative hypothesis postulated that meloxicam treated steers would have increased weight gain and feed conversion over the first forty-two days. After multiple dialogues with producers and practicing veterinarians an additional arm was added to the study design in the sixth chapter. A treatment group of meloxicam administration on arrival was added to compare effects to the meloxicam administration prior to transportation and control animals. We rejected our hypotheses. There was no main effect of therapeutic meloxicam treatment on reduction of disease. Weight gain and feed conversion were also unaffected by treatment. Within the same study design the subjects utilized in chapter 5 were also used to evaluate treatment effects on carcass at the end of the feeding period. The original idea of an increased health benefit with in the first forty-two days would translate to carcass merit differences at harvest. No differences at harvest were seen between either meloxicam treated groups
and the control animals. Due to the lack of significant findings performance data was incorporated into the fifth chapter rather than becoming a standalone chapter.

Collectively, these studies suggest a benefit of meloxicam’s use biologically with in the first week of the feeding period. The benefits include a reduction in the stress response of transportation. Other effects on immune function have implications but were not translated to disease. We confirmed the stressful nature of transportation on cattle that are shipped for long distances. In addition, the stressful nature of transport is more detrimental from a disease perspective on calves that are smaller in body weight. However, the justification for use to indirectly mitigate disease using a non-steroidal anti-inflammatory has been proven ineffective. As a standalone treatment meloxicam is not beneficial as a prophylactic transportation treatment to reduce bovine respiratory disease, further research is warranted to evaluate the modality of combinatorial or successive treatments after arrival for the reduction of disease in the feed yard. The remaining chapters highlight the process and research that substantiate this finding.

**Literature Cited:**


performance of beef steers relative to bulls castrated on arrival at the feedlot. *Journal of Animal Science*, 90, 1026-1039.


CHAPTER 2.
THE MULTIFACETED EFFECTS OF TRANSPORTATION ON CATTLE PHYSIOLOGIC PARAMETERS; A REVIEW

Modified from a manuscript submitted to Animal Health Research Reviews

N.K. Van Engen,* J.F. Coetzee†

Abstract (300 words)

The goal of this review is to present a concise and critical assessment of the literature related to physiologic responses in cattle that are subjected to transportation. Over two-thirds of United States cattle are transported. Understanding trends in circulating physiologic parameters is an important part of mitigating negative effects of transportation. For the producer, linking these effects after transportation to morbidity outcomes within the first 45 days on feed (i.e., especially development of bovine respiratory disease) is critical. Physiologic parameters in circulation are of primary importance and may have value for prediction of bovine respiratory disease (BRD) on arrival and for understanding of disease pathogenesis. The results of our literature survey indicated that post-transportation immune function, increased acute phase proteins, glucocorticoids, and inflammation are a pivotal starting point for understanding disease.

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These potential biomarkers may have utility in identifying disease for targeted therapeutics so that traditional protocols that rely heavily on metaphylaxis can be avoided. Additional research is needed to develop strategies for physiological marker identification, treatment methods, or predictive behaviors to prevent respiratory disease before and after transport. This review examines the significant deleterious effects of transportation handling and stress, and current immune system translation and non-antimicrobial mitigation strategies.

**Keywords:** transport, cattle, stress, immune system, acute phase proteins

**Introduction**

Transportation of cattle is a management intervention that can occur during significant bovine life events including sorting, weaning, processing, and slaughter (Schwartzkopf-Genswein and Grandin, 2014, Swanson and J., 2001). Transportation is a stressor that predisposes calves to the development of bovine respiratory disease (BRD). At least 7% of the cost of cattle production can be attributed to BRD (Griffin, 1997). The financial costs associated with BRD-induced shipping fever are extremely high. The United States Department of Agriculture estimates that the cattle industry loses 1 billion USD each year from shipping-associated BRD. Most of the approaches to respiratory disease management in cattle are limited to vaccination and antibiotics to decrease disease prevalence and severity (Penny, 2015). The physiological changes that occur during transportation begin with dehydration (Galyean et al., 1981), lack of feed intake (Cole et al., 1988, Galyean et al., 1981), tissue damage (Murata et al., 2004), fume inhalation (Wong et al., 2004, Wong et al., 2003), and physical and psychological stress.
These changes can result in immune system inhibition from prolonged exposure to stressful stimuli. Inflammation due to cytokine and acute phase protein responses are consistent components of BRD progression. Inflammation from BRD within the lung significantly affects performance through decreases in daily gain, dry matter intake, and feed conversion (Gifford et al., 2012). The additional increases in inflammation can result in increased pyrexia- and cachexia-related energy demand, which leads to protein resorption from skeletal muscle.

Though there is evidence for decreased immune function and increased inflammation, little intervention is performed in cattle prior to transportation (Duff and Galyean, 2007). Most, if not all, of conventional on-arrival metaphylaxis or vaccination, or both, occurs within the first weeks after arrival (White et al., 2015, Richeson et al., 2006). A recent diagnostic laboratory investigation revealed that during the 3-year period from 2009–2011, the prevalence of antimicrobial resistance in submitted isolates of *Mannheimia haemolytica* increased from 5% to 35% (Lubbers and Hanzlicek, 2013). Consumer concern about resistance in meat products resulted in the development of organic and all natural product niche markets (Sofos, 2008). Though development of resistance has been documented in respiratory pathogens after antimicrobial treatment, changes in common foodborne pathogens have not been consistently found to be different (Fox et al., 2008). Valid methods that can be used to identify the cattle most susceptible to disease while in the feed yard are needed. Some early detection behavior methods have been proposed to remotely detect cattle that are displaying typical behaviors that indicate the presence of respiratory disease, without the need for human interaction (White et al.,
2015). Previous research studies have also examined the use of trends in physiologic and metabolic parameters after the cattle arrive at their destination. There is no single biomarker that indicates BRD risk upon arrival and no mitigation strategy has been found to be solely effective in decrease of BRD incidence. Compiling a trend of increasing and decreasing biomarkers may be the best possible method for indicating problematic groups of cattle, and the use of non-antimicrobial methods to help decrease BRD prevalence for incoming cattle requires investigation. For this review, relevant transportation effects on BRD risk are depicted in Figure 1; the figure illustrates the complex connections within the cascade of events that result in clinical respiratory disease.

**Deleterious Effects of Transportation**

At the cellular level, tissue damage results in release of the membrane phospholipid bilayer and incites an inflammatory cascade at the site of injury through the conversion of lipid membrane into prostaglandins and other metabolites (Enyedi et al., 2016). During road transport, the truck environment and truck-associated parameters can have characteristics with an underlying predisposition to cause tissue damage, discomfort, and added stress. Environmental stressors such as climate, nutrition, and human intervention have critical roles in animal welfare (Bova et al., 2014). The truck design, stocking density, driver, road quality, and ventilation and ambient temperature of the hauling container are important factors to consider during an assessment of transport conditions (Broom, 2008). The pre-transport loading process can be the most stressful part of the trip, and the stress response then normalizes while the cattle are in transit (Pettiford et al., 2008). Loading stress can be associated with cattle that have high
temperaments or calm temperaments (Burdick et al., 2011). During loading or transit, movement of the cattle can increase the chances for soft tissue injury. Rough roads and improper handling can cause carcass bruising, which is detrimental to animal welfare and is an additional loss in product at the packer (Huertas et al., 2010). Damage that occurs when unloading large groups of cattle into a pen is located in the perianal and hip bone area of the carcass (Honkavaar et al., 2003). If the cattle are not destined for slaughter, the bruising nature of the shipment process can affect behavior. This trauma combined with the distance traveled may be associated with the animal’s responses to the transportation event. Compared with shorter distances, travel distances greater than 40 miles from the slaughter house are associated with a statistically significant increase in bruising risk (Jarvis et al., 1996). However, bruising is inevitable regardless of the travel distance. The increases in bruising can be monitored physiologically using increases in plasma creatine kinase concentrations. One study found that increases in creatine kinase occurred in veal calves during transport, but there was no correlation with the amount of muscle bruising at harvest (Grigor et al., 2004).

After arrival at the destination, normal cattle behavior is altered as cattle recuperate. Immediately after transport, heifers spend more time at the hay feeder; they also lay down more often 1 to 2 days after transport, compared with control heifers (Theurer et al., 2013). These findings are consistent with the loss of body weight in the form of rumen gastrointestinal contents and the physical exertion required to stand the entire transport period. A split 9-hour transport, 12-hour rest, and 9-hour transport study revealed that during the first and second 9 hours in transport, bulls spent significantly less time lying down compared with their non-transported counter parts; there were no
differences in lying or standing during the 12-hour rest period (Earley et al., 2013). Counterintuitively, non-transported bulls spent significantly more time in recumbency than bulls that were transported. Results from other observational studies have indicated that on arrival and following transport, young bulls spent less time acclimating or exploring the new home pen and more time interacting with each other (Cafazzo et al., 2012).

The transport truck’s compartment environments are not equal; there are compartment-associated differences in average daily gain and disease risk (White et al., 2009). Cattle in the forward sections or transported in spaces with less than 15 head have reduced odds of treatment. Vibration of the vehicle while in motion can affect the animals during transportation. The greatest vibration occurs on gravel roads. When cattle assume a position perpendicular to the direction of travel, they experience significantly less vibration (Gebresenbet et al., 2011). Vibration originating predominantly from the vertical axis of the trailer is dependent on tire pressure (Stevens and Camp, 1979). Many truck design characteristics, and the road conditions that are traversed to move livestock from point “A” to point “B,” cannot be readily changed but there are parameters that should be controlled. The driver’s behavior during transport affects the cattle’s behavior (Cockram and Spence, 2012). Driver tendencies can be defined by the need to accelerate or brake in certain situations, but most are less frequent during highway transit. There is a lower probability of sudden changes during highway driving. The role of the driver in the prevention of animal discomfort is important and easy to address.

Increased values for PCV and total protein concentrations are evidence of the dehydration that occurs during shipping (Jarvis et al., 1996). Dehydration is directly
related to disease due to the disruption in the mucociliary apparatus function of the 
respiratory epithelium (Ackermann et al., 2010). The ambient temperature in the 
transportation environment is a potential cause of dehydration. When the temperature is 
outside the animal’s normal critical limits, energy expenditure increases to maintain body 
temperature homeostasis. Most trailers that are hauling cattle depend on movement to 
provide ventilation; a stationary position results in stagnant conditions. Quantification of 
the trailer’s temperature climate is difficult to interpret when one temperature logger is 
used; multiple loggers should be used to obtain more accurate information (Goldhawk et 
al., 2014a). However, there are some management benefits from limited logger 
information. Time of year and ambient conditions are key inputs for decisions and 
associated risks during transport (Goldhawk et al., 2014b). In-transit temperatures >30°C 
have adverse effects on thermoregulation. Heifers shipped at high ambient temperature 
(>32°C ) had significant behavioral changes, weight loss, and peripheral 
vasoconstriction-associated nasal and rectal temperature decreases (Theurer et al., 2013). 
The most significant aspects of transport for consideration and avoidance are transporting 
unfit cattle and long-distance transportation without stops for food, water, and rest 
(Schwartzkopf-Genswein and Grandin, 2014). An unavoidable loss of bodyweight (i.e., 
shrink) occurs during transport. This effect usually occurs due to the lack of feed and 
water, and increased defecation and urination. Marques et al. found that food deprivation 
in transport is the major factor that accounts for lost performance (Marques et al., 2012). 
They transported bulls for 24 hours and allowed one period of rest at 12 hours with no 
access to food or water. The animals had statistically significant decreases in body 
weight, compared with control animals who were rested and received rations. The
decreased performance in this study was identical between groups of animals fasted for 24 hours, despite transportation effects. The effectiveness of a rest stop with access to food and water for avoiding the performance losses during transport was examined by (Cooke et al., 2013b). The results for use of a 2-hour rest stop at the half-way point of a 1,290-km journey with ad libitum hay and water access were not different from the results for straight-through transport. Only the non-transported control animals had greater feed to gain and average daily gain values. Even when transporting young calves for a duration of 19 hours, there is no benefit from a 1-hour feeding stop (Knowles et al., 1999). This finding is supported by other results that calves regain their original body weight by 24 to 72 hours post-transport (Knowles et al., 1997). The losses occur during a wide time interval. Most of the gut contents are lost during first 24 hours of transport, but the greatest rate of loss occurs within the first 12 hours (Knowles, 1999).

Shrink is a measure of performance, but it is also associated with the health of cattle that are transported. Loss of bodyweight in lighter weight cattle may be connected to increased morbidity and mortality, but increased hot carcass weight and average daily gain during the post-transport period (Cernicchiaro et al., 2012b). For the body weight loss variable, there were similar outcomes for morbidity, mortality, hot carcass weight, and average daily gain when distances traveled were compared (Cernicchiaro et al., 2012a). However, as the distance increased the likelihood of a negative effect health outcomes also increased. Cernicchiaro et al. found that procurement of cattle from specific regions of the United States correlated with increased post-transport morbidity. Other study findings indicated there were no correlations between the shrink that results from short- or long-term transportation and the development of respiratory disease.
(Ribble et al., 1995b). Ribble et al. found a strong correlation between comingling calves from different sources and subsequent development of BRD (Ribble et al., 1995a). These conflicting results indicate the multifactorial nature of the BRD process. However, it is highly likely that comingling, arrival weights, and shrink are associated with disease risk.

During the transportation process cattle are exposed to potentially irritating diesel fumes. Fumes can consist of organic compounds (e.g., aldehydes, benzenes, and polycyclic aromatic carbons) that are disruptive to the mucosal epithelium (Wierzbicka et al., 2014). Wong et al. found that exposure of rats to diesel exhaust can cause neurogenic pulmonary responses that can perpetuate lung inflammation (Wong et al., 2003). Diesel fume disturbance and disruption of the respiratory mucosal system can be a large contributor to respiratory impairment and subsequent disease (Riedl and Diaz-Sanchez, 2005). Other heavy metals (e.g., vanadium) that are released during exhaust excretion of diesel fuels cause inhibition of the innate defenses of the respiratory epithelium (Klein-Patel et al., 2006). Fumes and their effects on respiratory disease are difficult to study in cattle and are best interpreted through extrapolation of research findings from other mammalian species.

**Biomarkers of Stress**

Components of the bovine endocrine system and non-endocrine physiological parameters have been used to quantify the level of stress. (Arthington et al., 2003) Neural-hormonal biomarkers of interest have been identified (i.e., ACTH, cortisol, catecholamines, and iodothyronines). The rapid release and degradation of these hormones and the natural, circadian rhythm release during the day present a challenge to the use of these markers for biological assay (VanCauter et al., 1996).
Glucocorticoids (specifically cortisol) are released during stressful situations, but responses are delayed due to transcriptional downstream effects from cytoplasmic receptor complexes that interact with DNA. Glucocorticoid interference can inhibit signaling mechanisms (e.g., TLR-4) during dendritic cell maturation; these signaling mechanisms are critical for the innate defense response and lipopolysaccharide recognition (Rozkova et al., 2006). Other DNA transcription effects increase during glucocorticoid upregulation of the gluconeogenic capacity of the liver to use amino acids and fatty acid mobilization for energy creation (J.E., 2006). In addition to metabolic functions, cortisol has inhibitory effects on inflammation, vascular permeability to white cells, and the immune system (i.e., primarily for lymphocyte reproduction) (J.E., 2006). Although not studied extensively in cattle, the effects of circadian rhythms are important for the interpretation of cortisol levels over periods of time; there are variations in normophysiologic secretions throughout the day (VanCauter et al., 1996).

The hypothalamus pituitary adrenal (HPA) axis should be considered during investigation of transportation effects on cattle. Sustained stressor events result primarily in corticosteroid and aldosterone release (vonBorell, 2001). Cortisol is the main corticosteroid measured in studies of the stress response (Table 1). Cortisol can be used as a biomarker that is linked with behavior responses in cattle; anxiety is indicated by less time ruminating and increased vocalization (Bristow and Holmes, 2007). Cortisol identification methods include measurement in plasma, hair, and the fecal contents of calves during transport (Marti et al., 2017, Mostl et al., 2002, Arthington et al., 2003). Results for beef bulls indicated that 4.5 hours after the start of a 9-hr transportation event there was a 321% increase in cortisol concentrations, compared with a 24-hr baseline.
sample that was taken before transportation began (Buckham Sporer et al., 2008). Other
groups and reviews confirm that the cortisol increase that occurs during transportation
can be used as a marker of stress (Odore et al., 2011, Knowles, 1999, Gupta et al., 2007).
Ruling out a novel environment as a cause of stress is important. Arrival at an original
starting point location versus a naïve arrival location had minimal effects on cortisol
levels (Browning and Leite-Browning, 2013).

Effects revealed after longer transport distances can potentially cause a decrease
in the cortisol response that may be due to the extensive fasting or exhaustion associated
with the journey (Van Engen et al., 2014). Supporting results indicated that increased
transport time correlated with a decrease in the level of plasma cortisol concentration
(Bulitta et al., 2015, Gebresenbet et al., 2012). This change could be caused by the
animal’s acclimation to transport. However, attributing the cortisol response only to
transportation-related variables excludes other variables associated with the cortisol
response. Cattle handling and processing variables may also contribute to the stress
response. In addition to animal handling, the effects of the individual animal’s
temperament should be considered when cortisol is examined (Burdick et al., 2010).
Regardless of differences in animal handling style, temperament can confound the
outcome. Conclusions have greater validity when temperament is not a factor.

Counterintuitively with the HPA axis, corticotropin releasing hormone can have
alternate effects from cortisol. Corticotropin releasing hormone increases the
inflammatory response of macrophages in vitro and incites increased production of
inflammatory cytokines (Agelaki et al., 2002). ACTH did not increase prostaglandin
levels in pregnant cows (Geary, 2012). This result suggested that specific pathways of inflammation can be affected by physiologic fluctuations in CRH, ACTH, and cortisol. Regardless of circulating cortisol levels in response to stressors, it is important to note that there is a complex relationship between glucocorticoid synthesis and development of BRD (Senthilkumaran et al., 2013). Annexin A1 is a protein found in epithelial secretions. Concentrations of annexin A1 correlate with circulating cortisol concentrations. However, calves that do not develop BRD while in the feed yard have increased Annexin A1 concentrations, compared with calves that do develop BRD (Senthilkumaran et al., 2013). This finding emphasizes the complex associations between glucocorticoids, stress, and BRD.

A short startle response induces the hypothalamic adrenal medullary system (i.e., the fight or flight response) and causes increased sympathetic tone. Similar to cortisol, epinephrine and norepinephrine responses are correlated with animal temperament (Table 1). Compared with calm bulls, temperamental bulls have elevated epinephrine levels following transportation (Burdick et al., 2010). Two studies found that transportation alone increased norepinephrine (Odore et al., 2004) and epinephrine (Odore et al., 2004, Aktas et al., 2011), but temperament was not included in the analyses of the results of either of these studies. The short half-lives of epinephrine and of norepinephrine within the biological system negatively affects the value of these parameters as biomarkers for the identification of stress.

Tri-iodothyronine (T₃) has many and diverse functions in tissues; T₃ is a metabolically important hormone. The effects of periods of stress due to handling and transport on changes in T₃ levels have been investigated. The results indicated that there
was an increase in T₃ concentrations after transport and after handling events, but there were no differences between the post-transport and post-handling T₃ concentrations (Mitchell et al., 1988). Mitchell et al. (1988) suggested that the transport effect was confounded by the need to handle the animals after transport and that the effect of handling, not transport, was the cause of the T₃ increase. Further research on post-transport T₃ levels is needed, but one plausible hypothesis is that increased metabolism mobilizes nutrients during the fasting that occurs during transport.

Cortisol and catecholamines control metabolism in the liver and are included in pathways important for processing carbohydrates (gluconeogenesis), proteins, and lipids (lipolysis) (Gifford et al.). Investigations of specific hematological outcomes associated with circulating energy modifications are of interest because of the shrink and fasting effects of transport. Non-esterified fatty acids, glucose, and albumin levels in blood were elevated, and beta-hydroxybutyrate levels in blood were decreased, after transport (Earley and O'Riordan, 2006, Cafazzo et al., 2012, Earley et al., 2010). Other results indicate there is an increase in beta-hydroxybutyrate following transportation, which is relevant to a metabolic fasting state (Bernardini et al., 2012). Increases in these elements of a blood panel are consistent with the effects of cortisol during the stress response. Breed differences in cortisol response between a group of Brahman steers and a group of Hereford steers may account for the significant glucose increase in the Brahman, compared with the Hereford, group (Browning and Leite-Browning, 2013). Although glucose increases, the return to homeostasis occurs within 24 hours of the transport event (Earley et al., 2010). Lipid mobilization also occurs during the shrink and fasting states of transportation. Mobilization is linked with increased non-esterified fatty acid levels in
circulating blood after transportation (Cooke et al., 2013a). Non-esterified fatty acid increases are linked to catecholamine release (Agnes et al., 1990). A reduction in non-esterified fatty acid metabolism occurred in cattle transported for 1,290 km when there were two, 2-hour stops with feed and water (i.e., at each 430 km). However, this intervention reduced but did not eliminate the increase in the non-esterified fatty acid response (Cooke et al., 2013b).

Metabolic markers can be used for more than indicators of fasting. Lactate concentrations can be significantly elevated after transport and correlated with transport time (Chacon et al., 2005, Bulitta et al., 2015). Lactate is a marker for transport-associated muscle fatigue; longer distances and increased standing times can cause increased lactic acid production due to oxygen depletion and the inability to re-phosphorylate ADP (Sahlin, 1986). The circulating blood level of creatine kinase has also been studied as a marker of muscle damage. Most investigators have found elevated creatine kinase levels upon arrival (Bernardini et al., 2012, Earley et al., 2010, Grigor et al., 2004, Van de Water et al., 2003). However, the results of another study indicated that there were no significant changes in creatine kinase following transport (Cafazzo et al., 2012). Increases in creatine kinase can be used as an indicator for exertion associated with transport. However, fluctuations in creatine kinase levels do not always result in significant effects and can vary based on trip- and animal-associated differences.

Results from a castration trial indicated that the neuropeptide substance P (i.e., a derivative of the nociception response) can be used as a biomarker (Coetzee et al., 2008). Other implications of the peptide in physiology include effects on mast cell degranulation and migration of macrophages to the lung epithelium during acute respiratory infection.
Substance P has been examined during transportation events. Increases have been documented on arrival and at 4 hours after arrival, compared with baseline levels (Theurer et al., 2013), and at 24 hours post initiation of transportation (Van Engen et al., 2014). Discovery and expansion of the pain biomarker library in cattle is crucial for understanding of welfare issues.

**Immune System Translation**

Increased stress and glucocorticoid release engenders the loss of a host’s ability to prevent BRD. The immune system should be included in an examination of ways to prevent disease and potential pathways of pathogenesis.

Stress and inflammatory responses can mediate immune system signaling through changes in the peripheral blood cellular profile. Transportation is accountable for a stress-induced neutrophilia (Earley et al., 2006, Earley and O’Riordan, 2006). There are two different understandings of neutrophil functionality after transportation. Indications have been made for a reduction of neutrophil oxidoreductase activity at arrival and increases four hours after arrival (Murata et al., 1987). Others report reductions in phagocytic neutrophil intensity at 48 h post-transit (Burdick et al., 2011). Linking these changes has been investigated at an expression level. The increasing circulating neutrophil genetic expression level suggests an increase in antibacterial function. Neutrophilia upregulation and the corresponding bacteria killing properties occur through increases in tissue remodeling matrix metalloproteinase 9, and decreased expression of the apoptotic marker FAS and extravasation cell marker L selectin. (Buckham Sporer et al., 2008). Neutrophil phagocytosis and oxidative burst capacity increase, and beta 2 integrin essential for extravasation out of the vasculature decreases, after transport
Transport leads to neutrophils that are activated in circulation but with a diminished ability to enter tissue, and a neutrophilia occurs. Hulbert and colleagues also found that upregulation of phagocytosis creates resistance to a microbial threat in calm-disposition cattle compared with temperamental cattle. The numbers of neutrophils expressing a betaglycan gene correlated with decreased lung lesions and pulmonary adhesion, when the gene expression was elevated 7 days after transportation (Eitam et al., 2010). However, the increased betaglycan results were not statistically significant following a 9-hour transportation (Buckham Sporer et al., 2007). Betaglycan is of primary interest because it binds to TGFβ. TGFβ is involved in the development of lung pathology that results in fibrosis of lung parenchyma (Bartram and Speer, 2004). Betaglycan has potential as a genetic marker for BRD in calves that are arriving in the feed yard. Betaglycan measurement may be a tool that can be used to identify calves that would eventually succumb to the effects of chronic lung fibrosis.

Lymphocytes are the main adaptive immune functioning cells in the bovine. Transportation events are associated with decreases in the numbers of peripheral circulating lymphocytes (Earley and O'Riordan, 2006). These events have also been associated with decreased lymphocyte blastogenesis (Murata, 1989) and function (Murata and Hirose, 1991). Despite decreasing lymphocyte counts after transport, the level of circulation natural killer cells is positively correlated with the increasing post-transport levels of cortisol (Ishizaki and Kariya, 2010). A decrease in the ratio of lymphocytes to granulocytes is accounted for by an increase in circulating granulocytes (Kang et al., 2017). Post-transport lymphocyte changes in expression cause significant upregulation of the heat shock proteins 70A1A and 60, which has a cytoprotective effect (Eitam et al.,
Lymphocytes were taken from transported Holstein-Friesian bulls, cultured, and stimulated with concanavalin A; the decrease in IFNγ production suggested there was a decrease in immune function (Gupta et al., 2007). Other findings have suggested that IFNγ is not inhibited in specific T cell populations upon stimulation; in some cases it is enhanced by transportation when cells are stimulated in vitro (Van Engen et al., 2016). In transported pregnant dairy heifers, the change in the different populations of lymphocytes has been described as a shift to CD 8+ cells (Kang et al., 2017). Deciphering the age-related changes in T cell populations is critical when considering susceptibility to BRD complex because most of the circulating population in young calves consists of γδ T cells.

Most of the literature describing adaptive immunity in cattle following transportation discusses the humoral response. Polyclonal antibody concentrations increase within the first 15 days after a transport event (Razzuoli et al., 2016). This result suggests that calves can have an immunological response after a transportation event. Calves naïve to keyhole limpet hemocyanin were vaccinated against the antigen to determine the differences in immune responses between transport and non-transport animals for several antibody classes. After 10 days, transported calves had significant increases in IgG1 through a weaning*transport interaction. At 20 days, IgG1 was increased in the transport group compared with the not transported calves. However, there were no changes in IgA or IgM on either of those days (MacKenzie et al., 1997). Different vaccination choices (e.g., use of a modified live virus vaccine) can change the outcome of a calf’s immune system responses, depending on age at vaccination (Richeson et al.). Conversion of an antibody response indicated that was a higher BVDV
type 1 antibody titer at 14 days compared with delay vaccinates, without a compromise in performance during a 56-day receiving period (Richeson et al., 2009). Vaccination within the receiving period results in variability in performance outcomes, and positive and negative effects. The result of a comparison between cell mediated immunity on arrival and at 7 days after use of an on-arrival vaccination also supports the hypothesis of a maintained immune function response (Van Engen et al., 2016). The results suggest that transport does not have a substantial prolonged effect on a parenterally induced immune response. Immune function impairment associated with shipping stress could be confined to the lung and increases the likelihood of shipping fever. In contrast, stress is a result of prolonged and multifactorial events and transport alone is not sufficient to decrease immune function.

Inflammatory cytokine signals are released during reactions to infection, tissue injury, and stress. Lipopolysaccharide challenge in whole blood 48 hours after transport indicated there was a statistically significant 40% increase in tumor necrosis factor (TNF) α expression, followed by a statistically significant 59% decrease at 96 hours, compared with pre-transport baseline values (Carroll et al., 2009). TNF in serum alone has also been found to increase after transportation (Van Engen et al., 2014). An increase in proinflammatory cytokines (e.g., IL 6 and TNF α) can be attributed to a novel environment and can be modulated for 15 to 30 days after arrival (Razzuoli et al., 2016). The release of corticotropin releasing hormone is linked to the release of circulating immune system proinflammatory cytokines (Hulbert et al., 2011). Description of different cytokine profiles is warranted, but there is currently no single marker that is a valid indicator of shipping fever outcomes.
Acute phase proteins have been suggested as markers of post-transportation stressor events (Arthington et al., 2003). There is a crucial need to develop a valid method that can be used to identify cattle requiring or that will require prophylactic treatment for BRD. Acute phase proteins released from hepatocytes are potential markers, but they have poor functionality as identifiers for specific diseases (Tothova et al., 2014). They do provide indications of fulminant and subclinical inflammation and when used together may indicate that a disease process is imminent (Ceron et al., 2005). Haptoglobin, serum amyloid A, ceruloplasmin, and fibrinogen are the proteins routinely investigated for cattle. The investigation of acute phase proteins as markers for potential BRD has value. However, the results of a limited meta-analysis indicated that they have no diagnostic accuracy for, and are not valid indicators of, BRD (Abdallah et al., 2016). When the prevalence of disease is low, acute phase proteins have poor utility as a standalone diagnostic tool (Seppa-Lassila et al., 2017).

Circulating haptoglobin released from hepatocytes binds free hemoglobin and reduces oxidative damage (Murata et al., 2004). Calves inoculated with respiratory bacterial pathogens had marked increases in the acute phase protein haptoglobin; this protein is a potential diagnostic tool for detection of pulmonary inflammation in calves (Hanthorn et al., 2014). Haptoglobin has limited merit for detection of BRD on arrival to determine whether targeted prophylactic treatment should be used (Holland et al., 2011). Calves with detectable haptoglobin values had significantly higher severity scores and rectal temperatures at the first, second, and third treatments and were more likely to be treated sooner after arrival (Holland et al., 2011). The release of haptoglobin is a response to exogenous corticotropin releasing hormone; this result suggests that it is connected to
stress events (Cooke et al., 2012). Long transportation events lead to a significant increase in the release of haptoglobin (Table 2). Haptoglobin has poor utility as a biomarker that can be used to distinguish between chronic and acute inflammation (Bannikov et al., 2011). Use of haptoglobin and matrix metalloproteinase complexes may improve the validity of haptoglobin’s use as a biomarker. Matrix metalloproteinases are secreted from numerous immune cells (e.g., macrophages).

Serum amyloid A has also been implicated as part of acute inflammatory disease processes and has potential as a marker for respiratory disease (Alsemgeest et al., 1994). The production of serum amyloid A is directly related to inflammatory cytokine production, and is usually a response to viral infection (Yamada, 1999). Large increases in circulating serum amyloid A concentrations are associated with decreased antibody production (Benson and Aldobenson, 1982), enhanced monocyte chemotaxis (Badolato et al., 1994), and decreased neutrophil oxidative burst capacity (Linke et al., 1991). The serum amyloid A concentration can increase ten-fold by 48 hours after transportation (Lomborg et al., 2008). Even when cattle were exposed to only a 3-hour transportation event, there was a 46% increase in serum amyloid A concentration over the 7 days following transportation (Arthington et al., 2003). Serum amyloid A concentration is a sensitive marker for respiratory infection or, through interpretation, the inflammation of disease (Orro et al., 2011).

Ceruloplasmin is a tissue protectant produced during iron-mediated free radical injury. It has anti-inflammatory properties and has potential as an indicator of infection (Murata et al., 2004). The metalloenzyme concentration is correlated with the serum copper concentration and has been suggested as a useful indicator of nutritional copper
status for cattle (Pourjafar and Dehkordi, 2008). Investigations of events with infectious etiologies have found significant increases in young calves (<30 days of age) clinically infected with rotavirus (Rocha et al., 2016). One study found that there was a 28% increase in ceruloplasmin concentration at 7 days after shipment in one experiment; a 48% increase occurred during the 21-d post-transport period in another experiment (Arthington et al., 2003). The results of most investigations quantifying ceruloplasmin during transportation are consistent with the increasing trend found in the serum. The increase in ceruloplasmin has been associated with decreases in growth rates in cattle (Cooke et al., 2009). Other studies have found that ceruloplasmin concentration is negatively correlated with average daily gain and positively correlated with circulating cortisol level (Araujo et al., 2010).

Fibrinogen is a substrate that allows for fibrin formation; it is key in tissue repair by providing support and a binding matrix for the extravasation of cells associated with inflammation (Murata et al., 2004). Fibrinogen has been found to increase and decrease after transportation. After a long-distance transport by sea and road, calf fibrinogen levels were increased by 29% compared with the pre-transport values and were 22% higher than the control values (Earley et al., 2012). These increases occur within circulating serum. The results for fibrinogen measured within the lung during times of stress indicate that an opposite response occurs. Analysis of lung fluid extracted using bronchial alveolar lavage in stressed and non-stressed treatment groups revealed a marked decrease in fibrinogen 12 hours after exposure to the stressor was terminated; this result suggests there was a loss of permeability due to the stress-associated release of glucocorticoids and catecholamines (Mitchell et al., 2008). There is a stronger correlation between circulating
fibrinogen levels and bacterial respiratory pathogens (e.g., *M. haemolytica* and *Pasteurella multocida*) than to viral pathogens under field conditions (Nikunen et al., 2007). The critical role of fibrinogen in the bovine is a major focus of disease and clinical pathology interpretation for veterinarians.

Acute phase proteins are a potential hematological identifier of transport stress and predisposition to BRD. However, even though general increases occur, concentrations can vary between breeds and values be interpreted with caution (Qiu et al., 2007). Rather than using the change in a singular biomarker, reporting the magnitude of changes in multiple circulating markers could be used for decision making. The current understanding of acute phase proteins and other proteomics concentrates heavily on a small number that are found in high concentrations. The transition to liquid chromatography-mass spectrometry screening (LC-MS) for identifying novel proteins at lower concentrations may increase the number of potentially useful proteins.

**Mitigation Strategies**

Investigation of methods to alleviate the negative effects of transport and prevent morbidity and mortality and enhance receiving performance in the feed yard continues. Pre-transportation and on-arrival treatments and methods vary. Most methods are based on a nutritional, behavioral, and/or non-antimicrobial pharmaceutical approach.

Pre-transportation treatment using vitamin A, vitamin D, and vitamin E had no effect on shrink associated with transport (Jubb et al., 1993). Investigation of the use of vitamin E supplementation on arrival in a population of high-risk calves experiencing 64.5% morbidity revealed a decrease in the treatment costs associated with the cattle that were fed 2000 IU vitamin E (Carter et al., 2000). This article did not report any results
indicating increased weight gain or feed efficiency with treatment. However, a study of stressed heifers treated using vitamin E after shipment found that at days 14–28 there was an increased feed efficiency and average daily gain, but other performance and health variables were unaffected (Choat et al., 2000). Trace minerals such as chromium have also been administered on arrival as an additive feed supplement in the range of parts per million. Chromium supplementation results in increases in average daily gain, presumably through increased dry matter intake at differing levels of Cr supplementation. The decreases in morbidity that occurred from day 2 to 30 were greatest in the group supplemented with 0.2 ppm Cr (Moonsieshageer and Mowat, 1993).

Nutrition supplements in the form of energy, specific amino acids, and electrolytes that are given prior to transportation result in gains in hot carcass weight as cattle are transported to harvest (Grumpelt et al., 2015). Supplementation for caloric gain in arrival diets from rumen protected poly-unsaturated fatty acids exhibited negative effects on weight performance and intake. However, a decrease in circulating haptoglobin concentration was a beneficial effect (Araujo et al., 2010). The differences in supplementation of a linolenic or a linoleic acid can promote differences in specific inflammatory prostaglandin production (Yaqoob and Calder, 2007). Ultimately, the differences in the inflammatory mediators could change the immune response to an anti-inflammatory or inflammatory T cell response.

Electrolyte supplementation in cattle before transportation helps to mitigate changes in osmolality on arrival compared to control animals (Schaefer et al., 1992). Compiled evidence was insufficient to substantiate a reduction in stress however increased performance at harvest was noted through increased hot carcass weights and
decreased live weight loss for treated animals (Schaefer et al., 1997). Multiple-day transport durations on a boat are associated with electrolyte benefits in cattle (Beatty et al., 2007). Overall, the literature does not support a production or health advantage for live cattle entering a feed yard with electrolyte supplementation prior to transportation. Most electrolyte research results indicate that there is an added benefit at harvest (Schaefer et al., 1997).

Behavior methods aimed at decreasing morbidity have also been investigated. The use of trainer cows for incoming steers upon arrival after transport resulted in an increase in performance and health for some of the treatment groups, but overall the results between groups were not consistent enough to be conclusive (Loerch and Fluharty, 2000). Remote early disease identification was used to monitor behavior in calves within the first 45 days of arrival at the feedlot (White et al., 2016). This method shows promise for detection of calves before the traditional method of visual identification can be used. It also identified animals that had lower rectal temperatures. It might be useful for identification of animals during the early stages of the disease process (i.e., before cytokines are released in response to illness) (White et al., 2015).

Strategies based on the use of pharmacological techniques have also been investigated as options to mitigate negative effects (e.g., loss of performance, stress, and pain) and promote animal welfare. Some approaches used in older studies have aggressively targeted the stress response. Therapeutics involving the use of an adrenolytic agent (metyrapone) to suppress cortisol production prior to transportation have been studied (Agnes et al., 1990). Metyrapone treatment does not reduce the increase in non-
esterified fatty acid levels after transportation; the long-term effects were not described. Improved veterinary practices for transported animals include the use of nonsteroidal anti-inflammatory drugs (NSAIDs). Intravenous administration of flunixin meglumine (an FDA approved NSAID for the treatment of pyrexia in cattle) did not restore performance on arrival at the feed yard, but the results suggested that it contributed to a reduction in cortisol, haptoglobin, and ceruloplasmin until day 4 after transport (Cooke et al., 2013a). Flunixin treatment did result in a significant increase in non-esterified fatty acid concentrations that persisted through the fourth day after transport, compared with the non-treated transport group. The NSAID meloxicam may reduce effects of stress and inflammation. An inverse relationship between circulating plasma drug concentration and cortisol, neutrophil, and basophil counts has been reported (Van Engen et al., 2014). Beyond the suggestion of a decrease in stress and inflammation, there may also be an added benefit in terms of increased performance on arrival. Meloxicam-treated calves had greater and more efficient weight gain upon arrival. Meloxicam also reduced haptoglobin production, compared with an untreated transport group (Guarnieri et al., 2014). The translation of these added benefits has not been proven to carry through into the post-transport period or result in a decrease in morbidity. Additional investigation of pharmacological practices that are not associated with metaphylaxis antibiotic treatment is warranted.

**Conclusion**

Transport effects vary and so do the subsequent physiological responses. Other variation is attributed to differences in age, sex, temperament, and recent environmental exposure. Physiologic responses are influenced by multifaceted effects that ultimately begin with
dehydration, feed deprivation, tissue damage, and diesel fumes. These connections to stress related glucocorticoids and inflammatory cytokines require emphasis during research investigation and veterinary communication with producers. Drastic and prolonged changes in stress, acute phase proteins and cytokines leads to inflammation and immunosuppression. This cascade of factors is attributed to pathogen induced disease and decrease of feedlot health. There is a clear need to develop a model using these markers to predict BRD and develop therapeutic measures that concentrate on decreasing the aforementioned markers to promote active immune function without the use of antibiotics. This goal is attainable through the continued understanding of cattle physiologic responses and focusing research on addressing the variables discussed in this review. Moving forward, research projects conducted on cattle transportation should monitor for BRD and report findings. There is a need for connection of clinical related BRD morbidity to these markers.
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respiratory syncytial virus. *Comparative Immunology Microbiology and
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EFFECT OF PRETRANSIT MIXING ON FATAL FIBRINOUS PNEUMONIA
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AND SHRINKAGE IN CALVES ARRIVING AT A LARGE FEEDLOT.
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health, performance and serum IBR titer levels of newly-received stocker cattle.


Figure 1. Influence of transportation on bovine respiratory disease. A visual flow diagram depicting the intricacies of the role transportation has on generating bovine respiratory disease in the feed yard. Arrows connect the hierarchy of cascading effects incited by transportation starting with the visually observed and progressing to the underlying physiology with in the animal that results in respiratory disease outcomes. Boxes or arrows with a dash line represent changes monitored after transport.
*All arrows in table 1 reference transported animal’s stress biomarker response in comparison to either baseline value or a non-transported control group.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Transport Duration</th>
<th>Study Population</th>
<th>Stress Biomarker</th>
<th>Effect of transport*</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Price et al., 2015)</td>
<td>2 hours</td>
<td>10 Brahman cows</td>
<td>Cortisol</td>
<td>↑</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>(Romero et al., 2014)</td>
<td>4 hours</td>
<td>65 Zebu steers</td>
<td>Cortisol</td>
<td>↑</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>(Van Engen et al., 2014)</td>
<td>1,316 km</td>
<td>97 Brahman cross steers</td>
<td>Cortisol</td>
<td>↓</td>
<td>P&lt;0.01</td>
</tr>
<tr>
<td>(Browning and Leite-Browning, 2013)</td>
<td>4 hours</td>
<td>Brahman and Herford</td>
<td>Cortisol</td>
<td>↑</td>
<td>P&lt;0.01</td>
</tr>
<tr>
<td>(Cooke et al., 2013a)</td>
<td>1,280 km</td>
<td>45 Angus*Hereford</td>
<td>Cortisol</td>
<td>↔</td>
<td>P&lt;0.09</td>
</tr>
<tr>
<td>(Cooke et al., 2013b)</td>
<td>1,290 km</td>
<td>63 Angus*Hereford</td>
<td>Cortisol</td>
<td>↑</td>
<td>P&lt;0.01</td>
</tr>
<tr>
<td>(Falkenberg et al., 2013)</td>
<td>4 hour</td>
<td>22 Holstein heifers</td>
<td>Cortisol</td>
<td>↑</td>
<td>P&lt;0.01</td>
</tr>
<tr>
<td>(Theurer et al., 2013)</td>
<td>518 km</td>
<td>20 Crossbred heifers</td>
<td>Cortisol</td>
<td>↑</td>
<td>P&lt;0.01</td>
</tr>
<tr>
<td>(Bernardini et al., 2012)</td>
<td>19 hour</td>
<td>7 Holstein calves</td>
<td>Cortisol</td>
<td>↑</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>(Burdick et al., 2011)</td>
<td>4 hour</td>
<td>15 Brahman bulls</td>
<td>Cortisol</td>
<td>↑</td>
<td>P&lt;0.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Epinephrine</td>
<td>↔</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Norepinephrine</td>
<td>↔</td>
<td>NS</td>
</tr>
<tr>
<td>Reference</td>
<td>Transport Duration</td>
<td>Study Population</td>
<td>Acute Phase Protein</td>
<td>Effect of transport*</td>
<td>Significance</td>
</tr>
<tr>
<td>---------------------------------</td>
<td>--------------------</td>
<td>-----------------------------</td>
<td>---------------------</td>
<td>----------------------</td>
<td>--------------</td>
</tr>
<tr>
<td>(Odore et al., 2011)</td>
<td>45 min</td>
<td>32 piedmontese</td>
<td>Cortisol</td>
<td>↑</td>
<td>*P&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Epinephrine</td>
<td>↑</td>
<td>*P&lt;0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Norepinephrine</td>
<td>↑</td>
<td>*P&lt;0.05</td>
</tr>
<tr>
<td>(Buckham Sporer et al., 2008)</td>
<td>9 hours</td>
<td>12 Angus, 12 Fresian, 12 Belgian blue*Fresian bulls</td>
<td>Cortisol</td>
<td>↑</td>
<td>*P&lt;0.001</td>
</tr>
<tr>
<td>(Gupta et al., 2007)</td>
<td>12 hours</td>
<td>72 Holstein Fresian</td>
<td>Cortisol</td>
<td>↑</td>
<td>*P=0.0001</td>
</tr>
<tr>
<td>(Odore et al., 2004)</td>
<td>950 km</td>
<td></td>
<td>Cortisol</td>
<td>↑</td>
<td>*P&lt;0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Epinephrine</td>
<td>↔</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Norepinephrine</td>
<td>↑</td>
<td>*P&lt;0.05</td>
</tr>
<tr>
<td>(Arthington et al., 2003)</td>
<td>344 km</td>
<td>32 Brahman cross</td>
<td>Cortisol</td>
<td>↔</td>
<td>NS</td>
</tr>
<tr>
<td>(Bulitta et al., 2015)</td>
<td>12 hours</td>
<td>Mature cows and bulls</td>
<td>Cortisol</td>
<td>↓</td>
<td>*P&lt;0.0001</td>
</tr>
<tr>
<td>(Gebresenbet et al., 2012)</td>
<td>2, 4-6, 10-11 hours</td>
<td>Cows, bulls, and calves</td>
<td>Cortisol</td>
<td>↓</td>
<td>*P&lt;0.01</td>
</tr>
</tbody>
</table>

*All arrows are in table 1 reference transported animal’s stress biomarkers response in comparison to either baseline value or a non-transported control group.
**Table 2**

Summary of the scientific literature examining the changes in acute phase proteins during transportation

<table>
<thead>
<tr>
<th>Reference</th>
<th>Transport Duration</th>
<th>Study Population</th>
<th>Acute Phase Protein</th>
<th>Effect of transport*</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Cooke et al., 2013a)</td>
<td>1,290 km</td>
<td>63 Angus x Hereford</td>
<td>Haptoglobin</td>
<td>↑</td>
<td>P &lt; 0.04</td>
</tr>
<tr>
<td>(Guarnieri et al., 2014)</td>
<td>1,440 km</td>
<td>84 Angus x Hereford</td>
<td>Haptoglobin</td>
<td>↑</td>
<td>P &lt; 0.03</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ceruloplasmin</td>
<td>↑</td>
<td>P &lt; 0.04</td>
</tr>
<tr>
<td>(Cooke et al., 2013a)</td>
<td>1,280 km</td>
<td>45 Angus x Hereford</td>
<td>Ceruloplasmin</td>
<td>↑</td>
<td>P &lt; 0.03</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Haptoglobin</td>
<td>↑</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>(Van Engen et al., 2014)</td>
<td>1,316 km</td>
<td>97 Brahman x Angus</td>
<td>Haptoglobin</td>
<td>↑</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Fibrinogen</td>
<td>↔</td>
<td>NS</td>
</tr>
<tr>
<td>(Holland et al., 2011)</td>
<td>957 km</td>
<td>360 heifers</td>
<td>Haptoglobin</td>
<td>↑</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>(Earley et al., 2013)</td>
<td>18 hours</td>
<td>30 Charolais</td>
<td>Haptoglobin</td>
<td>↑</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>(Marques et al., 2012)</td>
<td>1,200 km</td>
<td>45 Angus x Hereford</td>
<td>Haptoglobin</td>
<td>↑</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ceruloplasmin</td>
<td>↑</td>
<td>P &lt; 0.04</td>
</tr>
</tbody>
</table>

*All arrows in table 2 reference transported animal’s acute phase protein response comparison to either baseline value or a non-transported control group.*
Table 2: Continued

<table>
<thead>
<tr>
<th>Reference</th>
<th>Transport Duration</th>
<th>Study Population</th>
<th>Acute Phase Protein</th>
<th>Effect of transport*</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Earley et al., 2012)</td>
<td>2 days</td>
<td>52 continental bulls</td>
<td>Haptoglobin</td>
<td>↑</td>
<td><em>P</em>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Fibrinogen</td>
<td>↑</td>
<td><em>P</em>&lt;0.01</td>
</tr>
<tr>
<td>(Averos et al., 2008)</td>
<td>27 hours</td>
<td>62 young bulls</td>
<td>Haptoglobin</td>
<td>↑</td>
<td><em>P</em>&lt;0.001</td>
</tr>
<tr>
<td>(Lomborg et al., 2008)</td>
<td>4-6 hours</td>
<td>6 Holstein cows &amp; 2 Holstein heifers</td>
<td>Haptoglobin</td>
<td>↑</td>
<td><em>P</em>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Serum Amyloid A</td>
<td>↑</td>
<td><em>P</em>&lt;0.01</td>
</tr>
<tr>
<td>(Buckham Sporer et al.,</td>
<td>9 hour</td>
<td>12 Angus</td>
<td>Haptoglobin</td>
<td>↓</td>
<td><em>P</em>&lt;0.001</td>
</tr>
<tr>
<td>2008)</td>
<td></td>
<td>12 Friesian</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>12 Belgian Blue x Friesian</td>
<td>Fibrinogen</td>
<td>↓</td>
<td><em>P</em>&lt;0.001</td>
</tr>
<tr>
<td>(Arthington et al., 2003)</td>
<td>344 km</td>
<td>32 Brahman cross</td>
<td>Haptoglobin</td>
<td>↓</td>
<td><em>P</em>&lt;0.04</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Fibrinogen</td>
<td>↑</td>
<td><em>P</em>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ceruloplasmin</td>
<td>↑</td>
<td><em>P</em>&lt;0.01</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Serum Amyloid A</td>
<td>↑</td>
<td><em>P</em>&lt;0.05</td>
</tr>
</tbody>
</table>

*All arrows in table 2 reference transported animal’s acute phase protein response comparison to either baseline value or a non-transported control group.*
CHAPTER 3.

IMPACT OF ORAL MELOXICAM ON CIRCULATING PHYSIOLOGICAL BIOMARKERS OF STRESS AND INFLAMMATION IN BEEF STEERS AFTER LONG DISTANCE TRANSPORTATION

Modified from a manuscript published in the *Journal of Animal Science*


Abstract

Transportation stress can result in significant economic losses to producers due to decreased animal productivity and increased medication costs associated with sickness such as bovine respiratory disease (BRD). Meloxicam (MEL) provides pain relief and anti-inflammatory effects in cattle for several days after a single oral treatment. Our

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*** Anatomy and Physiology, Kansas State University
hypothesis was that MEL administration before shipping would reduce the impact of long
distance transportation on circulating physiological biomarkers of stress and
inflammation in beef steers. Ninety-seven beef steers were blood sampled for baseline
biomarker determination and then randomly assigned to receive either 1 mg/kg MEL (n =
49) or a placebo (CONT) (n = 48) per os prior to a 1,316-km transportation event lasting
approximately 16 h. Calves were then blood sampled on arrival and 5 d later. Changes in
the hemogram, circulating plasma proteins, total carbon dioxide (TCO₂), fibrinogen,
substance P (SP), cortisol, haptoglobin (Hp)-matrix metalloproteinase-9 (MMP-9)
complexes and tumor necrosis factor alpha (TNFα) between treatments over time were
compared using a Mixed Effects Model with statistical significance designated as \( P <
0.05 \). ANCOVA was conducted to assess the relationship between circulating MEL
concentrations and biomarker changes over time. An increase in neutrophil, platelet,
monocyte, white blood cell and red blood cell counts occurred after transportation (\( P <
0.0001 \)) and a decrease in lymphocyte count was observed (\( P < 0.0001 \)). MEL treatment
reduced the stress-induced neutrophilia (\( P = 0.0072 \)) and circulating monocyte count (\( P =
0.013 \)) on arrival. Mean corpuscle hemoglobin (\( P = 0.05 \)), mean corpuscle volume (\( P =
0.05 \)) and lymphocyte count (\( P = 0.05 \)) was also greater in the CONT calves compared
with MEL calves after transportation. Furthermore, HP-MMP-9 complexes, TCO₂,
TNFα, plasma proteins and SP increased and cortisol decreased after shipping (\( P < 0.01 \)).
MEL treatment tended to reduce serum cortisol concentrations (\( P = 0.08 \)) and there was
evidence of a time-by-treatment interaction (\( P = 0.04 \)). An inverse relationship between
plasma MEL concentrations and circulation cortisol concentrations (\( P = 0.002 \)),
neutrophil (\( P = 0.04 \)) and basophil counts (\( P = 0.03 \)) was also observed. The results
suggest that MEL administration may reduce the impact of long-distance transportation on circulating physiological biomarkers of stress and inflammation in beef calves.

Introduction

Transportation of calves intended for beef production is a common practice. In the United States, at least 65% of calves are shipped to feedlots in Texas, Kansas, Nebraska and Colorado, usually by truck, due to a geographical separation between calf rearing and finishing facilities (Swanson and Morrow-Tesch, 2001; USDA, 2009). Immunosuppression after transportation predisposes calves to developing BRD, which commonly occurs in the first 45 d of arrival and is associated with 65 to 80% morbidity and 35-55% mortality (Cernicchiaro et al., 2012; Edwards, 1996; Speer et al., 2001). BRD is estimated to cost the beef industry $500 million annually (Miles, 2009).

Therefore, even a modest reduction in stress after transportation could have a significant economic benefit for livestock producers (Speer et al., 2001).

Transportation is associated with an increase in circulating pro-inflammatory cytokines in cattle leading to the initiation of an inflammatory reaction (Arthington et al., 2003). Previous studies support the hypothesis that extended exposure to a non-steroidal anti-inflammatory drug (NSAID) may reduce stress and improve performance after castration and dehorning (Baldrige et al., 2010; Coetzee et al., 2012b). Meloxicam is an NSAID approved in Canada for pain relief in calves after disbudding and is used as an anti-inflammatory for dogs in the United States. Meloxicam was found to reduce the incidence of BRD in calves after castration when administered on arrival at the feedlot (Coetzee et al., 2011). However, a similar effect was not observed in calves received as steers. We hypothesized that MEL administration to beef steers before shipping may be
effective at reducing the impact of transportation on stress biomarkers. The objective of this study was to assess stress biomarkers and inflammatory mediators in beef steers that received oral MEL or a CONT before long distance transportation.

**Materials and Methods**

Before the initiation of this experiment, all animal use, handling, and sampling techniques described were approved by the Iowa State University Animal Care and Use Committee (IACUC protocol # 5 – 12 – 7355 - B).

**Animals, Housing, Treatment Allocation, and Shipment**

This study was a randomized complete block design with cattle assigned to receive either oral meloxicam (MEL) or a whey protein placebo (CONT) prior to shipment based on bodyweight (Figure 1). Ninety-seven medium-large frame, horned & polled, Brahman, and Angus X Brahman crossbred steers aged 15 – 17 mo, weighing 201 to 465 kg were procured from the Brown Loam Experiment Station at the Mississippi State University research farm near Raymond, MS after a 32 wk backgrounding period. All calves received preweaning vaccinations for infectious bovine rhinotracheitis, bovine viral diarrhea types I and II, parainfluenza-3 virus, bovine respiratory syncytial virus (Pyramid 5, Boehringer Ingelheim, St. Joseph, MO) clostridial organisms (Ultra bac 7, Pfizer Animal Health, Madison, New Jersey), and *Mannheimia haemolytica* (One Shot, Pfizer Animal Health, Madison, New Jersey) and were dewormed at the facility with eprinomectin (Eprinex, Merial, Duluth, Georgia). Post weaning vaccinations consisted of a booster for all pathogens stated prior to the start of the backgrounding period.
Following weaning in October 2011, calves were held for 60 d in Brown Loam Research Facility in Raymond, MS feedlot. Calves were initially fed a diet of soy hull pellets at 2% of their BW followed by free choice hay and 20% crude protein molasses. Animals were then maintained on ryegrass pastures until late June when they were allowed access to crabgrass and Bermuda grass pastures prior to shipping. If pasture was limited at any point in time hay was provided. The hay in both instances contained mixed grasses but primarily consisted of Bermuda grass.

Baseline blood sample collection occurred at time point 0 which was immediately followed by administration of meloxicam or the placebo. Calves were ranked by BW and assigned to either a MEL or CONT-treated group by a randomization table generated using Excel (Microsoft Corp., Redmond, WA). To facilitate dose determination and to minimize disruption during processing, calves were subdivided into 3 groups based on weights determined at study enrollment. These groups were as follows: light (201 - 314 kg), medium (319- 370 kg), and heavy (380 - 465 kg) weight. At time zero (0 h) MEL tablets (MEL Tablets USP 15 mg [NDC 0378 – 1089 - 01], Mylan Pharmaceuticals Inc. Morgantown WV; Lot # 3032625) were administered to the three groups so that the average weight per group resulted in a mean dose of 1 mg meloxicam/kg BW. The doses were calculated using pretreatment BW from the previous wk in addition to adjusted weight gain during that wk. When weighed, no restrictions to food or water were implemented. The light group (n = 31) received an actual dose of 0.9 to 1.4 mg/kg BW. The medium group (n = 33) received an actual dose of 0.89 to 1.0 mg/kg BW, and the heavy group (n = 33) received an actual dose of 0.90 to 1.1 mg/kg BW. Calves in the CONT-treated group received an equivalent dose of whey protein supplement product.
(Health-watchers, inc. Bohemia, NY), a pharmaceutically inactive excipient used in the manufacture of MEL tablets. Both treatments were administered in gelatin capsule boluses (Torpac, Fairfield, NJ; Lot # 2634). Whey protein was also added as filler to the capsules containing meloxicam tablets so that operators remained masked to treatment group.

After treatment, calves were shipped approximately 1,316 km from Raymond, MS a feedlot facility near Tabor, IA in 2 truckloads carrying 48 and 49 calves respectively. Loading was coordinated for the trucks to arrive at the 24 h time point. Calves were blood sampled in Iowa on 10 July 2012 after a 16 h transportation event that concluded approximately 24 h after baseline blood collection. Calves were then maintained in an open pen 192 m² with an allowance of approximately 0.69 m of bunk space per animal and one water source per pen. All calves were housed in the same pen. Upon arrival calves were fed a typical receiving diet composed of dry corn, dry distiller's grain, corn silage, and a protein/vitamin/mineral supplement throughout the experiment. Feed and water were offered *ad libitum*. A final blood sample was collected at 5 d after arrival, 144 h after baseline sample collection at 0 h.

**Collection of samples**

Blood samples were collected at 0 h, 24 h, and 144 h via direct puncture of the jugular vein to assess complete blood count (CBC) and to determine plasma concentrations of MEL, total carbon dioxide (TCO₂), haptoglobin (Hp)-matrix metalloproteinase-9 (MMP-9) complexes, tumor necrosis factor alpha (TNFα), cortisol, and substance P (SP). Blood samples were collected in 6 ml evacuated tubes that
contained lithium heparin (Vacuette Heparin tubes, Greiner Bio-One, Monroe, NC) (MEL and cortisol concentrations), serum separator tubes (Vacuette SST tubes, Greiner Bio-One, Monroe, NC) (TCO₂, TNFα, Hp-MMP 9) or EDTA tubes (Vacuette K3 EDTA tubes, Greiner Bio-One, Monroe, NC) (CBC and SP concentrations). Benzamidine hydrochloride (Santa Cruz Bio Technology, Dallas, TX; Lot # D1510), was added to one of the EDTA tubes at 1 mM per mL of whole blood to reduce endogenous protease-associated degradation of SP in the samples. Blood samples were centrifuged for 15 min at 1,600 x g. All samples were processed within 30 min of collection. Immediately after centrifugation, plasma was harvested, placed in cryovials, and frozen at –70°C until analysis.

**Plasma Meloxicam Analysis**

Plasma concentrations of MEL were determined using high-pressure liquid chromatography (Surveyor MS Pump and Autosampler, Thermo Scientific, San Jose, CA, USA) with mass spectrometry detection (TSQ Quantum Discovery MAX, Thermo Scientific, San Jose, CA, USA) as previously described (Kreuder et al., 2012). The accuracy of the assay for MEL in bovine plasma was 99 ± 3% of the actual concentration while the coefficient of variation was 5% determined on 4 sets of replicates for each of the following concentrations: 15, 150, and 1500 ng/mL.

**Cortisol Analysis**

Plasma cortisol concentrations were determined by RIA using a cortisol kit (Siemens Coat-a-Count Kit, Siemens, Malvern, PA) according to manufacturer instructions. The range of detection was from 3 to 1000 ng/mL. The coefficient of
variation for intra assay variability was 10% and the inter assay variability was calculated < 15%

**Substance P Analysis**

Substance P concentrations were analyzed as described by Liu et al. (2008) using non-extracted plasma. Method validation using non-extracted plasma consisted of complete recovery (± 15 %) of a known SP concentration added to pooled baseline plasma. Plasma concentrations of SP were analyzed by RIA using double antibody system with a primary antibody vs. SP (1:60,000, purchased from Phoenix Pharmaceuticals, Burlingame, CA (Lot # H-061-0) and 125-I-substance P (20,000 cpm) purchased from PerkinElmer Inc (Waltham, Massachusetts, USA). EDTA (13 mM). Immunoassays have been utilized previously to determine SP concentrations and have been validated for use with bovine plasma (Coetzee et al., 2008; Theurer et al., 2013). The assay had a determination range of 5 to 160 pg/ml. The coefficient of variation for intra assay variability was at 11 % and the inter assay variability was calculated at 4 %.

**Total serum carbon dioxide analysis**

Total carbon dioxide concentration (T CO₂) was analyzed using the serum collected from steers at 0 h and 24 h. Samples were centrifuged immediately after collection and all analyses were completed within 48 h after collection to minimize the effect of sample handling on total CO₂ concentrations (Tinkler et al., 2012). The samples were analyzed using Nova 4, a total CO₂ analyzer (Nova Biomedical, Waltham Massachusetts), following manufacturer’s instructions. Data analysis was completed
through the use of a known carbon dioxide standard kit (Verichem Laboratories, Providence, Rhode Island).

**TNFα Analysis**

Tumor Necrosis factor α was analyzed using a bovine TNFα ELISA test that was previously validated by Farney et al. (2011). The optical density was then measured at 450 and 550 nm using a plate reader (PowerWaveXS; BioTek Instruments Inc., Winooski, VT). The corrected difference values were fit to the standard curve by point-to-point regression. Values of detection ranged from 3.9 to 250 pg/mL with intra-assay and inter-assay critical variances of 14.1 % and 15.9 %, respectively.

**Hp-MMP 9 Analysis**

An ELISA specific for bovine neutrophil haptoglobin in complex with matrix-metalloproteinase 9 (Hp-MMP 9) was performed as described previously (Bannikov GA et al., 2011) with minor alterations. Standard concentrations ranged from 1.78 ng/mL to 456 ng/mL which represent the upper and lower limits of quantitation of the assay respectively. The concentration of Hp-MMP 9 was determined using the linear portion of the equation of the line described by absorbance of the calibrators at 450 nm and the known concentration of these calibrators. Any sample remaining outside of the range of the standard curve ( > 456 ng/mL) were diluted further and re-analyzed. Between plate variability of calibrators from five plates was less than 3 % (median = 1.8 %; range 0.98-2.7 %). The average correlation coefficient determined by linear regression of the
absorbance versus concentration of the calibrators was 0.91 (range 0.85 to 0.95). The analytical sensitivity of the assay was 3.5 ng/mL.

Clinical pathology analysis

The Iowa State University Clinical Pathology Laboratory determined the CBC using the ADVIA 120 analyzer (ADVIA 120, Siemens, Malvern, PA) using a program specific for bovine blood. This automated process measures total white blood cell count, white blood cell differential, red blood cell count, platelet counts, hemoglobin, fibrinogen, mean corpuscle volume (MCV), mean corpuscle hemoglobin concentration (MCHC), mean corpuscle hemoglobin (MCH), red blood cell distribution width (RDW), and hematocrit.

Statistical Analysis

The statistical analysis of the study was performed using SAS (Version 9.2, SAS Institute, Inc., Cary, NC). Responses were analyzed using linear mixed models with repeated measures. Animal was the subject of repeated measures whereas Truck was included in the model as a random effect. Two structures of fixed effects were considered. One is analyses of variance (ANOVA) structure with treatment group, time and their interaction; the other is an analysis of covariance type (ANCOVA) with MEL concentration (continuous) and time (categorical) included in the model. Furthermore, MMP values were analyzed following quantification of MMP as either having the presence (“yes”) or absence (“no”) of the protein in measurable quantities. A mixed effect logistic regression model that contained repeated measures was used to describe the data.
Model assumptions were considered to be appropriately met based on diagnostics conducted on studentized residuals. Estimated least square means and corresponding standard errors, or 95% confidence intervals, are presented. A significant difference was considered to be present when \( P \leq 0.05 \), and a marginal difference was considered to exist if \( 0.05 < P \leq 0.10 \). Relevant pairwise comparisons were conducted when the significance of the interaction term was \( P \leq 0.10 \) using Tukey-Kramer or Bonferroni adjustments, as appropriate in each case, to avoid inflation of Type I error rate due to multiple comparisons.

**Results and Discussion**

Meloxicam is an NSAID of the oxicam class that is approved in the European Union and Canada for adjunctive therapy of acute respiratory disease, diarrhea, and acute mastitis and the alleviation of pain associated with disbudding in calves when administered at 0.5 mg/kg intravenously (IV) or subcutaneously (SC) (EMEA, 2009). Meloxicam administered to cattle by any route constitutes extra-label drug use (ELDU) because there are currently no analgesic drugs specifically approved to provide pain or stress relief in livestock in the United States (Smith and Modric, 2013). Under the Animal Medicinal Drug Use Clarification Act, 1994 (AMDUCA), ELDU is permitted only under veterinary supervision “when the health of an animal is threatened, or suffering or death may result from failure to treat” provided specific conditions are met (US Food and Drug Administration, 1994). In the absence of FDA-approved analgesic compounds in food animals, use of oral meloxicam tablets for alleviation of pain or stress in cattle can be considered under AMDUCA. To our knowledge, this is the first published report evaluating the effect of a long-acting NSAID on the stress response in cattle after long-
distance transportation. Further studies are needed to assess if this translates to improved health and performance on arrival at the feedlot.

Plasma meloxicam concentrations

None of the calves had detectable plasma meloxicam concentrations in samples collected at the baseline time point. Mean (±SEM) plasma meloxicam concentrations in MEL-treated calves ranged from 1,868 ± 92.16 ng/mL on arrival at the feedlot (24 h after dosing) to 38.38 ± 6.18 ng/mL at 144 h after dosing. There was no difference in plasma meloxicam concentrations between the 3 different weight categories of calves that were used for dose determination ($P = 0.90$). There were also no meloxicam detected in calves assigned to the CONT group.

The pharmacokinetics of meloxicam after oral administration at 1 mg/kg indicate that a peak plasma concentration ($C_{\text{max}}$) of approximately 3 μg/mL occurs at around 12 h after administration ($T_{\text{max}}$) with an elimination half-life ($T_{\frac{1}{2}}$) of about 28 h (Coetzee et al., 2009, 2011). In the present study, the mean meloxicam concentration determined at 24 h after oral administration was less than the peak concentration previously reported. However, the mean concentration of meloxicam recorded at 144 h represents a $T_{\frac{1}{2}}$ of approximately 24 h and is consistent with the level expected after approximately 5 to 6 plasma elimination half-lives. Taken together the results of this study suggest that meloxicam administered at 1 mg/kg provided circulating meloxicam concentrations for up to 5 d after oral administration.
**Body weight (BW)**

Calves experienced a characteristic decrease in BW after the transportation event known as “shrink.” This weight loss was reported as a time effect ($P < 0.001$) with no treatment effect observed between groups ($P = 0.67$) (Figure 2). Furthermore, there was no association between plasma MEL concentrations and shrink ($P = 0.78$) (Table 4). These results are consistent with previously reported studies evaluating weight loss following transportation from a North American review on animal welfare (Schwartzkopf-Genswein et al., 2012). The observed weight loss in this study is consistent with previous reports in the literature. However, the calves in our study showed an 8% loss in BW where as Arthington and others (2003) reported a 2 to 3% loss in BW after a 3 h transportation event and Buckham Sporer et al. (2008) reported shrinkage of 10% in calves (approx. 230 kg) transported for over 9 h. This supports the hypothesis that percentage of weight loss during transportation is likely associated with the duration of the transportation event as was previously suggested (Cernicchiaro et al, 2012).

**Cortisol**

Serum cortisol concentrations were affected by time ($P < 0.0001$) in both the MEL and CONT groups. The concentration of cortisol decreased from baseline to 24 h after transportation and increased marginally at the 144-h sample collection (Figure 3). The present study provided evidence of a time-by-treatment interaction on cortisol response ($P = 0.04$) and an inverse relationship between circulating cortisol concentrations and MEL concentrations ($P = 0.0017$) (Table 4).
The decrease in cortisol observation contradicts previous published reports that demonstrate an increase in cortisol ranging from 5.8% in 2 to 3 wk mixed breed calves transported for 8 to 24 h in duration (Knowles et al., 1997) to 311% in 4 to 6 mo old dairy calves transported for 4 h (Murata et al., 1987). Elevated cortisol concentrations in serum are maintained after exogenous ACTH stimulation for approximately 2 h (Lay et al., 1996). The longer travel time in the present study may have allowed calves to become acclimated to their surroundings, thus reducing blood cortisol levels while on the truck in transit. Moreover, the sample obtained at 24 h may not have coincided with peak cortisol concentrations following transportation.

The observed time by treatment effect was likely due to the combined anti-inflammatory and analgesic effect of NSAIDs in addition to a proposed direct modulation of ACTH release resulting in a reduction in circulating cortisol concentrations (Watson et al., 2009). Recently, Geary (2012) reported that circulating cortisol concentration in cows receiving the NSAID flunixin meglumine was numerically less than control cows after injection of ACTH to mimic transportation stress. It is likely that this study, which only comprised 20 animals, lacked sufficient statistical power to detect a significant difference between groups. Taken together the relationship between NSAID administration and stress reduction after transportation warrants further investigation in order to determine if this results in improved health and performance of calves after long-distance transportation.
**Haptoglobin-Matrix Metalloproteinase 9 (Hp-MMP 9) complexes**

The majority of the serum samples contained < 15 ng/mL Hp-MMP 9 complexes (253 / 288 samples). Therefore, in addition to conducting a repeated measures analysis on the positive results, we also chose to classify samples as positive or negative based on the cutoff of <15 ng/mL (negative) and > 15 ng/mL (positive). Overall 2.1 % of the calves were positive at first sampling time, 15.6 % were positive at 24 h after arrival and 18.9 % were positive at 4 d after arrival. However, using this analysis there was no effect of treatment ($P = 0.97$), time ($P = 0.88$) or time-by-treatment interaction ($P = 0.59$).

In the calves that did demonstrate quantifiable increases in Hp-MMP 9 complexes, a time effect ($P = 0.0061$) was observed in both treatment groups (Figure 4) but no treatment effect ($P = 0.88$) was observed. There was also no evidence of a relationship between MEL concentrations and logHp-MMP-9 ($P = 0.74$) (Table 4).

Matrix metalloproteinases regulate the degradation of extracellular matrix protein components and modulates cytokine activation through in-vivo cleavage. Bannikov et al., (2007) reported that neutrophil MMP-9 is covalently complexed with neutrophil synthesized haptoglobin suggesting that this is a potentially useful biomarker indicating activation and degranulation of neutrophils (Theurer et al., 2013). Increases in plasma Hp-MMP-9 concentration over time are consistent with other studies reporting significantly elevated plasma Hp-MMP-9 concentrations in cattle with acute inflammation (Bannikov et al., 2011). A number of disorders commonly diagnosed in cattle are characterized by a significant mobilization and activation of neutrophils (Slocombe et al., 1985). The present study documents for the first time that plasma
concentrations of Hp-MMP 9 are associated with physiologic alterations during transportation.

Fibrinogen

Circulating fibrinogen concentration was not significantly affected by transportation ($P = 0.17$) or MEL administration ($P = 0.41$) (Table 1). There was also no association between MEL concentrations and fibrinogen ($P = 0.42$) (Table 4).

Fibrinogen maintains homeostasis by providing a substrate for fibrin formation and tissue repair (Murata et al., 2004). Fibrinogen results for cattle during transportation remain equivocal. A 133% increase in fibrinogen was reported in a study with 32 calves that were transported for 3 h (Arthington et al., 2003). Conversely, a 28% reduction in fibrinogen over 48 h after transportation was reported in a study involving 36 calves transported for 9 h (Buckham-Sporer et al., 2008). Therefore the usefulness of fibrinogen as a biomarker of inflammation after transportation requires further investigation.

Plasma Protein

Plasma protein concentrations increased over time after transportation ($P < 0.0001$) most notably at the 24 h sampling time point ($P < 0.001$) as illustrated in Table 1. Plasma protein was unaffected by treatment ($P = 0.32$) and there was no association between plasma MEL concentrations and plasma protein ($P = 0.32$) (Table 4).

Hyperproteinemia was anticipated after transportation as a result of dehydration due to inadequate water intake and water loss during shipping. This observation is in agreement with previous reports (Parker et al., 2003; Earley et al., 2006a; Earley and O’Riordan, 2006b). However, another study reported a decrease in plasma protein after a
9 h transportation event (Buckham Sporer et al., 2008). This was attributed to altered protein metabolism associated with transportation or differences in the nutritional status of animals immediately before transportation. It was also reported that breed may influence plasma protein concentrations but these effects could not be established in the present study.

*Tumor Necrosis Factor alpha (TNFα)*

A significant increase in circulating TNFα concentrations was observed after transportation ($P < 0.001$) (Figure 5). However, there was no effect of MEL administration on TNFα concentration ($P = 0.79$). There was also no association between plasma MEL concentrations and TNFα concentrations ($P = 0.96$) (Table 4).

Cytokines are signaling proteins produced primarily by macrophages in response to infection, tissue injury or stress. Elevated levels of TNFα increase vascular permeability of endothelial cells, which allows for the increased entry of immunoglobulin, complement and other cells to damaged tissue (Janeway et al., 2012). An increase in cytokine production after transportation was anticipated based on a recent publication that reported a 40% increase in TNFα at 48 h after a 4h transit event (Hulbert et al., 2011). Although a recent study indicated a reduction of TNFα in calves with BRD treated with MEL and oxytetracycline, a treatment effect was not observed in the present study (Bednarek et al., 2003). The direct mechanism behind this effect is poorly understood and requires further investigation.
**Substance P**

An increase in plasma SP concentrations was observed after transportation \( P < 0.0026 \) (Figure 6), but there was no treatment effect \( P = 0.13 \) or time by treatment interaction \( P = 0.45 \). There was also no association between plasma MEL concentrations and SP \( P = 0.89 \) (Table 4).

Substance P is an 11-amino acid neuropeptide associated with areas of the neuroaxis involved in the integration of pain, stress, and anxiety (Devane et al., 2001; Coetzee et al., 2008). The present study supports the results of a recently reported 46% SP increase following 4 h of transportation (Theurer et al., 2013). Although an inverse linear relationship of meloxicam and SP has been recently reported in a dehorning study (Coetzee et al., 2012), MEL administration did not reduce SP in the present study. The absence of an effect could be associated with a reduced magnitude of SP release after transportation compared with dehorning.

A wide variety of physical and psychological stressors can stimulate release of SP. Pulmonary exposure to diesel exhaust fumes, ozone, allergens, carbon dioxide and cigarette smoke can elicit SP release in airways in rats (Wong et al., 2004a; Wong et al., 2004b). The role of SP in the pathogenesis of BRD has yet to be elucidated. In experimental *M. haemolytica* infection in sheep there was strong immunoreactivity for SP on macrophages infiltrating into the inflamed areas on d 1 post-infection (Ramirez-Romero et al., 2001; Grubor et al., 2004). Furthermore, exposure of alveolar macrophages to SP resulted in enhanced phagocytosis and TNF production compared to non-stimulated controls (Rogers et al., 2006). An increase in vascular permeability in
response to SP exposure has also been demonstrated (Ragsdale et al., 2008). Taken together these studies indicate that transportation increases SP release and that this may be associated with the development of pulmonary inflammation that may contribute to the pathogenesis of BRD. However, administration of an NSAID appeared to be ineffective at directly mitigating SP release after shipping in the present study.

Total CO$_2$

There was an increase in total CO$_2$ (TCO$_2$) during transit ($P < 0.0001$) (Table 1) but there was no treatment effect ($P = 0.51$) or association between plasma MEL concentrations and TCO$_2$ ($P = 0.90$) (Table 4).

An increase in TCO$_2$ after shipping has been previously reported as a result of a decrease in potassium, chloride and water from sweating (Stockham et al., 2008). However, in a study investigating the partial pressure of carbon dioxide (pCO$_2$) after transportation, no difference was reported for transported steers compared to control animals (Parker et al., 2003). TCO$_2$ of serum or plasma provides a measure of acid-base balance due to metabolic changes. The increase in TCO$_2$ observed in the present study is likely indicative of either a metabolic or respiratory alkalosis (Dehkordi and Dehkordi., 2011). Metabolic alkalosis is most frequently associated with digestive disturbances in ruminants. Respiratory alkalosis is caused by hyperventilation, which may be stimulated by pulmonary disease or heat stress. Both types of alkalosis may therefore be present during long distance transportation where animals are subjected to food deprivation and exposed to changes in environmental temperature.
Hematology

Time effects were observed for all hematological data \((P < 0.05)\), except for basophil count \((P = 0.84)\) and mean corpuscle hemoglobin concentration (MCHC) \((P = 0.45)\) (Table 3). Treatment effects were observed for total leukocyte counts \((P = 0.01)\), mean corpuscle volume \((P = 0.05)\), mean corpuscle hemoglobin \((P = 0.05)\), and lymphocyte count \((P = 0.05)\) (Table 2). A time by treatment interaction was evident for monocyte count \((P = 0.04)\) and a trend towards an interaction was observed for red blood cell distribution width (RDW%) \((P = 0.07)\) and neutrophil count \((P = 0.10)\).

A stress leukogram, characterized by a mature neutrophilia, lymphopenia, and monocytosis, was observed in both groups following transportation. The administration of MEL appeared to attenuate the development of a stress leukogram as evidenced in the response variables discussed below.

A neutrophilia \((P < 0.0001)\) observed across both groups after transportation (Figure 7) is consistent with results from previous studies (Blecha et al.,1984; Buckham Sporer et al., 2008; Burdick et al., 2011). The increase in the mean number of circulating neutrophils recorded in the CONT group upon arrival at the feedlot was greater than in the MEL group \((P = 0.0072)\) (Table 2). Neutrophil count was also inversely proportional to plasma MEL concentrations providing further evidence of a treatment response \((P = 0.04)\) (Table 5).

A stress neutrophilia is the result of the corticosteroid-induced release of mature cells from the bone marrow and the reduced margination and migration of neutrophils out of the vasculature (Benschop et al., 1996; Jones et al., 2007). Several studies have
determined the functional capacity of the immune response using \textit{in vitro} tests after transport. Murata et al. (1987) observed that nitroblue tetrazolium reduction activity of neutrophils was initially reduced by 10\% on arrival followed by a 36\% increase at 4 h after transport. Similarly, Burdick and others (2011) found that cattle had decreased phagocytic neutrophil intensity at 48 h post-transit and that calm bulls had more active neutrophils with greater phagocytosis and oxidative burst intensities to \textit{E. coli} than temperamental bulls at 96 h. These findings indicate that, although the relative number of neutrophils is increased after transportation, the functional capacity of these cells is decreased leading to immune compromise. The mitigation of the stress neutrophilia in the present study and the negative association between MEL concentrations and neutrophil count suggests that MEL may have reduced some of the negative impacts of transportation stress on immune function. Further studies evaluating immune cell function are planned to characterize this effect further.

Lymphocyte counts decreased from before shipping to arrival in both the CONT ($P < 0.0001$) and MEL ($P < 0.0005$) groups (Table 2). A further reduction in lymphocyte count was observed between arrival and 120 h later in both the MEL ($P = 0.01$) and CONT ($P = 0.0001$) groups. The reason for the observed treatment effect of MEL was likely due the greater lymphocyte count in the CONT calves before shipping because no time by treatment interaction was evident (Figure 8).

Exposure to corticosteroids causes redistribution of circulating lymphocytes into lymphoid compartments resulting in the development of a lymphopenia (Burton and Kehrli., 1996). Murata et al. (1987) reported a 90\% decrease in T-lymphocyte numbers of 4 to 6 mo old calves transported 4 h but no change in the number of B lymphocytes.
Buckham Sporer et al. (2008) reported a 20% increase in leucocyte count from 4.5 to 14 h after initiation of a 9 h transportation event followed by a decrease ranging from 7 to 24% at 24 and 48 h, respectively. Hubert et al. (2011) reported an 86% increase in the neutrophil: mononuclear cell ratio at 24 h post-transit. This observation is similar to the results of the present study and is consistent with the development of a stress leukogram after transport.

Monocyte counts increased from before shipping to arrival in the CONT ($P < 0.0001$) but not the MEL ($P = 0.18$) group (Table 2; Figure 9). Exposure to corticosteroids also causes mobilization of marginated monocytes within the vasculature resulting in the development of a monocytosis (Latimer and Prasse., 2003). Burdick et al. (2011) reported an 8% increase in peripheral mononuclear cells after a 4 h transportation event ($P = 0.056$). This observation is similar to the results of the present study and is consistent with the development of a stress leukogram after transport. The concurrent mitigation of both the monocytosis and neutrophilia in the present study provides further support for the hypothesis that MEL reduced some of the negative impacts of transportation stress on immune function.

Basophilia in mammalian blood smears is seldom dramatic but an increase in circulating basophil count has been associated with stress in birds (Latimer and Prasse., 2003). In the present study there was no effect of time or treatment on basophil count (Table 2). However, there was a negative association between circulating MEL concentration and basophil count ($P = 0.03$) (Table 5). To our knowledge there have been no studies that have specifically investigated the effect of transportation on basophil count. Given the absence of an overall effect of treatment and transportation despite a
negative association with MEL, basophils appear to have limited utility in assessing stress in cattle.

A treatment effect was evident for both MCH \((P = 0.05)\) and MCV \((P = 0.05)\) with an observed decrease in MEL calves compared to CONT calves (Table 3). Although values remained in the laboratory’s reference range for normal limits, this effect may be the result of decreased synthesis of prostaglandin E2 (PGE\(_2\)) associated with cyclooxygenase inhibition after NSAID administration. PGE\(_2\) has demonstrated both enhancement and maintenance of haemopoietic stem cell activity and increases in MCH and MCV are usually associated with hematopoiesis (North et al., 2007; Hogatt et al., 2009). Therefore, compounds that decrease PGE\(_2\) synthesis could potentially decrease stem cell activity resulting in a decreased MCH and MCV.

A time-by-treatment interaction was evident in RDW\% \((P = 0.07)\) with the distribution width being less in CONT calves compared with MEL calves \((P = 0.03)\) on arrival (T24) (Table 3). There was no effect of time on RDW\% in the MEL-treated group. However, a weak positive association between MEL concentrations and RDW\% \((P = 0.07)\) was observed.

RDW \% is a measure of variation in the size of the red blood cells. It is a calculated parameter representing the co-efficient of the variation of the Mean Corpuscular Volume (MCV) (Subhashree et al., 2013). It is thus not surprising that a difference in RDW \% was observed in the present study because MCV was less in MEL-treated calves \((P = 0.05)\). In a previous study Fazio and others (2011) observed an increase in RDW \% in sheep transported 125 km by road for 3 h but no explanation was
offered for why this observation occurred. It has been suggested that an increase in RDW % can be used as a biomarker to identify pulmonary compromise but the conflicting results presented in these reports suggest that additional studies are needed to investigate this relationship further.

The overall results of this study suggest that long distance transportation was associated with a significant increase in neutrophil, platelet, monocyte, white blood cell and red blood cell count after shipping and a decrease in lymphocyte count. Furthermore, MMP-9 complexes, TCO₂, TNFα, plasma proteins and SP increased and cortisol concentrations decreased after shipping. MEL treatment reduced the stress neutrophilia and monocytosis after shipping. Circulation cortisol, neutrophil and basophil concentrations were also inversely proportional to plasma MEL concentrations. Further studies, including examination of the effect of MEL administration on cell function, calf health and performance, are needed to fully elucidate the overall impact of NSAID administration on the negative effects of long-distance transportation. These findings may have implications for developing strategies to reduce the impact of transportation stress on the health and performance of feedlot cattle

**Literature Cited**


Table 1. Comparison between the least squares (LS) means ± SE serum chemistry biomarkers at 0, 24 and 144 h after treatment (trt) with 1 mg/kg meloxicam (MEL) or a Placebo (CONT) before transportation.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal Range</th>
<th>Trt</th>
<th>Group</th>
<th>Time, h</th>
<th>P-values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LS Means ± SE</td>
<td>LS Means ± SE</td>
<td>LS Means ± SE</td>
<td>Trt</td>
<td>Time</td>
</tr>
<tr>
<td>Plasma Protein, g/dL</td>
<td>6.9-7.7</td>
<td>CONT</td>
<td>7.02 ± 0.07</td>
<td>7.40b ± 0.07</td>
<td>7.00a ± 0.07</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MEL</td>
<td>7.02a ± 0.07</td>
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<tr>
<td>Total carbon dioxide, mM</td>
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<td>CONT</td>
<td>22.77a ± 0.33</td>
<td>24.98b ± 0.34</td>
<td>0.51 &lt;0.0001</td>
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<tr>
<td></td>
<td></td>
<td>MEL</td>
<td>22.43a ± 0.33</td>
<td>24.81b ± 0.33</td>
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<tr>
<td>Fibrinogen, mg/dL</td>
<td>100-500</td>
<td>CONT</td>
<td>438.11 ± 24.68</td>
<td>462.39 ± 24.68</td>
<td>483.8 ± 25.09</td>
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<tr>
<td></td>
<td></td>
<td>MEL</td>
<td>426.23 ± 24.30</td>
<td>477.62 ± 24.12</td>
<td>433.85 ± 25.54</td>
</tr>
</tbody>
</table>

a,b Different superscripts indicate significant differences between time points in a row after transportation ($P < 0.05$).
Table 2. Comparison between the least squares (LS) means ± SE of white blood cell parameter data at 0, 24 and 144 h after treatment (trt) with 1 mg/kg meloxicam (MEL) or a placebo (CONT) before transportation at 0 h, after transportation at 24 h and at 144 h thereafter.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal Range</th>
<th>Trt Group</th>
<th>Time, h</th>
<th>P-values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>0</td>
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<tr>
<td>Platelet, 10^3 cells/μL</td>
<td>100 - 800</td>
<td>CONT</td>
<td>389.47 ± 23.25</td>
<td>392.62 ± 23.25</td>
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<td>MEL</td>
<td>395.53 ± 22.80</td>
<td>409.78 ± 22.80</td>
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<td>Neutrophil, 10^3 cells/μL</td>
<td>0.6 - 4.0</td>
<td>CONT</td>
<td>1.89a ± 0.23</td>
<td>3.88ab ± 0.23</td>
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<td></td>
<td>MEL</td>
<td>1.75b ± 0.23</td>
<td>3.18bc ± 0.23</td>
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<tr>
<td>Lymphocyte, 10^3 cells/μL</td>
<td>2.5 - 7.5</td>
<td>CONT</td>
<td>7.32ab ± 0.41</td>
<td>6.31b ± 0.41</td>
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<tr>
<td></td>
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<td>MEL</td>
<td>6.43a ± 0.41</td>
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<td>Neutrophil: Lymphocyte Ratio, 10^3 cells/μL</td>
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<td>MEL</td>
<td>4.51ab ± 0.70</td>
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<td>Monocyte, 10^3 cells/μL</td>
<td>0.03 - 0.83</td>
<td>CONT</td>
<td>0.39a ± 0.03</td>
<td>0.55ab ± 0.03</td>
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<td>MEL</td>
<td>0.38a ± 0.03</td>
<td>0.44ab ± 0.03</td>
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<td>Basophils, 10^3 cells/μL</td>
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<td>CONT</td>
<td>0.08 ± 0.01</td>
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<tr>
<td></td>
<td></td>
<td>MEL</td>
<td>0.07 ± 0.01</td>
<td>0.07 ± 0.01</td>
</tr>
</tbody>
</table>

a-c Different superscripts indicate significant differences between time points in a row after transportation (P < 0.05).

x.y Different superscripts indicate significant differences between treatment groups in the related CONT and MEL columns after transportation (P < 0.05).
Table 3. Comparison between the least squares (LS) means ± SE of red blood cell parameter data at 0, 24 and 144 h after treatment (trt) with 1 mg/kg meloxicam (MEL) or a placebo (CONT) before transportation at 0 h, after transportation at 24 h and at 144 h thereafter.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal Range</th>
<th>Trt Group</th>
<th>Time, h</th>
<th>P-values</th>
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</tr>
<tr>
<td>Red Blood Cell, (10^9) cells/µL</td>
<td>5.0 -10.0</td>
<td>CONT</td>
<td>8.4±0.14</td>
<td>8.59±0.14</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MEL</td>
<td>8.8±0.14</td>
<td>8.79±0.14</td>
</tr>
<tr>
<td>Hemoglobin, g/dl</td>
<td>8.0 -15.0</td>
<td>CONT</td>
<td>11.75±0.15</td>
<td>12.00±0.15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MEL</td>
<td>11.83±0.15</td>
<td>11.85±0.15</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>24.0 -46.0</td>
<td>CONT</td>
<td>33.12±0.43</td>
<td>33.94±0.43</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MEL</td>
<td>33.42±0.42</td>
<td>32.52±0.42</td>
</tr>
<tr>
<td>Mean Corpuscle Volume, fl</td>
<td>40.0 -60.0</td>
<td>CONT</td>
<td>39.27±0.45</td>
<td>39.69±0.45</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MEL</td>
<td>38.17±0.44</td>
<td>38.33±0.44</td>
</tr>
<tr>
<td>Mean Corpuscle Hemoglobin, pg</td>
<td>11.0 -17.0</td>
<td>CONT</td>
<td>13.95±0.18</td>
<td>14.03±0.18</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MEL</td>
<td>13.51±0.18</td>
<td>13.55±0.18</td>
</tr>
<tr>
<td>Mean Corpuscle Hemoglobin Concentration, g/dl</td>
<td>30.0 -35.0</td>
<td>CONT</td>
<td>35.52±0.16</td>
<td>35.25±0.16</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MEL</td>
<td>35.40±0.16</td>
<td>35.37±0.16</td>
</tr>
<tr>
<td>Red blood cell distribution width, %</td>
<td>8.0 -15.0</td>
<td>CONT</td>
<td>18.78±0.27</td>
<td>18.42±0.27</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MEL</td>
<td>19.00±0.27</td>
<td>18.95±0.27</td>
</tr>
</tbody>
</table>

a-c Different superscripts indicate significant differences between time points in a row after transportation (P < 0.05).

x,y Different superscripts indicate significant differences between treatment groups in the related CONT and MEL columns after transportation (P < 0.05).
Table 4. Intercept and slope of chemistry biomarkers and bodyweight change with SE for the correlation between meloxicam (MEL) plasma concentration and the outcome variables.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Intercept</th>
<th>Slope Gradient Estimate</th>
<th>SE</th>
<th>P - Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bodyweight, kg</td>
<td>354.34</td>
<td>0.08</td>
<td>0.30</td>
<td>0.78</td>
</tr>
<tr>
<td>Log Cortisol, ng/mL</td>
<td>37.19</td>
<td>-1.51</td>
<td>0.47</td>
<td>0.0017</td>
</tr>
<tr>
<td>Substance P, pg/mL</td>
<td>26.06</td>
<td>0.16</td>
<td>0.78</td>
<td>0.89</td>
</tr>
<tr>
<td>Plasma Protein, g/dL</td>
<td>6.96</td>
<td>-0.01</td>
<td>0.01</td>
<td>0.32</td>
</tr>
<tr>
<td>Fibrinogen, mg/dL</td>
<td>454.96</td>
<td>3.03</td>
<td>3.76</td>
<td>0.42</td>
</tr>
<tr>
<td>Haptoglobin-matrix metalloprotease-9 complexes, ng/mL</td>
<td>1.42</td>
<td>-0.02</td>
<td>0.05</td>
<td>0.74</td>
</tr>
<tr>
<td>Total carbon dioxide, mM</td>
<td>24.91</td>
<td>-0.01</td>
<td>0.06</td>
<td>0.90</td>
</tr>
<tr>
<td>Tumor necrosis factor-alpha, pg/mL</td>
<td>168.05</td>
<td>0.09</td>
<td>1.91</td>
<td>0.96</td>
</tr>
</tbody>
</table>
Table 5. Intercept and slope of hematological biomarkers with SE for the correlation between meloxicam (MEL) plasma concentration and the outcome variables.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Intercept</th>
<th>Slope Gradient Estimate</th>
<th>SE</th>
<th>P - Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basophils, $10^3$ cells / µL</td>
<td>0.08</td>
<td>0.00</td>
<td>0.00</td>
<td>0.03</td>
</tr>
<tr>
<td>Neutrophil, $10^5$ cells / µL</td>
<td>2.58</td>
<td>-0.07</td>
<td>0.03</td>
<td>0.04</td>
</tr>
<tr>
<td>Hemoglobin, g/dL</td>
<td>11.34</td>
<td>-0.03</td>
<td>0.02</td>
<td>0.06</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>32.08</td>
<td>-0.09</td>
<td>0.05</td>
<td>0.07</td>
</tr>
<tr>
<td>Red blood cell distribution width, %</td>
<td>19.05</td>
<td>0.03</td>
<td>0.02</td>
<td>0.07</td>
</tr>
<tr>
<td>White Blood Cell, $10^5$ cells / µL</td>
<td>9.05</td>
<td>-0.07</td>
<td>0.05</td>
<td>0.12</td>
</tr>
<tr>
<td>Red Blood Cell, $10^6$ cells / µL</td>
<td>8.37</td>
<td>-0.02</td>
<td>0.01</td>
<td>0.20</td>
</tr>
<tr>
<td>Monocyte, $10^3$ cells / µL</td>
<td>0.47</td>
<td>-0.01</td>
<td>0.00</td>
<td>0.27</td>
</tr>
<tr>
<td>Mean Corpuscle Volume, fl</td>
<td>38.52</td>
<td>-0.02</td>
<td>0.02</td>
<td>0.27</td>
</tr>
<tr>
<td>logMPV, fl</td>
<td>2.07</td>
<td>0.01</td>
<td>0.01</td>
<td>0.30</td>
</tr>
<tr>
<td>Neutrophil: Lymphocyte Ratio, $10^5$cells / µL</td>
<td>2.54</td>
<td>-0.05</td>
<td>0.06</td>
<td>0.42</td>
</tr>
<tr>
<td>Platelet, $10^5$ cells / µL</td>
<td>508.83</td>
<td>2.78</td>
<td>3.60</td>
<td>0.44</td>
</tr>
<tr>
<td>Mean Corpuscle Hematocrit, gm/dL</td>
<td>35.35</td>
<td>0.01</td>
<td>0.02</td>
<td>0.73</td>
</tr>
<tr>
<td>Mean Corpuscle Hemoglobin, pg</td>
<td>13.61</td>
<td>0.00</td>
<td>0.01</td>
<td>0.79</td>
</tr>
<tr>
<td>Lymphocyte, $10^5$ cells / µL</td>
<td>5.45</td>
<td>-0.01</td>
<td>0.03</td>
<td>0.80</td>
</tr>
</tbody>
</table>
Figure 1. Flow chart outlining the timeline of study events that occurred at time points 0 h, 24 h and 144 h. Treatment with either meloxicam at 1 mg/kg or a placebo was administered at time 0 h after baseline blood sample collection. Follow-up blood sample collection for determination of biomarkers and drug concentrations occurred after a 16 h (1,316 km) transportation event and 120 h thereafter.
Figure 2. Mean, ± SE for BW change (kg) after treatment with 1 mg/kg meloxicam or a placebo at 0 h followed by a 16 h (1,316 km) transportation event. Letters that are the same indicate data points that are not significantly different ($P < 0.05$).
Figure 3. Mean, ± SE for serum cortisol concentrations after treatment with 1 mg/kg meloxicam or a placebo at 0 h followed by a 16 h (1,316 km) transportation event. Letters that are the same indicate data points that are not significantly different ($P < 0.05$).
**Figure 4.** Mean, ± SE for plasma log haptoglobin (Hp)-matrix metalloproteinase-9 (MMP-9) complex concentrations after treatment with 1 mg/kg meloxicam or a placebo at 0 h followed by a 16 h (1,316 km) transportation event. Letters that are the same indicate data points that are not significantly different ($P < 0.05$).
Figure 5. Mean, ± SE for serum tumor necrosis factor -alpha (TNFα) concentrations after treatment with 1 mg/kg meloxicam or a placebo at 0 h followed by a 16 h (1,316 km) transportation event. Letters that are the same indicate data points that are not significantly different ($P < 0.05$).
Figure 6. Mean, ± SE for plasma substance P concentrations after treatment with 1 mg/kg meloxicam or a placebo at 0 h followed by a 16 h (1,316 km) transportation event. Letters that are the same indicate data points that are not significantly different ($P < 0.05$).
**Figure 7.** Mean, ± SE for circulating neutrophil counts after treatment with 1 mg/kg meloxicam or a placebo at 0 h followed by a 16 h (1,316 km) transportation event. Letters that are the same indicate data points that are not significantly different \((P < 0.05)\).
Figure 8. Mean, ± SE for circulating lymphocyte counts after treatment with 1 mg/kg meloxicam or a placebo at 0 h followed by a 16 h (1,316 km) transportation event. Letters that are the same indicate data points that are not significantly different ($P < 0.05$).
**Figure 9.** Mean, ± SE for circulating monocyte counts after treatment with 1 mg/kg meloxicam or a placebo at 0 h followed by a 16 h (1,316 km) transportation event. Letters that are the same indicate data points that are not significantly different ($P < 0.05$).
CHAPTER 4.
IMPACT OF ORAL MELOXICAM AND LONG-DISTANCE TRANSPORT ON CELL MEDIATE AND HUMORAL IMMUNE RESPONSES IN FEEDLOT STEERS RECEIVING MODIFIED LIVE BVDV BOOSTER VACCINATION AT ARRIVAL

Modified from a manuscript published in the journal of *Veterinary Immunology and Immunopathology*


Abstract

The objective of this study was to investigate the impact of oral meloxicam (MEL) and long-distance transportation on cell-mediated immunity (CMI) in preconditioned steers receiving a booster vaccination on arrival. We hypothesized that steers treated with MEL at 1 mg/kg body weight, 6 hours before night-time transport, would be less immunocompromised on arrival (day 0) and after 7 days, and that CMI following vaccination with a modified live bovine viral diarrhea virus (BVDV) recall

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antigen would be increased. Brahman crossbreed steers, 13 to 17 months of age (n=87), were randomly assigned to one of four treatment groups: MEL, transported (MTR) (n=22), MEL, non-transported (MNT) (n=22), lactose placebo, transported (CTR) (n=21), and lactose placebo, non-transported (CNT) (n=22). MTR and CTR steers were transported for approximately 16 hours non-stop on a truck from Mississippi to Iowa (approximately 1,300 km), whereas steers in the MNT and CNT groups remained in Mississippi as non-transported controls. Body weight was measured and jugular blood was collected at −1, 0, and 7 days from all steers at the same time, regardless of location. Multi-parameter flow cytometry (MP-FCM) was used to identify T-cell subsets and detect the expression of three activation markers (CD25 [interleukin (IL)-2 receptor], intracellular interferon-gamma (IFNγ), and interleukin-4 (IL-4) after in vitro stimulation with BVDV recall antigen. Plasma cortisol concentration was measured on day −1, 0, and 7 as a marker of transport-associated stress. Serum antibody titer to BVDV was assessed on day −1 and day 7 post-booster vaccination. Whole-blood samples were analyzed using MP-FCM on days 0 and 7. Results were log transformed and analyzed using repeated measures of analysis of variance. Compared with non-transported controls, transport led to an increase in BVDV-induced expression of CD25, IFNγ, and IL-4 in CD4+, CD8+, and γδ+ T-cell subsets (P<0.05). MEL treatment mitigated the transportation-associated increase in CD25 expression by peripheral blood mononuclear cells (PBMCs), CD4+, and γδ+ T cells. CMI outputs for the MTR group were less than those of the CTR group (P<0.05); however, the MTR and NT groups did not differ (P>0.10). A treatment*transport interaction was noted for the increase in IL-4 expression by CD8+ T cells after transport, with a significant difference between the CTR and MTR groups at
day 7. In conclusion, the use of oral MEL prior to transport appears to have inhibitory or homeostatic effects, but further research is needed to validate the effect of MEL treatment on specific T-cell subsets in transported cattle.

**Introduction**

Stressful events predispose young calves to bovine respiratory disease (BRD). Transportation is one of the most stressful events in the life of a young calf (von Borrell, 2013; Fike and Spire, 2006; Tarrant 1990). Stress can alter immune function, potentially compromising the welfare of the animal by increasing the likelihood that it will develop BRD (Buckham Sporer et al., 2008). Most advising veterinarians recommend that producers use disease prevention protocols, such as vaccination or antimicrobial metaphylaxis, to reduce the likelihood of respiratory problems developing upon arrival (Nickell and White, 2010). Vaccination on arrival has been shown to be beneficial in promoting a rapid increase in antibody (Ab) titer in response to type 1 strain bovine viral diarrhea virus (BVDV) (Richeson, et al., 2009). Viral infections, such as BVDV, have been linked to increased risk of secondary bacterial infection in BRD, leading to increased morbidity and mortality (Edwards, 2010). BVDV is known to compromise the immune system in naïve calves. Diligent use of vaccination practices can decrease the incidence of BVDV infection and the subsequent development of BRD (Fulton et al., 2005).

Transport-associated stress increases the levels of pro-inflammatory and stress-related biomarkers in cattle (Arthington et al., 2003; Cooke et al., 2013). Preemptive administration of a long-acting nonsteroidal anti-inflammatory drug (NSAID), such as meloxicam (MEL), may reduce the negative impact of transport-associated stress on
physiological biomarkers in cattle (Van Engen et al., 2014; Guarnieri Filho et al., 2014). MEL has been approved in Europe for use in cattle as an anti-inflammatory drug, and in Canada for analgesia at the time of calf disbudding procedures. As alternatives to widespread antimicrobial use in food animals are being sought, changes to current treatment and management protocols will be needed. These changes could entail combinatorial pretreatment with NSAIDs, arrival vaccination, and limited metaphylaxis to prevent BRD.

The objective of the present study was to examine the effect of MEL treatment and long-distance transportation on cell-mediated immunity (CMI). CMI responses were evaluated in MEL-treated steers 0 and 7 days after vaccination. The CMI responses were then compared with those of MEL-treated and untreated steers not subjected to transport. Lymphocyte responses to BVDV type II Ag were evaluated using in vitro T-cell stimulation and multi-parameter flow cytometry (MP-FCM) analysis using fluorescence-labeled antibodies.

**Materials and methods**

The Iowa State University animal care and use committee (IACUC) approved all procedures described and performed during this study (IACUC# 5-12-7355 B).

**Animals**

A total of 87 uniform Brahman crossbred feedlot steers (353 ± 37 kg; 13 to 17 months of age; from the Brown Loam Branch Experiment Station, Raymond, Mississippi) were used in this study. All steers were free of visual or clinical signs of disease, and were vaccinated twice (before and after weaning) against *Clostridium* spp.
(Ultra bac 7, Zoetis, Madison, NJ), MLV BVDV types I and II, parainfluenza-3 virus, infectious bovine rhinotracheitis virus, bovine respiratory syncytial virus (Pyramid 5, Boehringer Ingelheim, St. Joseph, MO), and *Mannheimia haemolytica* (One Shot, Zoetis, Madison, NJ).

**Study design**

This study was a 2×2 factorial trial that randomly assigned 87 steers into 4 study groups: MEL, transported (MTR) (*n*=22); MEL, non-transported (MNT) (*n*=22); placebo, transported (CTR) (*n*=21); and placebo, non-transported (CNT) (*n*=22) (Figure 1).

Baseline variables included body weight, plasma cortisol concentrations, MEL concentrations, and serum Ab titer. Plasma and serum samples for baseline determinations were collected on day −1. The MEL-treated groups received MEL (Meloxicam tablets, Aurobindo Pharma Limited, Dayton, NJ) administered orally at 1 mg/kg body weight, whereas the placebo-treated groups received an equivalent weight in lactose placebo administered orally, 3 hours before groups MTR and CTR were loaded onto the truck. Oral administration was accomplished using a balling gun and a single gelatin capsule (Torpac, Fairfield, NJ) containing the entire treatment dose.

Transportation commenced in the late afternoon, and the animals arrived at the Tri County Steer Carcass futurity feed yard, Lewis, Iowa, the following morning, after an approximately 16-hour, nonstop trip. Upon arrival (day 0), plasma and whole blood for the CMI assays were collected from animals in all groups at approximately the same real time at both locations. After sample collection, all animals received a single dose of a combined modified live virus vaccine for infectious bovine rhinotracheitis, BVD Types I and II, parainfluenza-3, and bovine respiratory syncytial virus (BoviShield 5; Zoetis,
Madison, NJ). On day 7, body weight was determined, and plasma, serum and whole blood was collected from animals in all groups at both locations at approximately the same real time.

Sample collection

Blood samples were collected from the jugular vein. Three 10-mL vacutainer tubes (BD Vacutainer, Franklin Lakes, NJ; EDTA tubes, lithium heparin tubes, and serum separator tubes) were used to collect blood samples to be centrifuged for serum and plasma. Whole-blood samples (13.5 mL) were collected in 20-mL plastic syringes with 1.5 mL of 2 × acid citrate dextrose (ACD; 0.15 M sodium citrate, 0.076 M citric acid monohydrate, 0.287 M dextrose). The needles were removed and the syringes capped. These samples were processed according to the CMI materials and methods section. Blood samples for serum and plasma were processed within 30 min of collection by centrifugation at 1,600 × g for 15 min. The serum and plasma were immediately frozen on dry ice and stored at −80°C until tested. On days 0 and 7, the whole blood collected from animals in the MNT and CNT groups was transported overnight at room temperature (22°C) to Ames, Iowa by the study investigators, whereas whole blood collected from animals in the MTR and CTR groups in Iowa was transported to the laboratory and held in the laboratory at room temperature (22°C) for the same duration. All whole-blood samples were submitted for the CMI assay and tested at the same time.

Weights were determined using scales on site at the Brown Loam research facility and at Tri County Steer Carcass futurity feed yard. Scales were certified and calibrated at each site using standardized test weights. Furthermore, researchers collecting the data
verified that the scale was in proper working condition prior to data collection using test weights.

**Cortisol analysis**

Plasma cortisol was determined using a commercially available Coat-a-Count radioimmunoassay kit (Siemens, Malvern, PA), as previously described by Van Engen *et al.* (2014). Briefly, $^{125}$iodinated cortisol was used in the analysis procedure to determine its concentration using a gamma counter. As described in the manufacturer’s instructions, 25 µl of plasma was added to each polypropylene tube containing Abs immobilized within the tube wall. One milliliter of $^{125}$iodinated cortisol stock solution was added, incubated for 45 minutes at 37°C, and decanted. The standard curve provided by the manufacturer had an $r^2$ value >0.98, the assay’s detection range was 3 to 500 ng/mL, and the inter- and intra-assay variability was 13% and 11.5%, respectively.

**MEL concentration analysis**

The plasma concentration of MEL was determined using high-pressure liquid chromatography (Surveyor MS Pump and Autosampler; Thermo Scientific, San Jose, CA) with mass spectrometry detection (TSQ Quantum Discovery MAX; Thermo Scientific). A 100-µL volume of 1 M trichloroacetic acid was added to 200 µL of each sample of plasma, spiked plasma, and quality control plasma, following the addition of piroxicam (10 µL of a 10 ng/µL stock) as an internal standard. The samples were vortexed for 5 s and then centrifuged for 20 min at 2,000 × g to sediment the precipitated material. A 150-µL portion of the supernatant was transferred to an injection vial fitted with a glass insert containing 100 µL of 1.9% ammonium hydroxide in 25% aqueous acetonitrile. The injection volume was set to 12.5 µL. Mobile phase A consisted of 0.1%
formic acid in water, and mobile phase B consisted of 0.1% formic acid in acetonitrile. The flow rate was 0.250 mL/min. The mobile phase began at 40% B, and samples were eluted with a linear gradient to 95% B over 5 min; the composition was maintained at 95% B for 1.5 min, and then the column was re-equilibrated with 40% B. Separation was achieved with a solid-core C18 column (KinetexXB-C18, 100 mm × 2.1 mm, 2.6-µm particle size, Phenomenex, Torrance, CA) maintained at 40°C. Piroxicam eluted at 3.3 min, and MEL eluted at 4.5 min. Four meloxicam and three piroxicam transitions were monitored during mass spectrometry analysis via selected reaction monitoring mode. The quantifying ions were 72.99, 88.01, 114.99, and 140.98 m/z for MEL and 77.97, 94.98, and 120.98 m/z for piroxicam. Plasma blanks, calibration spikes, QC samples, and bovine plasma samples were batch processed using a method developed in the Xcalibur software (Thermo Scientific, Waltham, MA). The processing method automatically identified and integrated each peak in each sample and calculated the calibration curve based on a weighted (1/X) linear fit. The concentration of MEL in the experimental plasma samples was calculated by the Xcalibur software based on the calibration curve. Results were then viewed using the Quan Browser feature of the Xcalibur software. A total of 12 calibration spikes were prepared in bovine plasma, covering a concentration range of 5 to 20,000 ng/mL. QC samples were prepared at concentrations of 15, 150, and 1,500 ng/mL. QC samples were prepared in duplicate with each set of samples. Calibration curves exhibited a correlation coefficient ($r^2$) exceeding 0.998 across the entire concentration range. The QC samples at 150 and 1,500 ng/mL were within 2–9% of their nominal values. The low QC at 15 ng/mL differed from its nominal value by 13–15%. 
Serum virus neutralization test

Sera from each sample were submitted to the Veterinary Diagnostic Laboratory at the College of Veterinary Medicine at Iowa State University. The serum virus neutralization tests for BVDV type II virus used the 125 genotype 2 strain as antigen (Ag).

CMI assay

Whole-blood samples were transferred from capped syringes to 15-mL centrifuge tubes and then centrifuged at 1,000 × g for 20 min. The buffy coats were collected, red blood cells were lysed and peripheral blood mononuclear cells (PBMCs) were pelleted, isolated and washed as previously described (Platt et al., 2008). MP-FCM was performed to assess the memory response of T-cell subsets to BVDV type II. A 200-µL aliquot of each PBMC suspension, containing 10⁶ cells, was added to each of the four wells of a 96-well flat-bottomed tissue culture microtiter plate. Two wells received 50 µL of RPMI++ (RPMI containing 15% fetal bovine serum and 1.5% penicillin/streptomycin solution) as non-Ag-stimulated controls. The other two wells received 50 µL of a suspension of BVDV type II (Strain 890, 4.2×10⁶ TCID₅₀/mL, propagated in MDBK cells; Ridpath, J., National Animal Disease Center, Ames, IA). The plates were incubated at 37°C in a humidified 5% CO₂ incubator. On day 4 of the incubation, PBMCs in all wells were stained and analyzed by MP-FCM, and the results were analyzed as described earlier (Platt et al., 2009).

Statistical analysis

SAS software (version 9.2, SAS Institute, Inc., Cary, NC) was used for statistical analyses. Repeated measures analysis of variance was used to compare the main effects
of meloxicam administration and of transportation over time, and their respective
interactions. Statistical significance was designated as a $P$ value <0.05. Cellular
responses were evaluated using an expression index (EI), as defined by the equation
shown in Figure 2. The EI numerator refers to cells stimulated with BVDV recall Ag, and
‘unstimulated’ refers to cells from the same animal not exposed to the BVDV recall Ag.
The EI for each treatment group was analyzed as the response variable, assuming a
Gaussian distribution. This method was used for all parameters investigated in this study.
Variables that did not exhibit normal distribution were log transformed.

**Results**

*Plasma MEL concentration*

Prior to treatment (at day $-1$), no steers had detectable concentrations of MEL in
plasma. On day 0, the plasma MEL concentration ranged from 1.39 to 3.72 μg/mL, with a
mean (±SD) of 2.79 (±0.55) μg/mL. On day 7, the concentration ranged from 0.00 to 0.20
μg/mL, with a mean (±SD) of 0.02 (±0.03) μg/mL. All steers in both the TR and NT
groups that were designated to receive MEL tested positive for the drug, and all controls
tested negative.

*Change in body weight*

The weight of all steers was monitored throughout the study and changed
significantly as a result of interactions between time and transport ($P<0.0001$). However,
the effect of MEL treatment alone was not significant ($P=0.667$). The weight of steers in
both the MTR and CTR groups had decreased on day 0, upon arrival following transport.
No significant change in weight was observed in steers not subjected to transport. By day
7, the transported steers had recovered their lost weight and showed an additional weight increase as a group (Figure 3).

**Cortisol concentration**

A time*transport interaction ($P=0.036$) was noted with respect to cortisol concentration, with an observed increase on day 0 in TR steers; however, neither transportation alone ($P=0.119$) or MEL administration alone had an impact on plasma cortisol concentrations ($P=0.340$).

**Titer of BVDV-neutralizing antibodies**

The Ab titer results for both the TR and NT groups for both MEL and CONT animals combined were log$_2$ transformed (Figure 4). The titer increased over time in both groups between day −1 and day 7 ($P<0.001$). No effects associated with transport ($P=0.600$) or treatment were observed ($P=0.645$).

**CMI responses**

Three different cell types were analyzed to assess the effects of TR and MEL on CMI, namely CD4$^+$ T cells, CD8$^+$ T cells, and γδ$^+$ T cells. Data are also shown for total PBMCs without differentiation of the cell type. Furthermore, three expression indices were calculated for each cell type namely, CD25, interferon-gamma (IFNγ), and interleukin (IL)-4.

A time effect was observed for all groups of cells ($P<0.001$), except in the case of PBMCs expressing CD25 and γδ$^+$ T cells expressing IFNγ ($P>0.05$). In terms of the main effects, the primary outcome of this research trial was evidence of an increase in overall peripheral blood CMI responses ($P<0.05$) following transportation (Table 1).
Furthermore, a main effect of meloxicam treatment was observed, as evidenced by changes in CD25 expression on PBMCs, CD4⁺, and γδ⁺ T cells (Table 1). Specifically, CMI outputs in the MTR group were lower than in the CTR group but similar to the NT groups. In addition, a treatment*transport interaction was noted with respect to IL-4 expression in CD8⁺ T cells. Specifically, expression increased after transport, with a difference observed between the CTR and MTR groups on day 7. The individual significance of these effects is highlighted in Table 1.

**PBMCs**

There was evidence of a time*transport interaction in the expression of CD25 on PBMCs ($P=0.051$) (Table 1). Specifically, CD25 expression on PBMCs in the CTR group was significantly elevated on day 7 compared with all other groups ($P<0.05$) (Figure 5). PBMC expression of IFNγ also supported a time*transport interaction ($P=0.047$) (Table 1). Instead, PBMC IFNγ expression in the MTR group was elevated compared with the MNT group at 7 days after the administration of the MLV BVDV ($P=0.045$). A significant effect of transport was also noted for IL-4 expression by PBMCs was higher in the CTR group compared with the CNT group ($P=0.047$) on day 0.

**CD4⁺ T cells**

Transport was associated with a significant increase in CD25 expression on CD4⁺ T cells in the CTR group compared with the MNT group on day 7 ($P=0.005$) (Figure 6). Furthermore, there was a time*transport interaction for mean IFNγ EI ($P=0.0024$) and IL4 ($P=0.007$) expression in CD4⁺ T cells (Table 1). A transport*time interaction was also evident for IL-4 expression, as indicated by differences on day 0 between the CTR and MNT groups ($P=0.007$). Specifically, the expression of IL-4 in the CTR group was
increased on arrival in comparison with that in the MNT group ($P=0.010$). No differences in IL-4 expression were observed between groups on day 7.

**CD8$^+$ T cells**

Transport affected the expression of CD25 on CD8$^+$ T cells on day 7 following arrival and administration of the MLV BVDV vaccine. Specifically, CD25 expression at day 7 was higher in the CTR group than in all other groups ($P<0.05$) (Figure 7). No difference in CD25 expression was observed among groups at arrival on day 0. IFN$\gamma$ expression by cytotoxic T cells was elevated in the CTR group compared with the MNT group on day 7 ($P=0.0095$). Overall there was a time*transport interaction for IL-4 expression ($P=0.0237$). Within the CD8$^+$ T-cell population, the expression of IL-4 on day 7 mimicked the trend in the expression of IFN$\gamma$ on CD8$^+$ T cells. Expression of IL-4 by CD8$^+$ T cells in the CTR group was increased in comparison with the other 3 groups at day 7 ($P<0.05$) (Figure 8).

**$\gamma\delta^+$ T cells**

A time*transport interaction was observed in the expression of CD25 on $\gamma\delta^+$ T cells ($P=0.0486$). Specifically, CD25 expression on $\gamma\delta^+$ T cells was elevated on day 7 in the CTR group compared with all other groups ($P<0.05$) (Figure 9). No differences were observed in CD25 expression on $\gamma\delta^+$ T cells on day 0 between groups. Similarly, there was no difference in the expression of IFN$\gamma$ by $\gamma\delta^+$ T cells on day 0 or day 7; however, a time*transport interaction was observed ($P=0.0087$). Specifically, IL-4 expression increased in the CTR group compared with the MNT group ($P=0.049$). No other significant differences were observed in IL-4 expression by $\gamma\delta^+$ T cells between groups.
Discussion

Assessing stress in cattle by visual inspection can be challenging. In this study, we examined the impact of TR, MEL, and their interaction on different aspects of the immune system over time. To our knowledge, no studies examining the effects of MEL administration on CMI prior to long-distance transport of cattle, or their corresponding CMI responses following arrival and administration of MLV BVDV have been reported. Manipulation of immune responses through vaccination is a common practice that has shown great value in preventing disease. We hypothesized that the concurrent administration of meloxicam would impact the host immune response at the time of vaccination and enhance the vaccine response. We found that the use of oral MEL prior to transport appears to have inhibitory or homeostatic effects on at least one EI for each immune cell reported, and has no effect on antibody titer response to MLV BVD at day 7. The in vivo effects of MEL on immune function in cattle are poorly understood; evidence from studies in mice suggests that MEL administration following vaccination in order to reduce the pain associated with an adjuvant does not interfere with the immune response (Kolstad et al., 2012).

Pharmacologically, MEL reaches its highest concentration (i.e., $C_{\text{max}}$) in cattle 12 h after administration, with a plasma half-life of 28 h (Coetzee et al., 2009). Therefore, in the present study, MEL concentration is presumed to have peaked during transport; therefore, six plasma elimination half-lives would have transpired by day 7. The mean plasma $C_{\text{max}}$ of 3.10 μg/mL (range 2.64–3.79 μg/mL) is reported and a median effective concentration for meloxicam in the plasma, applied from a lameness study in horses, is approximately 0.2 μg/mL (Coetzee et al., 2013). Although the drug concentration is low
on day 7, the pharmacokinetics of the anti-inflammatory effects of the NSAID suggests long lasting therapeutic drug concentrations for part of the week and during this time the body is responding to the immunization on day 0. MEL is not specifically approved for use in cattle in the United States; however, extra-label use is allowed under the Animal Medicinal Drug Use Clarification Act (United States Food and Drug Administration, 2014) for analgesia, because there are currently no compounds specifically approved for this purpose.

Weight loss is an unavoidable consequence of long-distance transportation of cattle. We hypothesized that weight loss would be evident in animals in the TR groups, whereas the weight of cattle in the NT groups would remain relatively constant. Our results indicate that this hypothesis was correct and consistent with results reported elsewhere (Cooke et al., 2013). Body weight declined following transportation in our study, and MEL treatment had no effect on weight loss or resumption of weight gain upon arrival at the feed yard. In cattle, weight loss is greatest within the first 12 h during transportation, and the greatest losses are typically seen in cattle that are fed roughage diets versus concentrates (Knowles, 1999). These results are typical of any production scenario and are unaffected by MEL treatment. However, one research group recently reported that oral MEL administration is beneficial in preventing a decrease in dry-matter intake 1 week following transportation, and in preventing an overall decline in performance following transportation (Guarnieri Filho et al., 2014). These conflicting results may have been due to differences in study design, specifically the breed, age, duration of the transportation event, and sampling times over the course of the study. Differences in diet composition at the different locations in which the present study was
conducted may have had an impact on the differences in performance observed on day 7. However, most changes were probably due to increased rumen feed content.

Based on previous research, we hypothesized that steers in the TR groups would have significantly higher plasma cortisol concentrations than those in the NT groups (Knowles et al., 1997; Murata et al., 1987; Buckham Sporer et al., 2008). The effect of time*transportation interaction reported herein support this hypothesis. However, determining the difference between the two main effects is challenging because this interaction was likely due to the lack of a transportation event on day -1. We also hypothesized that MEL-treated steers would have lower plasma cortisol concentrations than their CONT counterparts in both the TR and NT groups. However, no differences were observed at any time between the MEL-treated and CONT groups. There are many potential confounders that could trigger adrenocorticotropic hormone production and subsequent cortisol release during transportation. Our finding of an increase in cortisol levels associated with transportation agrees with many reports in the literature, but contradicts our previous findings (Van Engen et al., 2014). Previously, we found that relative to baseline, cortisol concentrations decrease in plasma after TR. Our previous experiments may have been influenced by stress resulting from limited handling events, whereas the steers used in the current study may have been better acclimatized to processing, and the NT groups were comparable. Increased cortisol concentrations can have numerous implications, but in the present study, we interpreted increased cortisol as a stress marker and indicator of potential negative immunological effects. Glucocorticoids may suppress the immune system (Roth and Kamberle, 1982). Using human gene profiling, Gallon et al. (2002) concluded that glucocorticoids can upregulate
the innate immune system and inhibit the adaptive immune system. At day 0, the TR group had no statistically significant difference in cortisol levels compared to the NT group. The limited sampling points may have missed changes in endogenous glucocorticoid levels that could have effected immunological CMI patterns. Further research is needed to evaluate the effect of MEL treatment on specific T-cell subsets in transported cattle that have significantly higher cortisol in comparison with a control population.

We hypothesized that an increase in the expression of CMI markers (e.g., CD25, IFNγ, and IL-4) would be observed in the TR groups compared with the NT groups, but that immune function in MEL-treated calves would be maintained due to the use of NSAIDs, which would reduce changes in physiological biomarkers that are associated with the stress of transportation (Van Engen et al., 2014; Cooke et al., 2013). Our results suggest that the expression of CD25 and IL4 was lower in the MTR group compared with the CTR group, particularly at day 7. EI in the MTR was equivalent to that in both the MNT and CNT groups (Figures 7 and 10). These results appear to support our hypothesis that MEL would maintain immune function at a level similar to that of the NT controls. However, because shipping resulted in a higher expression of CMI markers in TR steers compared with NT steers on arrival and at 7 days post-arrival (Figure 5 and Figure 7), an alternate interpretation of the data could be that MEL reduced the overall CMI response, resulting in there being no difference between MEL and NT calves on Day 7. Further studies to delineate these outcomes are warranted.

In the present study, we were able to look at the effects of transport and meloxicam on cellular memory. Expression of CD25 on CD4+ T cells are interpreted as a
marker of a memory response. In evaluating the memory response at day 0 and day 7 using the expression index, the equation takes into account CD4+ cells that constitutively express CD25 and are understood to be T regulatory (T_{reg}; CD4+CD25+) cells (Seo et al., 2007). The cells are evaluated in the denominator of the EI and are compared to stimulated CD4 cells expressing CD25 in the numerator of the equation (Figure 2). Thus, the CD4+CD25+ cell EI was considered a marker of the memory response toward BVDV in this study. The memory response was higher in the CTR group (day 0), and MEL had no effect on this cell population between individual treatment groups. However, on day 7, there was a significant difference between the MNT and CTR groups (Figure 6). A study examining the effects of MEL exposure on the proliferation of CD4+ T cells in vitro demonstrated an increase in CD4+CD25+ T cells (Maslanka and Jaroszewski, 2012).

These results, combined with our findings, support the hypothesis that MEL does not inhibit immune responses in transported animals upon arrival. Conversely, the treatment effect observed at day 7 is notable: after a full week of exposure to the anti-inflammatory effects of MEL, the level of CD25 expression in the MTR group was comparable to that of the NT group. This observation supports the conclusion that MEL administration decreases the CMI response at day 7.

CD25 expression on γδ+ T cells was similar in the NT and MTR groups, but greater in the CTR animals relative to the other groups. Bovine γδ+ T cells have multiple immune functions and play innate, adaptive, and regulatory roles (Guzman et al., 2011). In human, murine, and bovine models, cells that constitutively express CD4+CD25+ are considered T_{reg} cells, which can exert immunosuppressive effects. However, Hoek et al. (2008) reported that the γδ+ T cell subpopulation expressing FoxP3 may not exert similar
effects in cattle, whereas other γδ⁺ T-cell subsets in cattle do suppress the immune system. Given that CD25 expression may not necessarily be an indicator of regulatory function for γδ T cells in cattle, and our analysis did not distinguish between γδ⁺ T-cell subsets, more research is needed to identify which specific subsets were impacted by MEL administration prior to transportation, so that the overall effect on the immune system can be elucidated.

Contrary to our hypothesis that IFNγ expression would be lower in TR steers on day 0 compared with NT or MEL-treated steers, none of the T-cell subsets exhibited a decrease in IFNγ associated with TR or treatment. However, there was an increase in IFNγ production by PBMCs in CTR animals compared with CNT animals upon arrival, suggesting that TR causes an increase in IFNγ expression. This conflicts with results reported by Gupta et al. (2007), who found a significant increase in cortisol levels and reduction in IFNγ production by cultured lymphocytes in Holstein-Friesian bulls after a 12-h transport event. This disparity is likely associated with differences in the age, sex, and breed of the animals used in these two studies. IFNγ expression is important in Ag-specific memory responses, and is necessary for T helper 1 responses to viruses (Platt et al., 2008). The T helper 1 response is key for both the stimulation of Ab production and the recall response to BVDV. We also found that MEL had no significant effect on IFNγ expression, a finding that is in agreement with those reported by Maslanka et al. (2012), who described the effects of MEL on CD8⁺ lymphocytes in vitro. Overall, their findings suggested that in contrast to dexamethasone, which exhibits immunosuppressive effects, MEL is safe for use in treating infection-associated inflammation in veterinary practice. Bednarek et al. (2003) suggested that the inhibition of cyclooxygenase-2 has multiple
effects mediated through inhibition of prostanoids, potentially resulting in the normalization of tumor necrosis factor-alpha levels and increased production of IFNγ in lung bronchial alveolar macrophages. The absence of this effect in the present study may be because we studied immune cells outside the lungs that may react differently following stress, such as that associated with long-distance transport.

IL-4 causes the differentiation of naïve T helper cells into T helper 2 cells, which play a pivotal role in the development of humoral responses to Ag through the stimulation of B cells (Estes et al., 1995). As previously mentioned, the stress associated with TR led us to hypothesize that IL-4 expression by all cell populations would be lower in the TR group compared with the NT group. The interaction between MEL and TR on IL-4 expression was unexpected, and renders it difficult to differentiate the two main effects for circulating CD8⁺ cells. The significant increase in IL-4 expression in the CTR group compared with all other groups on day 7 suggests that MEL may assist in the maintenance of normal IL-4 expression by circulating CD8⁺ T cells after transport. The MTR EI was similar to that of both the MNT and CNT groups; however, based on our sampling time points, it is difficult to draw inferences regarding the kinetics of IL-4 expression by CD8⁺ cells and the potential effects this may have on humoral responses through T-cell regulation.

Overall, transportation can cause significant changes in immune function in cattle. Though this is the initial interpretation of our results, drawing inferences regarding the kinetics of T-cell populations is both difficult and risky. Within the first week after vaccination, T cells migrate to the lymphoid tissue to undergo clonal expansion in response to the vaccine Ag (Murphy et al., 2012). Thus, to extrapolate a response based
on measurements made on days 0 and 7 would be difficult, because we examined blood lymphocytes, and it would take much longer to detect humoral indications of clonal expansion. Other factors, such as the breed, age, or sex of the animals may influence the effect of stress. These factors make it difficult to ascribe a causal relationship, but our results do provide a basis for directing further investigations of the effects of transportation stress and the observation that MEL either does or does not influence immune function.

We initially hypothesized that there would be an increase in BVDV Ab titer due to the recall response, irrespective of treatment with MEL. Sampling 7 days after vaccination represents a rapid turnaround for Ab-response examination and thus makes interpretation difficult. However, in this case, the log2-transformed Ab-response data suggest that neither MEL, nor transportation-induced stress, had an immediate effect on vaccination recall response. Richeson et al. (2009) examined the effect of vaccine administration immediately following transportation, and reported that anti-BVDV type I Ab reached a high titer sooner than animals vaccinated after 14 days, without compromising average daily weight gain or growth. Other investigations also confirm our findings that MEL did not affect vaccination response to respiratory pathogens (Rodrigues et al., 2015).

**Conclusions**

In conclusion, long-distance transportation affects both physiological homeostasis, and impacts CMI in beef steers. No differences in CMI were observed in the present study between MNT and CNT steers, however, some effects associated with the stress of transportation were observed and are not yet fully understood. Our study
provides evidence that the early increase in Ab titer to BVDV in response to vaccination is not affected by MEL or transportation. Changes in the CMI response demonstrated effects due to transportation and treatment with MEL. The results of this study indicate that CMI responses in the MTR were equivalent to those in NT for most outcomes, and that CMI responses were elevated in the CTR group compared to the other groups. These two outcomes suggest that MEL either has an inhibitory effect on CMI when transported or has a homeostatic effect when compared to NT controls. Further investigation is necessary to assess if there is an effect on disease outcome on arrival in cattle at high risk for BRD.

**Literature Cited**


immunosuppression by bovine CD4(+) and CD8(+) T cells. Infection and Immunity 75, 260.


Table 1. Mean (±SEM) expression indices for various immune cells: PBMCs, CD4+ T cells, CD8+ T cells, and γδ+ T cells.

Expression index was defined as: [the percent of cells expressing the indicated marker after stimulation with BVDV Ag multiplied by the mean MP-FCM fluorescence intensity], divided by [the percent of unstimulated cells expressing the indicated marker multiplied by the mean MP-FCM fluorescence intensity]. Cell markers of interest were CD25, IFNγ, and IL-4.

Sampling occurred on days 0 and 7. Each primary group (transported and non-transported) consisted of meloxicam (MEL)-treated (1 mg/kg) and untreated (CONT) calves. Groups with a single superscript (a, b) indicates a significant difference ($P<0.05$). Each superscript letters are for comparison within each row. NS indicates the difference was not significant ($P>0.05$).

<table>
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<th>Cell subset</th>
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Table 1 continued
Effect of MEL and transport on immunological cell markers

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Figure 1. Flow chart outlining the study timeline. Calves in the transport and non-transport groups were administered either 1 mg/kg meloxicam (MEL) or a placebo (CONT) orally on day −1, after baseline blood sample collection. Follow-up blood sample collection for determination of biomarkers, drug concentrations, and CMI data occurred after a 16-h (1,316 km) transport event (day 0) and 7 days later. Modified live vaccine administration occurred after blood sampling on day 0.
expression index = \frac{\% \text{ positive cells stimulated} \times \text{mean fluorescence intensity}}{\% \text{ positive cells unstimulated} \times \text{mean fluorescence intensity}}

Figure 2. Equation for calculating the expression index using flow cytometry data.

“Stimulated” refers to cells stimulated with BVDV recall antigen, and
“unstimulated” refers to a sample of cells from the same animal that were not
stimulated with BVDV recall antigen. The percentage of both stimulated and
unstimulated positive cells was multiplied by the corresponding geometric mean
fluorescence intensity.
Figure 3. Mean (±SEM) body weight (kg) after treatment with 1 mg/kg meloxicam (MEL) or a placebo (CONT) on day -1, followed by either a 16-h (1,316 km) transport event (TR) or non-transportation (NT). Comparisons were made across time and between transport groups. Different letters indicate statistically significant differences ($P<0.05$). Interaction between time and transport ($P<0.0001$) is present however, the effect of MEL treatment alone was not significant ($P=0.667$).
Figure 4. Mean (±SEM) log₂-transformed type 2 BVDV antibody concentration. MLV to BVDV was administered on arrival at day 0, after sample collection. Comparisons were made across time and between groups (MTR, CTR, MNT, CNT). Different letters indicate a significant difference \( (P<0.05) \). Time was the only main effect of significance, and titer increased for both groups between day \(-1\) and day \(7\) \( (P<0.001) \).
Figure 5. Mean (±SEM) index of CD25 expression on PBMCs. The expression index is defined as the percentage of cells expressing CD25 after stimulation with BVDV Ag divided by the percentage of unstimulated cells expressing CD25. The percentage of positive cells in both the numerator and denominator was multiplied by the mean flow cytometry fluorescence intensity. Different letters indicate a significant difference ($P<0.05$). Test slices were separated by day and significance based on treatment ($P=0.0019$) and transport ($P=0.0231$) main effects. Comparisons were made between individual treatment groups (MTR, CTR, MNT, CNT).
Figure 6. Mean (±SEM) index of CD25 expression on CD4\(^+\) T cells. The expression index is defined as the percentage of cells expressing CD25 after stimulation with BVDV Ag divided by the percentage of unstimulated cells expressing CD25. The percentage of positive cells in both the numerator and denominator was multiplied by the mean flow cytometry fluorescence intensity. Different letters indicate a significant difference (\(P<0.05\)). Test slices were analyzed by day and significance was interpreted based on the treatment (\(P=0.0433\)) and transport (\(P=0.0007\)) main effects. Comparisons were made between individual treatment groups (MTR, CTR, MNT, CNT).
Figure 7. Mean (±SEM) index of CD25 expression on CD8+ T cells. The expression index was defined as the percentage of cells expressing CD25 after stimulation with BVDV Ag divided by the percentage of unstimulated cells expressing CD25. The percentage of positive cells in both the numerator and denominator was multiplied by the mean flow cytometry fluorescence intensity. Different letters indicate a significant difference ($P<0.05$). Test slices were analyzed by day and significance was interpreted based on a transport effect ($P=0.0231$). Comparisons were made between individual treatment groups (MTR, CTR, MNT, CNT).
Figure 8. Mean (±SEM) index of IL-4 expression in CD8+ T cells. The expression index was defined as the percentage of CD8+ T cells expressing IL-4 after stimulation with BVDV Ag divided by the percentage of unstimulated CD8+ T cells expressing IL-4. The percentage of positive cells in both the numerator and denominator was multiplied by the mean flow cytometry fluorescence intensity. Different letters indicate a significant difference ($P<0.05$). Test slices were analyzed by day and significance was interpreted based treatment*transport interaction ($P=0.0237$). Comparisons were made between individual treatment groups (MTR, CTR, MNT, CNT).
Figure 9. Mean (±SEM) index of CD25 expression on γδ⁺ T cells. The expression index was defined as the percentage of γδ⁺ T cells expressing CD25 after stimulation with BVDV recall Ag divided by the percentage of unstimulated γδ⁺ T cells expressing CD25. The percentage of positive cells in both the numerator and denominator was multiplied by the mean flow cytometry fluorescence intensity. Different letters indicate a significant difference \( (P<0.05) \). Test slices were analyzed by day and significance was interpreted based treatment \( (P=0.0052) \) and transport \( (P=0.0056) \) main effects. Comparisons were made between individual treatment groups (MTR, CTR, MNT, CNT).
CHAPTER 5.

THE EFFECT OF PRE-TRANSPORTATION OR ARRIVAL MELOXICAM ADMINISTRATION TO CALVES ENTERING THE FEEDYARD ON MORBIDITY, BIOMARKERS AND CARCASS CHARACTERISTICS

A manuscript formatted for modification and submission to the *American Journal of Veterinary Research*

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Abstract

Objective: To investigate the use of meloxicam as a pre-transport or on arrival therapeutic to; mitigate disease outcomes of bovine respiratory disease (BRD), impact

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§§ Department of Animal Science Oklahoma State University
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biomarker outcomes associated with BRD, change performance over the first 42 days on feed, and influence carcass characteristics at harvest.

**Animals:** Multi-sourced, crossbred steer calves (n= 168), consisting of mainly British and British-Continental breeds, were purchased from a sale barn in central Kansas.

**Procedures:** Calves were processed prior to transportation and again on arrival. Subjects were randomized to three separate treatments: pre-transport meloxicam (PMEL), arrival meloxicam (AMEL), and a control group receiving inactive excipient (CONT). Animals were weighed and blood was collected pre-and post-transport. Haptoglobin (HP)-Matrix Metalloproteinase (MMP)-9, Cortisol, and Substance P were quantified. Nasal swabs were collected to assess total protein and Annexin A1. Weights were taken again at 42 days and at harvest. BRD was monitored using the depression, appetite, respiration and temperature system (DART). Harvest parameters were collected at the plant using a standardized grading system.

**Results:** Meloxicam did not have an effect on BRD morbidity over the course of the study and there was no effect on performance at 42 days (P>0.10). Of the calves that did succumb to BRD, no differences were seen in severity of disease (P>0.10). Substance P, HP- MMP9, total protein and annexin A1 levels were increased on arrival (P ≤ 0.05) and no treatment effect or interaction was noted in the statistical model (P>0.10).

**Conclusions and Clinical relevance:**

Meloxicam administration to steer calves prior to or on arrival as a prophylactic treatment does not mitigate BRD, or the severity of BRD for incoming calves and does not aid in a performance benefit at 42 days or over the entire feeding period.
Abbreviations:

ADG, average daily gain;

BRD, bovine respiratory disease;

BW, body weight;

DMI, dry matter intake;

G:F, gain to feed;

HCW, hot carcass weight;

HP, haptoglobin;

MMP, matrix-metalloproteinase;

NSAID, non-steroidal anti-inflammatory drug;

QG, quality grade;

YG, yield grade;

Introduction

Cattle transported long distances, at a lighter BW, are at a greater risk of morbidity and subsequent mortality due to bovine respiratory disease. In combination these two parameters are a means of classifying cattle into a “high risk” category of developing BRD. Cattle at high risk for BRD are less adept at handling stressors of transportation resulting in a decreased immune function. Physical and psychological stress over the length of transportation results in changes of circulating physiologic
parameters. Specifically, markers of interest that have been connected to inflammation and BRD. Glucocorticoids and their endogenous effects on annexin A1 have been implicated as a marker of BRD. In addition, acute phase proteins are also suggestive as important post transportation markers for the transportation stress. Furthermore investigation these markers to disease and the effects of therapeutics on these markers is warranted and necessary.

Previous reports have suggested a benefit of reduced lung consolidation when nonsteroidal anti-inflammatory drugs (NSAIDs) are administered as an adjunct to antibiotics. European legislation requiring the reduction of antibiotics has incited research for early intervention with NSAIDs to reduce the use of antibiotics and have reported success. Recent investigation directed at the use of NSAIDs in cattle prior to transportation focused on performance, and physiologic outcomes. Flunixin meglumine, a labeled antipyretic in cattle, was proven ineffective as a potential drug for increased performance on arrival. However, use of an alternative NSAID, meloxicam, prior to transportation has seen benefit in performance during the first week of arrival. In addition meloxicam has reduced the transport associated acute phase protein responses and reduced the stress leukogram response. No investigation has focused on the use of NSAIDs in high risk cattle administered prior to transportation to evaluate the effects on disease outcomes or performance past 21 days of the feeding period. We hypothesized that treatment with meloxicam prior to or on arrival after transportation would result in reduction of disease and increased performance in the first 42 days of the feeding period. In addition, we also hypothesized that the initial positive treatment effects would translate to improved carcass characteristics at harvest.
Materials and Methods

Before the initiation of this experiment, all animal use, handling, and sampling techniques described were approved by the Oklahoma State University IACUC (# AG-16-1).

Animals

Crossbred steer calves (n= 168) consisting of mainly British and British-Continental breeds were assembled in Kansas. In two separate events one week apart, an order buyer purchased calves deemed high risk from the Kingsville livestock market with initial BWs averaging 251 ± 24 kg. Animals were acquired from multiple sources and comingled with in the holding facility of the sale barn for an undetermined amount of time. Prior to enrollment calves were visually appraised and deemed healthy. During enrollment, duplicate ear tags with individual identification were assigned and placed in both ears and research data/samples were collected. The animals were loaded on a road transportation semi rig on August 11th and 18th 2016. Two truckloads of 89 and 79 calves respective to each mentioned day. Pen assignments were randomized and consisted of calves that came in on their study day and were from the same treatment group. No mixing occurred to account for behavior disruptions. Distance traveled from the livestock market to the research feedlot was 533 km. After arrival at the yard the calves were allowed to rest for 18 hours and then processed for sample collection.

Study Design

A randomized complete block design with three parallel arms was adopted for the clinical trial. Calves were randomized for treatment at the sale barn according to individual
identification in the ear tag. Treatment groups consisted of a pre-transportation meloxicam (n= 62) with on arrival placebo (PMEL), pre-transportation placebo (n=53) with on arrival meloxicam (AMEL), and controls (n=53) that received placebo both pre-transportation and on arrival. Meloxicam\(^a\) (255 mg) dosing per os (PO) was calculated based on the estimated weight average of 255 kg to one mg per kg of BW. During processing and enrollment at the sale barn, BW, nasal swabs, and blood were collected. Bolus administration then occurred with appropriate treatments and verification of swallowing was confirmed before release.

Cattle were restrained in a processing chute with a rope halter; blood was acquired via jugular venipuncture into vacutainer tubes. Processing occurred on site in the sale barn. Centrifugation of blood was performed at 3,000 g for 15 minutes. Plasma or serum was immediately drawn off of the centrifuged vacutainer tube with a micropipette and transferred to a micro-centrifuge tubes in duplicate. Samples were then frozen on dry ice and transported frozen to the laboratory. Samples were transferred to a freezer and stored at -80° C.

Nasal swabs were collected by inserting a sterile 14 cm culture swab, into the nasal cavity until resistance was met and then rotating against the nasal mucosa. Swabs were collected from the right and left nasal cavity and the two swabs from one animal were combined/pooled as follows. Using non-sterile medical gloves, the two swab tips were cut off and both together were placed in a single 1.5 mL micro centrifuge tube containing 500 μl PBS, and frozen on dry ice and transported to the laboratory where they were stored at -80° C.
On arrival at the Oklahoma State feedlot, cattle were processed in the first 18 hours of arrival. Samples for blood and nasal swabs were collected in the same manner as previously described. Calves were then given their second treatment on arrival. Calves in the PMEL and CON treatment groups received a placebo bolus. The remaining calves in the AMEL group received meloxicam boluses. The calves with in the three treatments were randomized and sorted to pens (n=18). PMEL, AMEL, and CON were allotted to an equal number of pens (n=6). Pens were identical in nature and each calf was receiving identical rations. Specific pens shared waterers with no treatment indication that required separation of groups from water sources. Pens consisted of only one of the three treatments. On day 42 calves were weighed for performance purposes. Study animals remained in their respective pens throughout the feeding period until their specified harvest date on April 7, 2017.

Processing

The morning of on arrival processing, all calves received commercially available vaccines against bovine herpes virus -1, BVDV (types 1 and 2), parainfluenza virus three, bovine respiratory syncytial virus in a 5-way vaccine and clostridal pathogens in a 7-way. All calves were administered a growth promoting implant containing 80 mg trenbolone acetate and 16 mg estradiol. The anti-parasitic protocol required an oral drench and injectable wormer. Later in the feeding period, all steers on trial also received a second implant of 4 mg of estradiol and 20 mg of trenbolone acetate on day 70.
Feeding management

Pens were an open dirt yard that measured 12.2m X 30.5m with the bunk lining 12.2 m of the pen. Feeding occurred twice daily throughout the feeding period at set times. Rations were formulated to meet or exceed the nutritional requirements of each calf. Water was made available in each pen ad libitum.

Bovine respiratory disease assessment

Criteria for identifying cattle with clinical signs of BRD was designated to individuals who were blinded to treatment, trained to detect BRD, and were consistently available to monitor calves. A severity scoring system was used to rank animals that were deemed clinical for BRD. The scale of the score was on a 1 to 4 and was attributed as follows: 1-mild, 2-moderate, 3 severe and 4- moribund. The scale of the score was based off of subjective clinical signs each trained individual was adept at identifying. Specifically, those signs included: animals that were off feed, gaunt in appearance, signs of dyspnea consisting of open mouth breathing with extension of the head and neck, hanging of the head in a depressed manner or a glassy appearance in the eye. If a severity score was assigned to an animal, it was removed from the pen for further evaluation. Most importantly the evaluation of interest was rectal temperature. The temperature criteria for treatment was set at 40°C when a severity score reached severe. After the treatment decision was made, calves were returned to their home pen for further observation. A mandatory post treatment interval was assigned based on antibiotic treatment.

Antimicrobials chosen were on label products for the treatment of BRD and given at the label recommended dose. Therapeutic regimen was the same for every animal that was
pulled and met the treatment criterion. A maximum of three antibiotic treatments were used per calf before they were deemed a treatment failure. Each antibiotic had an observed post-treatment interval that required observance prior to any further therapeutic intervention. First treatment was tilmicosin at 1.5 mL/100 lbs BW, second treatments was florfenicol at 6.0 mL/100 lbs. BW, and the third treatment was ceftiofur 1.5 mL/100 lbs. BW for 1st, 2nd and, 3rd BRD treatments. All antibiotics were administered SQ in the neck with the exception of exceed being administered SQ into the base of the ear. All dosing instructions followed the product label.

**Performance at harvest**

During the finishing phase cattle performance management, and carcass characteristics were performed in a manner consistent with previous publication. Cattle that are colored (primarily non-black hided) were sent to Dodge City, KS commercial facility 434 km from the research station to be harvested. All black hided cattle were harvested in Arkansas City, Kansas 109.76 km in distance from the research facility. Oklahoma State University personnel qualified and standardized in quantifying carcass characteristics. Data generated in plant included: hot carcass weight (HCW), Ribeye area, marbling score, fat over the 12th rib, yield grade (YG), and quality grade (QG).

**Cortisol Analysis**

Circulating plasma was analyzed for cortisol and numerated via radio-immunoassay using a commercially available kit. Guided by the instructions provided by the manufacturer the quantifiable range of the assay was from 2.5 ng up to 300 ng / mL. The average standard curve ($R^2$) is 99.8. Validity of the assays for each run was confirmed
with internal standards at 10 and 100 ng / mL. The coefficients of inter-assay and intra-assay variability were at < 8 % and < 12.5 % respectively.

**Substance P Analysis**

Substance P concentrations were determined prior to shipment and on arrival. In each EDTA blood tube, 200µg benzamidine was added 48 hours prior to the start of the study for protease inhibition. Whole blood extracted from the jugular vein was inverted 3 times to guarantee homogenization with the protease inhibitor and EDTA. The samples were centrifuged, frozen and stored as described previously in the design section. Time frame of processing was 30 minutes for all samples.

Substance P levels were determined using previously published methods described by using non-extracted plasma. Detection ranged for the assay was between 5 and 320 pg/mL. The coefficient if of variation for the intra-assay variability was 9.26% and the inter-assay variability was calculated to be 18.28%. The average R^2 for the calibration curve was 0.986.

**Annexin A1 Analysis**

Nasal swabs were passed into the nostrils of cattle as described previously in the study design portion of the materials and methods. A random subset of calves (PMEL=14, AMEL=19, CONT=18) were analyzed. The supernatant was collected and then frozen at -80˚C. Annexin A1 levels were measured and concentrated similarly to a previously described manuscript, with modifications as follows. Samples were concentrated by centrifugal filtration with a 3 kDa molecular weight-limit device, purified, and protein concentrations were measured. Two fluid samples with intermediate concentrations of
annexin A1 were used as standards; these were used on each gel to facilitate comparison of test samples between gels and blots. Samples containing 10 μg of protein were loaded on sodium dodecyl sulfate polyacrylamide gels and separated at 200 V for 1 hour, then proteins were visualized and total protein in each lane was quantified by densitometry.

Proteins were transferred to a membrane that was used for western analysis. The membrane was blocked with 5% BSA for 1 hour, then incubated with rabbit anti-human annexin A1 antibody overnight at 4°C, washed, and incubated with peroxidase-conjugated goat anti-rabbit immunoglobulin as secondary antibody, for 30 minutes, washed for 1 hour, and visualized by chemiluminescence. Bands (37 kDa), corresponding to that previously shown for annexin A1 were quantified by densitometry. Data are shown as the density of the band on the western blot adjusted for the background density of the blot, normalized to the corresponding adjusted total protein value obtained by densitometric analysis of the sodium dodecyl sulfate polyacrylamide gels -PAGE gel. Quantitative data were confirmed by visual inspection of the blots and gels.

Haptoglobin- Matrix Metalloproteinase -9 Analysis

The Hp-MMP 9 ELISA was performed as described. The capture antibody was a monoclonal anti-bovine MMP 9 (clone 10.1; native bovine neutrophil MMP 9 antigen) and wells were blocked by the addition of 300 μL of SuperBlock T20. All plates were prepared at the same time to minimize variation between plates.

The serum used as a standard was prepared from an ill bovine that previously had a verified Hp-MMP 9 of 913 ng/mL in a prior manuscript. This previous aliquoted
standard was thawed on ice and sonicated for three consecutive one minute intervals,
vortexed and serially diluted to create a standard curve as follows: 228, 114, 57, 28, 14, 7,
and 3.5 ng/mL. Blank wells contained all reagents, with serum from a healthy steer,
diluted 1:10 in TBS+Tween 20. Affinity chromatography and Hp-MMP 9 ELISA of this
animal's serum demonstrated it was free of demonstrable Hp-MMP 9 (Lakritz J,
unpublished observations).

Serum samples from experimental animals were diluted 1:10 with Tris-buffered saline (pH 7.5) to which 0.05% Tween-20 was added prior to analysis by ELISA. Diluted standards and serum samples from experimental animals (100 μL) were placed into wells of a 96 well plate in duplicate (16 standards, 40 serum samples/plate) for 2 hours on a plate shaker at room temperature. After washing pre-diluted rabbit-anti-bovine Haptoglobin-HRP conjugate, were allowed to bind to haptoglobin that is bound to MMP 9 in the wells on a plate shaker for 1 hour. After washing wells 5 times in Tris-buffered saline (pH 7.5) with 0.05% Tween-20, 100 μL of TMB substrate was added/well and color development was allowed for 20 minutes. After 20 minutes, 100 μL 0.1 N hydrochloric acid was added/well to stop the enzymatic reaction. Standard and sample absorbance was determined on a micro-plate reader at 450 nM. All samples whose absorbance at 450 nm or greater than the highest standard were re-diluted to 1:50 with the Tris-buffered saline (pH 7.5) to which 0.05% Tween-20 was added diluent and re-assayed (56 samples total). Sample concentrations were determined by linear regression of the known standard concentration versus absorbance value, using the intercept and slope calculated from the linear regression and corrected for the dilution of the sample (10-fold or 50 fold).
Statistical Analysis

Analysis was performed using statistical software. Experiment setup was analyzed as a randomized complete block design and the fixed effects of the model were treatments PMEL, AMEL, and CONT for all data investigation. BW was defined by four groups and also used as fixed effects. Group one was weights less than 233 kg, group two was defined by BW between 234 kg – 239 kg, group three was defined by 250 kg - 266 kg and lastly group four was any BW greater than 267 kg. These fixed effects were categorical in nature and associations between treatment and BW at arrival with-in pen BRD morbidity, severity score, were tested using a generalized linear mixed model fitted as a binomial distribution. Severity score was assessed as ordinal ranging as follows: 0= normal, 1=mild, 2=moderate, 3=severe and moribund. These were fitted with a multinomial distribution, cumulative logit link and restricted pseudo-likelihood.

Treatment and BW associations were accounted for at arrival and investigated as the fixed effects for the remaining outcomes of interest. Performance and carcass outcomes of ADG, DMI, HCW, REA, G:F, marbling, dressing percent were tested using linear mixed models with a Gaussian distribution, identity link, and residual pseudo-likelihood. These data were continuous in nature. Random effects of interest for the study were pen, arrival date, and harvest site for the carcass outcomes. Random intercepts for pen and arrival pen were fitted as well as an unstructured covariance structure for animals, to account for the hierarchical structure of the data which consists of repeated measures of cattle over time, cattle nested within pens, and pens nested within arrival date.
Continuous values for cortisol, substance P, HP-MMP 9 complexes, nasal swab total proteins and annexin A1 required natural logarithmic transformation to meet the normality and homoscedasticity of the residuals. Generalized linear mixed model models were fitted including main effects for treatment and study day and a two-way interaction between treatment and study day, using a Gaussian distribution, identity link and residual pseudolikelihood estimation. Random effects for pen and arrival date were included. Initially, all models were fitted using a Laplace likelihood approximation technique in order to quantify overdispersion (Pearson $\chi^2$/df). Final models, when feasible, were fitted using a residual pseudo-likelihood approximation procedure. Akaike and Bayesian Information criteria were used to compare model fit. Model assumptions were tested in all models and residuals were investigated using graphical tools. The Tukey-Kramer multiplicity correction was used for prevention of multiple treatment group comparison Type I error. 95% confidence intervals for means were calculated. Significance was indicated by $P$ values less than or equal to 0.05 and statistical trends are indicated by $P$ values between 0.05 and 0.10.

**Results**

Eleven animals were taken off trial. Five of the original enrollment claves died of heat stress before pen assignment. In the first week two succumbed to joint infections. Three months later one more study subject died of joint infection. The remaining three where removed late in the feeding period due to laminitis, lameness and respiratory
issues. No mortality was evident in the group as a consequence of acute BRD. This is depicted as percent mortality in table one and mortality was not analyzed statistically.

The mean total respiratory morbidity treatment average of all trial pens was 26.26%. Within the pens, morbidity ranged from 0% to 63%. Evaluation of the average first treatment respiratory morbidity percentage between PMEL, AMEL, and CONT was as 20.22%, 21.02%, and 23.08% respectively. These values had no statistical differences between them ($P=0.8674$). Of note, all pens that experienced 0% morbidity ($n = 3$) were assigned to meloxicam treatment. Further evaluation of first treatment in morbidity over days one through ten and days one through thirty are depicted in table 1. The number of total days in the yard until first treatment for BRD was needed, relative to day 0, was termed day of treatment. This time to first treatment in days did not differ between groups ($P=0.7092$). The severity of sickness for calves pulled to be treated in the feed yard was assessed. These severity scores of the different groups were not different ($P = 0.6342$). In addition to assessing the subjective severity of sickness, rectal temperature was also taken for animals that were pulled. No differences in rectal temperature between the groups AMEL, PMEL, and CONT were seen in the calves identified during clinical evaluation ($P=0.7863$).

Evaluation of live performance data occurred over the first 42 days of the trial (Table 2) and again at the final day of the feeding period. Weights between treatment groups did not differ on individual day comparisons prior to transportation, on arrival or at day 42 of the study period ($P > 0.10$). For all groups, there was no significant difference in weight between day -1 and day 0. There was an increase in BW over time from day 0 to day 42 ($P = 0.0001$). Similar to this finding further comparisons
highlighted significant differences between day 0 and day 42 from the final BW \((P \leq 0.001)\). The ADG, in kg per day, from day 0 to day 42 for AMEL, PMEL, and CONT were 1.80, 1.75 and 1.77 respectively with a standard error of 0.07. These values did not differ statistically between treatment groups \((P = 0.5615)\). Despite the overall lack of differences between the treatment groups for most variables, a treatment effect was detected between AMEL and the CONT groups for DMI \((P = 0.011)\). Within the model, BW was split into 4 categorical variables as previously stated in the statistical analysis section. BW was also had a significant \((P < 0.0001)\) on DMI and there was an interaction between treatment and the BW effects \((P < 0.0006)\). As each BW category increased from 1 to 4 there as an increase in the DMI for each group.

Harvest performance parameters for all three treatments (PMEL, AMEL, and CONT) are reported in Table 3. The total number of days in the feed yard did not differ between pens and treatment groups. This was not analyzed statistically and the average for all was 235 days on feed. The end BW comparison between PMEL, AMEL and CONT were similar \((P = 0.6332)\) and incorporated into calculation of ADG over the entire feeding period. Using initial BW of each group on arrival \((P = 0.7381)\), there was no significant difference of ADG over the entire feeding period \((P = 0.3480)\). Data pertaining to carcass characteristic parameters, HCW, HCW/final BW, ribeye area, fat over the 12th rib, QG, YG, and marbling score, were recorded at the harvest facilities. The HCW was very similar between groups and there was not differences that were observed through statistical models \((P= 0.8587)\). When accounting for BW on arrival at day 0 in the model there was significant effect \((P= 0.0009)\). Significance differences were between weight group 4 from 1 and 2. In line with the lack of treatment difference for
the HCW and the final BW, the dressing percentage was also similar between groups ($P = 0.5854$). Changes in the marbling score and the measured fat over the 12th rib were also unaffected by specific treatments at arrival. $P$ values for these respective parameters were 0.6531 and 0.5792. Ribeye area and the YG of the carcass also remained unchanged between PMEL, AMEL, and CONT ($P > 0.10$).

Circulating biomarker means are presented in Table 4. All biomarkers were natural log transformed for statistical analysis and then back transformed for interpretation in table 4. Cortisol levels in circulating plasma did not differ over time ($P = 0.5194$) or by treatment ($P = 0.7839$) and there was no interaction between time and treatment ($P=0.6973$). Substance P concentrations increased over time from day -1 to day 0 ($P < 0.0001$) with no treatment effect ($P = 0.1474$). The interaction between time and treatment was not significant ($P=0.9076$). In circulation, Hp-MMP-9 increased over time ($P= 0.0015$) however there was no difference due to treatment ($P= 0.7563$). The interaction between time and treatment was not significant ($P=0.2879$).

Total protein in swabs of the nasal cavity were significantly increased on arrival ($P= <0.001$) and there was no treatment effect ($P=0.7440$). The interaction between time and treatment was not significant ($P=0.7669$). Annexin A1 concentration per mg of protein were significantly decreased on arrival ($P=0.0004$) and there was no treatment effect ($P= 0.2603$). The interaction between time and treatment was not significant ($P=0.3160$). When accounting for total protein in annexin A1 and viewing annexin levels
as mg per mL of nasal secretion, there is no significant change from over time ($P = 0.8472$) or by treatment ($P = 0.8472$).

**Discussion**

The utility of a prophylactic therapeutic, meloxicam, is to benefit the health of the animal through reduction of pain and stress during or after transport. With current approved drugs on the market in cattle, no approved therapeutic is labeled for pain or stress relief. For cattle, meloxicam has become an intensively investigated extra label drug use NSAID. As described in previous literature, there are benefits during surgical procedures to prevent disease\(^{16}\) and reduction of pain in common procedures of bovine production medicine.\(^{17-19}\) Meloxicam is a member of the oxicam drug class and preferentially inhibits cyclooxygenase II activity, which has a physiologic role in prostaglandin production.\(^{20}\) Oral administration of meloxicam is pharmacokinetically proven to have high bioavailability and a half-life of 26 hours in the ruminating bovine.\(^ {21}\) These properties of the drug are advantageous when administering a dose prior to or after transport as well as during other production practices. Recent work indicates that meloxicam dosed prior to transport has benefit in reducing the stress response associated with transport\(^ {9}\) and decreasing the acute phase protein response while preventing performance losses on arrival.\(^ {8}\)

With a non-integrated beef system, economic, geographic and spatial limitations of feed yard infrastructure results in long distance transportation for most calves.\(^ {22}\) This necessity for cattle shipment is an additional factor for increased likelihood of BRD as well as other contributing factors such as colostrum management at birth,\(^ {23}\) weaning stress\(^ {24}\) and comingling.\(^ {25}\) In the present study, we were able to account for and locate
calves that were comingled from multiple sources and transported over a long distance. Due to the origination of lightweight calves from a sale barn, we suspected that the weaning management was minimal. The environment was ideal for the transmission of respiratory pathogens from infected to naïve animals with additional stressor events to decrease the innate and adaptive immune response to BRD.\textsuperscript{2,26} When purchased, these calves were deemed high risk, however they did not encounter the same pattern of high morbidity that we would have anticipated. Calves identified as high risk can have group morbidity reach upwards of 65-80\% of the incoming comingled herd.\textsuperscript{27} Our incoming cohort only experienced a total number of treatments ranging from 23.08 \% to 29.03 \% with a SEM of 7\%. However, there is still a potential benefit despite the lack of statistical significance when investigating NSAID therapy as a sole option for preventative mitigation of BRD.

Past literature has described the benefit of administering NSAIDs during the advent of a painful or stressful stimulus to alleviate acute stress responses.\textsuperscript{9} In a field study, meloxicam used as a standalone treatment for BRD resulted in greater weight gain over the feeding period and a decrease in lung lesions at slaughter when compared to control animals.\textsuperscript{28} Additionally, the kinetics of meloxicam allows a singular administration to provide three days of therapeutic benefit in comparison to three consecutive days of flunixin meglumine treatment.\textsuperscript{29} Meloxicam provides added benefits for receiving performance.\textsuperscript{8} Presumably this additional performance on arrival is attributed to pain and stress relief. Due to these literature findings, we hypothesized that meloxicam administration, either prior to transport or on arrival, would decrease the severity of BRD morbidity for incoming high-risk calves through the reduction of pain, stress and added
performance. This trial allowed us to reject this hypothesis. There was no difference between animals that receive the NSAID before or after transportation on the incoming first treatment for BRD. When treatments are isolated to the first 10 days, there is also no difference. Additionally, those animals that do become clinical do not have any reduction of temperature when treated with meloxicam prior to shipment or on arrival. Severity score was similar between the PMEL AMEL and CONT groups. Most of the respiratory disease encountered with in the feed yard was mild to moderate in severity. A study cohort with more moderate defined sickness was desired. Descriptions of an induced BRD model lack an effect of NSAID treatment on behavior as well as clinical signs. Differences of severity in BRD were one of the suggested means of masking of potential positive or negative effects. As authors, we would not advise the use of meloxicam as a pre transport or on arrival standalone management practice for BRD and would suggest the combination with and antibiotic approved for use against BRD.

Meloxicam falls under the animal medicinal drug use clarification act of 1994 as extra label drug use. However, it is of importance to stress that the sole justifiable use of meloxicam for production purposes is not warranted. In addition, these drugs may not be used in feed and a violation residue is prohibited. We hypothesized that the use of meloxicam to reduce pain and stress prior to transport or on arrival would have beneficial effects on ADG, DMI and G:F at 42 days. Justification for this hypothesis originated from previous research suggesting dairy calves treated with meloxicam, prior to the stress of dehorning, spent more time standing at the feed bunk and gained better. Over the study period, all three groups gained significant weight during the first 42 days. There was no added treatment benefit for ADG or G:F between day 0 and 42. Previous authors
have indicated a meloxicam effect for decreasing the shrink associated losses in the first week and additional benefits for increased ADG and G:F over the 21 days after arrival when comparing meloxicam treated groups to the transport controls. We would agree with these authors’ results for the first 21 days, but they did report data beyond the designated 21 days. The focus of this trial was a singular 42 d data collection. The results indicate that compensatory gain negates the potential original performance benefit. DMI had a significant interaction between weight and treatment. Likely this interaction does not translate to a substantial effect of treatment but rather is more a function of the weight at arrival. This is supported by a lack of difference in the measured parameters connected to DMI; ADG and G: F. Flunixin meglumine, another NSAID labeled for pyrexia in cattle, has also been investigated at cattle receiving after long distance transportation. This therapeutic option did not improve any receiving performance of the feeder cattle over 28 days. This is not surprising considering events such as castration can decrease the ADG and G:F with in the first 14 days however detecting effects at day 28 is not possible.

Carcass performance in feedlot cattle is influenced with on arrival decision making. These initial processing decisions, such as metaphylaxis or vaccination can make a difference at harvest. Disease related inflammation can have negative effects on harvest through partitioning of energy sources to catabolic processes associated with immune function and the acute phase proteins. The most notable partition of immunologic energy in the feed yard is accounted for in the combat of BRD. We hypothesized that the decrease in inflammation through use of a NSAID would be beneficial in reducing subclinical inflammation associated with transport and aid in
partitioning of energy to enhance carcass outcomes. When evaluating all of the carcass data we reject our hypothesis; NSAID treatment on arrival or prior to transport does not influence the majority of carcass performance. Other investigators have described a lack of benefit on carcass characteristics when using NSAID as ancillary therapy to BRD treatment in feedlot cattle.\textsuperscript{35} There may be a difference in outcome based on the time of administration prophylactically versus at diagnosis. Benefits at harvest can lead to premiums if cattle are marketed appropriately on grid pricing.\textsuperscript{36} Benefits of a singular oral meloxicam dose during a time of inflammation have improved performance in other production practices, most notably increased lactation in the dairy cow.\textsuperscript{37} In addition the hypothesis of an increased performance at arrival as described by Guarnieri et al., could potentiate the lingering benefits if the project were performed in a different cohort. However, we acknowledge the lack of investigation with in the 7 and 21 days due to constraints of the study design and personnel.

Addressing biomarkers prior to and after transportation scenarios has been done extensively. In this manuscript we choose biomarkers based on indication for stress,\textsuperscript{38} pain,\textsuperscript{39} and correlation to BRD.\textsuperscript{13,14}

Cortisol is a commonly investigated glucocorticoid hormone that is used as an indicator of stress during and after transportation.\textsuperscript{40} Previous investigation confirmed that the cortisol release was inversely proportional to meloxicam concentrations and was critical in reduction of the stress leukogram.\textsuperscript{9} We hypothesized that treatment with meloxicam would again have a reduction of cortisol and there would be a correlation of the reduction. We however reject this hypothesis for calves in this trial. Cortisol confounding by natural circadian rhythm\textsuperscript{41} and differences in the types and breeds can be
attributed. Despite the lack of observed changes in cortisol over time and the lack of a treatment outcome we would not attribute this to a lack of stress during transport.

Subjectively and non-statistically interpreting the cortisol levels, CONT animals did have a higher cortisol on arrival (40.17 ± 1.10 ng/mL) when compared to PMEL treated animals (35.22 ± 1.10 ng/mL) and AMEL animals (37.91 ± 1.10). Indication of the singular sampling time points as the potential confounder along with study subjects should be taken into consideration.

Substance P is a neurotransmitter stored in the dorsal horn of the spinal cord and released in response to cutaneous noxious stimuli. In calves, plasma concentrations of substance P have been considered a potential biomarker of pain following castration. Circulating increases have also been inversely proportional to meloxicam concentrations after scoop dehorning. Suggesting an analgesic benefit of NSAID therapeutics. Transportation has been described to increase the circulating neuropeptide. We hypothesized an increased substance P at arrival. The present study’s cohorts had an increase in substance P over time confirming our hypothesis. This agrees with the previous trials. We also hypothesized a decrease in substance P for animals in the PMEL group when compared to AMEL and CONT. We rejected this hypothesis. No treatment effects were noted. When comparing circulating substance P levels, the concentrations of substance P in our study is drastically less than those involving incisional castration (506.43 ± 38.11) and even drastically less than uncastrated controls (386.42 ± 40.09 pg/mL). Despite the differences in analysis method and the ability to draw conclusion through comparison out right, the percent change of these two studies is also markedly different than ours. Potentially, there is less efficacy for meloxicam in a scenario that
does not have a parameter of incisional damage for a pain response. Interpretation of the neuropeptide in a transport scenario as a marker of pain is still warranted from an animal welfare standpoint. Some literature supports the hypothesis that there is more likely a stress response with transport rather than a pain response\textsuperscript{24} however a combination of the two is still highly plausible based on cattle handling, disposition, and transport methods.

HP- MMP-9 complexes released in response to acute inflammation are useful diagnostically at identifying pulmonary inflammation; as described in a previous challenge model.\textsuperscript{43} Haptoglobin has been investigated in transport studies as part of the acute phase protein response during weaning and transport of cattle.\textsuperscript{25,44} However, the best indicator of inflammation is a complex of HP-MMP-9 in comparison to either HP or MMP-9 individually.\textsuperscript{14} With an indicator of inflammation associated with disease, we hypothesized that there would be an increase of HP MMP-9 over time and a decrease in the HP-MMP-9 levels for animals treated with meloxicam. We were correct in our hypothesized statement and a significant increase over time for circulating HP-MMP-9 occurred. However, there was no effect of meloxicam to reduce the biomarker in circulation. This is consistent with previous work.\textsuperscript{9} Though HP-MMP-9 has been validated as a marker of pulmonary inflammation, using haptoglobin alone as early indication of BRD on arrival has limited utility in deciding BRD treatment methods.\textsuperscript{12} In this trial, due to past results of meloxicam’s minimal effect these results were not surprising, but necessary to identify responses of different animal cohorts to relay the universal nature of the change in beef cattle after transportation. Reasons for lack of identifying BRD may have been constrained by the lower percentage of BRD compared to Holland et al. ’s investigation of haptoglobin as a predictor and our lack of high
severity scores. Using this marker would still have utility but timing and degree of illness are likely crucial.

Annexin A1 are proteins conserved heavily throughout the animal kingdom and are functionally, on a molecular level, membrane stabilizers. In cattle, stress induction drastically increases annexin A1 in bronchial alveolar lavage fluid. Release of annexin A1 has been associated with a reduction in pro-inflammatory effects through phospholipase A2 and cyclooxygenase II inhibition. Increases in levels of annexin A1 have also been described as indicators of resistance to respiratory disease. Due to the high correlation of annexin A1 to glucocorticoids Senthilkumaran et al. suggested a complex relationship between stress glucocorticoids annexin A1 and susceptibility to pneumonia. From this, we hypothesized an increase in annexin A1 levels on arrival and a treatment effect decreasing the levels of annexin A1. We rejected this hypothesis and instead saw a decrease in the number of annexin A1 proteins with in the nasal swab sample. However, this was confounded by the rise in total protein in the nasal swab and skewed the identification of annexin A1. The annexin A1 was originally reported as annexin A1 per mg of protein. When accounted for and annexin was viewed independently per mL of nasal fluid, there was no difference between groups. To account for this, we multiplied the μg of annexin per mg of total protein the mg total protein per mL of nasal fluid extract to result in μg annexin per mL nasal fluid extract. This lack of change may have been due to the high levels already present when initial sampling began on day -1. Alternatively, no changes in cortisol between both sampling timepoints may also be involved. The lack of other sampling days to prevent BRD assessment confounding and the small subset of samples may have attributed to a lack of meaningful
treatment findings. Total protein in the nasal swab was significantly increased on arrival compared to pre-transit samples. This was suspected due to previous reports of increased proteinaceous nasal secretions after stress and inflammation. Similar to assumptions with HP-MMP9, annexin A1 as a marker has utility but reiteration of timing and degree of illness is important.

In conclusion, meloxicam administered prior to transportation or on arrival has no influence on the number of BRD cases. Incoming calves that do develop clinical BRD do not differ in time to treatment and rectal temperature compared to untreated controls. Additionally, there was no effect on ADG or G:F at 42 days on feed. ADG at the final day of the feeding period and carcass characteristics are undistinguishable between groups. Circulating biomarkers are affected by the event of transportation. These trends signify inflammation and align with previous publications and research. No treatment effects on transportation biomarkers were noted. The lack of significance could be limited by sampling timepoints. However, the few sampling timepoints were constraints of budget, personnel, and intentional to limit disruption of days monitoring for disease. Meloxicam is still a valuable option as an ancillary treatment and would be beneficial in tandem with antibiotic therapeutics.
a Meloxicam Tablets, 15mg per tablet, USP; Aurobindo Pharma USA, Dayton, NJ
b Titanium 5; Elanco Animal Health, Greenfield, IN
c Vision 7; Merck Animal Health, Madison, NJ
d Component TE-IS implant; Elanco Animal Health, Greenfield, IN
e Safeguard; Merck Animal Health, Madison, NJ
f Dectomax; Zoetis Animal Health, Florham Park, NJ
g Revalor XS; Merck Animal Health, Madison, NJ
h Micotil; Elanco, Greenfield, IN
i Nuflor; Merck, Summit, NJ
j Excede; Zoetis, Florham Park, NJ
k Corti-cote [125 - I] Radioimmunoassay Kit; MP Biomedical, Eschwege, Germany
l Amicon Ultra-15, Millipore, Billerica, MA, USA
m Ready Prep 2-D Cleanup Kit; Bio-Rad Laboratories, Hercules, CA, USA
n Nanodrop 2000C, Thermo Scientific, Rockford, IL, USA
o TGX Stain-Free Fast Cast Acrylamide solutions, BioRad, USA
p ChemiDoc, BioRad, Mississauga, ON, Canada
q Image Lab software, Bio-Rad, Laboratories, Hercules, USA
r TransBlot Turbo PVDF Membrane, BioRad, Mississauga, ON, Canada
s 1:375; H00000301-D01P, Novus Biologicals, Oakville, ON, Canada
t 1:3000; DakoCytomation, Glostrup, Denmark
u Clairity ECL western blotting substrate, BioRad, Mississauga, ON, Canada
v Thermo Scientific, Pierce, Rockford, IL, USA
w Tris-buffered saline (TBS) 1X Cold Spring Harbor Protocols, 2009; doi:10.1101/pdb.rec11830
x Immunology Consultants Laboratory, Portland, OR, USA RHPT-10A; 1:5,000 dilution
y Kirkegaard & Perry Laboratories, Gaithersburg, MD, USA; 50-76-11

LITERATURE CITED


32. Tennant TC, Ives SE, Harper LB, et al. Comparison of tulathromycin and tilmicosin on the prevalence and severity of bovine respiratory disease in feedlot cattle in


Table 1. Mean values for total number of animals requiring treatment for BRD, breakdown of the percentages of first treatment and those treated with in the first 30 days, severity score, rectal temperature and average day of treatment for pulls.

<table>
<thead>
<tr>
<th>Variable</th>
<th>AMEL (n= 6 pens)</th>
<th>PMEL (n= 6 pens)</th>
<th>CONT (n=6 pens)</th>
<th>SEM</th>
<th>P-value§</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Mortality (%)</td>
<td>1.70</td>
<td>3.50</td>
<td>1.10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Morbidity (%)†</td>
<td>24.53</td>
<td>29.03</td>
<td>25.13</td>
<td>7.00</td>
<td>0.8033</td>
</tr>
<tr>
<td>First treatment morbidity (%)</td>
<td>21.02</td>
<td>20.22</td>
<td>23.08</td>
<td>6.95</td>
<td>0.9579</td>
</tr>
<tr>
<td>Day 1-10 (%)</td>
<td>15.09</td>
<td>16.13</td>
<td>17.30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 1-30 (%)</td>
<td>16.98</td>
<td>20.96</td>
<td>19.23</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day of treatment ¶</td>
<td>8.27</td>
<td>10.63</td>
<td>16.39</td>
<td>7.05</td>
<td>0.7092</td>
</tr>
<tr>
<td>Severity score ‡</td>
<td>1.75</td>
<td>1.25</td>
<td>1.72</td>
<td>0.41</td>
<td>0.6342</td>
</tr>
<tr>
<td>Rectal temperature (°C)</td>
<td>40.37</td>
<td>40.44</td>
<td>40.61</td>
<td>0.29</td>
<td>0.7863</td>
</tr>
</tbody>
</table>

§Significance was defined at $P \leq 0.05$. †Total morbidity percent refers to the total number treated per group and includes those animals treated twice with in the first 100 days. ¶Day of treatment refers to the mean day for animals pulled and treated. ‡Severity score ranged from 1(mild), 2(moderate), 3(severe) to 4 (moribund). — indicates that groups were not analyzed statistically.
Table 2. Mean values of live performance variables at the pen level separated by allotted treatment

<table>
<thead>
<tr>
<th>Variable</th>
<th>Time (d)</th>
<th>AMEL</th>
<th>PMEL</th>
<th>CONT</th>
<th>SEM</th>
<th>P-value$^$</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW (kg)</td>
<td>-1</td>
<td>253.53</td>
<td>252.36</td>
<td>248.39</td>
<td>9.99</td>
<td>0.9302</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>254.76</td>
<td>251.29</td>
<td>245.33</td>
<td>8.57</td>
<td>0.7381</td>
</tr>
<tr>
<td></td>
<td>42</td>
<td>330.67</td>
<td>325.17</td>
<td>319.17</td>
<td>10.31</td>
<td>0.7368</td>
</tr>
<tr>
<td>ADG (kg/d)</td>
<td>0-42</td>
<td>1.80</td>
<td>1.75</td>
<td>1.77</td>
<td>0.07</td>
<td>0.8888</td>
</tr>
<tr>
<td>DMI (kg/d)</td>
<td>0-42</td>
<td>7.37$^a$</td>
<td>7.53</td>
<td>7.62$^b$</td>
<td>0.05</td>
<td>0.011</td>
</tr>
<tr>
<td>Ratio of ADG to DMI (G:F)</td>
<td>0-42</td>
<td>0.2415</td>
<td>0.2338</td>
<td>0.2328</td>
<td>0.011</td>
<td>0.7957</td>
</tr>
</tbody>
</table>

Treatment administration occurred prior to transport on day -1 and again on day 0 arrival processing.

$^\$: Significance was defined at $P \leq 0.05$. letter differences indicate significance between groups.
Table 3. Mean values for the total days in the feed yard, the initial and final BW, ADG, and carcass characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>AMEL mean (±sem)</th>
<th>PMEL mean (±sem)</th>
<th>CONT mean (±sem)</th>
<th>P-value$^§$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total days in the feedlot</td>
<td>235.00</td>
<td>—</td>
<td>235.00</td>
<td>—</td>
</tr>
<tr>
<td>Initial BW (kg)</td>
<td>254.76 (± 8.57)</td>
<td>251.29 (± 8.57)</td>
<td>245.33 (± 8.57)</td>
<td>0.7381</td>
</tr>
<tr>
<td>Final BW (kg)</td>
<td>662.33 (± 10.89)</td>
<td>656.71 (± 10.89)</td>
<td>640.66 (± 10.89)</td>
<td>0.6332</td>
</tr>
<tr>
<td>ADG (kg/d)</td>
<td>1.72 (± 0.03)</td>
<td>1.73 (± 0.03)</td>
<td>1.68 (± 0.03)</td>
<td>0.4223</td>
</tr>
<tr>
<td>HCW (kg)</td>
<td>393.95 (± 11.08)</td>
<td>397.59 (± 11.00)</td>
<td>396.02 (± 11.02)</td>
<td>0.8587</td>
</tr>
<tr>
<td>HCW/final BW (%)</td>
<td>60.70 (± 0.84)</td>
<td>61.71 (± 0.79)</td>
<td>61.83 (± 0.86)</td>
<td>0.5854</td>
</tr>
<tr>
<td>Ribeye area (cm$^2$)</td>
<td>84.19 (± 4.15)</td>
<td>79.63 (± 4.15)</td>
<td>76.90 (± 4.15)</td>
<td>0.3715</td>
</tr>
<tr>
<td>Fat over 12th rib (cm)</td>
<td>1.35 (± 0.14)</td>
<td>1.40 (± 0.14)</td>
<td>1.30 (± 0.14)</td>
<td>0.5792</td>
</tr>
<tr>
<td>USDA QG</td>
<td>3.04 (± 0.05)</td>
<td>3.08 (± 0.05)</td>
<td>2.89 (± 0.05)</td>
<td>—</td>
</tr>
<tr>
<td>USDA YG</td>
<td>3.19 (± 0.31)</td>
<td>3.44 (± 0.31)</td>
<td>3.25 (± 0.31)</td>
<td>0.3630</td>
</tr>
<tr>
<td>Marbling score</td>
<td>471.15 (± 23.59)</td>
<td>463.37 (± 23.23)</td>
<td>454.47 (± 23.84)</td>
<td>0.6531</td>
</tr>
</tbody>
</table>

$^§$Significance was defined at $P \leq 0.05$ and trends are indicated by $P \leq 0.10$. Quality grade was indicated by translating standard (1), select (2), Choice (3), and Prime (4) to numeric values and averaging. — indicates that groups were not analyzed statistically.
Table 4. Back transformed mean values for biomarkers analyzed on days -1 and 0.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Time (d)</th>
<th>AMEL mean (±sem)</th>
<th>PMEL mean (±sem)</th>
<th>CONT mean (±sem)</th>
<th>Treatment</th>
<th>Time</th>
<th>Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Annexin A1 (ng/mL)</td>
<td>-1</td>
<td>10.90 (± 1.19)</td>
<td>11.68 (± 1.21)</td>
<td>12.08 (± 1.18)</td>
<td>0.8525</td>
<td>0.0004</td>
<td>0.3192</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>8.72 (± 1.19)</td>
<td>7.35 (± 1.21)</td>
<td>6.29 (± 1.18)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Protein (ng/mL)</td>
<td>-1</td>
<td>45.45 (± 8.05)</td>
<td>57.09 (± 8.68)</td>
<td>49.04 (± 7.88)</td>
<td>0.3749</td>
<td>&lt;.0001</td>
<td>0.838</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>64.44 (± 8.05)</td>
<td>82.52 (± 8.68)</td>
<td>74.68 (± 7.88)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cortisol (ng/mL)</td>
<td>-1</td>
<td>36.96 (± 1.10)</td>
<td>35.78 (± 1.09)</td>
<td>35.96 (± 1.10)</td>
<td>0.7839</td>
<td>0.5194</td>
<td>0.6973</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>37.91 (± 1.10)</td>
<td>35.22 (± 1.09)</td>
<td>40.17 (± 1.10)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HP- MMP9</td>
<td>-1</td>
<td>0.54 (± 0.35)</td>
<td>1.09 (± 0.33)</td>
<td>0.74 (± 0.35)</td>
<td>0.7563</td>
<td>0.0015</td>
<td>0.2879</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>1.79 (± 0.35)</td>
<td>1.52 (± 0.33)</td>
<td>1.39 (± 0.35)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Substance P (pg/mL)</td>
<td>-1</td>
<td>76.91 (±1.29)</td>
<td>78.54 (±1.29)</td>
<td>70.12 (±1.29)</td>
<td>0.1474</td>
<td>&lt;.0001</td>
<td>0.984</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>87.28 (±1.29)</td>
<td>89.30 (±1.29)</td>
<td>80.55 (±1.29)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

§Significance was defined at $P \leq 0.05$. Similar letters between groups indicate no significant differences between groups.
Annexin A1 and Total Protein were determined form nasal swabs of calves at designated time points. Cortisol, Hp-MMP9 and Substance P were all quantified from blood samples.
CHAPTER 6.

GENERAL CONCLUSIONS

The results of the research trial described in chapter 3 was successful at indicating a stress and inflammation response during long distance transportation for beef steers. This was identified as an increase in circulating cortisol, substance P, TNF α, Hp-MMP9 complexes and hematologic changes indicative of a bovine stress leukogram. Administration of an oral meloxicam dose of 1 mg per kg with 15 mg tablets was able to reduce the hematologic stress leukogram response. The remainder of the dissertation focused on this targeted dose and route of administration. The reduction in hematologic neutrophilia and monocytosis after shipping is suggestive of having a positive impact for the bovine after transportation. When meloxicam concentration levels were quantified in plasma, there was an inverse proportion of drug concentration to cortisol, neutrophils, and basophils. These results prove that meloxicam has benefit at reducing biomarkers of stress in cattle during transport.

This transport associated stress and inflammatory finding is consistent with a majority of the literature (Arthington et al., 2003, Browning and Leite-Browning, 2013, Buckham Sporer et al., 2008, Kang et al., 2017). When compared to other trials investigating NSAID therapeutic benefit in the midst of transport, similar results were concluded. Flunixin meglumine was also effective at reducing the cortisol response as well as inflammation; acute phase proteins (Cooke et al., 2013). Though this was accomplished, performance was not influenced by flunixin meglumine. Conversely, different investigators who also have interest in meloxicam during transportation
displayed a performance benefit with in the first week (Guarnieri et al., 2014). Though there was evidence of potential positive benefits of NSAID use in the scenario of transportation to reduce the stress responses, immune function with NSAID administration and transportation were unclear.

In our second trial, we addressed the issue of immune function after transport and the effects of meloxicam. Calves were immunologically challenged on arrival with a commercially available MLV (Bovi-Shield Gold, Zoetis). Adaptive immune system was monitored on arrival and one week following the vaccination. This was accomplished through in-vitro stimulation of PBMC’s with BVDV antigen and MP-FCM analysis as describe previously by Platt et al., (2009). Transportation affected circulating PBMC’s on arrival and one week after an on-arrival vaccination. Humoral response to BVDV was increased after one week and there was no difference between groups. Meloxicam did not have an effect on CMI when calves were in the non-transport group. In most cases the responses of meloxicam treatment CMI were similar in expression of CD 25, IL 4 and IFN γ to the non-transport controls. One week after arrival and vaccination, CMI differed between the control transport group and the meloxicam treated transport group. Treatment mitigated the transportation-associated increase in CD 25 expression by PBMCs, CD4⁺, and γδ⁺ T cells. Though there was a difference in transported groups, the meloxicam treated transport group consistently did not differ from non-transported groups. As a function of meloxicam’s effect on PBMCs, we cannot determine the type of effect after the conclusion of the research trial. There are two viable options either in stressful situations meloxicam is inhibitory or maintains homeostatic function of the cells.
Regardless of direction, the best way to confirm this was through a field trial investigating disease outcomes.

This was the first research trial to investigate meloxicam’s effects on the adaptive immune system after a transportation event. Others support our findings that in a non-stressful state, meloxicam does not have inhibitory effects on the immune system (Maslanka and Jaroszewski, 2013). Other proof of concept was validated in previous work proving that meloxicam as well as flunixin has no effect on cellular apoptosis in bovine T cells (Maslanka et al., 2010). The PGE$_2$ production inhibits the CD 25 expression on T cells (Maslanka et al., 2014) and also prevents IFN $\gamma$ production in $\gamma\delta$ T cells (Przybysz et al., 2016). This finding substantiates the idea that meloxicam would be homeostatic rather than inhibitory through the mechanism of action to inhibit COX II preferentially and decrease the enzyme’s production of prostaglandins. Taking the data from both chapters 3, 4 and previous research we inclined to assume that meloxicam is beneficial for specific stress and immune associated aspects of the bovine during transportation. However, though these hypotheses were elucidated, we needed a method to substantiate a production translation and investigate a clinical outcome that could be drawn from meloxicam’s use in a stressful situation to combat disease. Focus of interest would be an alternate hypothesis that meloxicam would aid in prevention of the most common and costly clinical outcome of transportation; BRD.

Finally, we investigated the efficacy of meloxicam in a production setting to look at the potential effects of meloxicam on clinical outcomes. A randomized, blocked, 3 arm parallel study design with no crossover was implemented. To add a practical component
to the study design, the third arm of the study. On arrival meloxicam treatment was added as the third arm. Most veterinarians and producers have a difficult time accessing cattle prior to transportation. Because of this limitation, we felt the addition was justified in our research. With mitigation of the stress response and presumptive homeostatic effects on the immune system using an oxicam class NSAID, we hypothesized meloxicam would reduce the BRD associated morbidity and decrease DART outcomes. Execution of the project allowed us to reject our hypothesis. After evaluation of the clinical outcomes we had no changes in the morbidity between meloxicam treated groups and the control groups over the entire trial period. Of the cattle that were pulled for BRD sickness through the DART system, no differences were seen in the clinical outcomes measured in sick cattle. The performance in the first 42 days showed no difference in ADG or G:F. Most of parameters at harvest were not significantly different between groups. The ADG and final body weights were not different. The HCW, dressing percent, YG, marbling score, ribeye area, and fat over the 12th rib also remained unchanged between treatment groups. Lastly, of the biomarkers analyzed, no treatment effects were elucidated. Treatment with meloxicam pre-transport ended in a higher substance P on arrival. HP-MMP-9 and Annexin A1 all had significant time effects. The only correlation to BRD was the population of calves with a lighter body weight were more prone to develop BRD. This was then included in the model as a fixed effect. This was the case without an effect of treatment.

Contradictory to our initial hypothesis, meloxicam did not benefit calves entering the feed yard in the aid to prevent clinical BRD. There was no benefit in the first 42 days of the feeding period. Other published research suggests meloxicam was beneficial as aid
in performance over the first week of the feeding period through gain and feed efficiency (Guarnieri et al., 2014). Administering a therapeutic for performance benefits is not a justifiable use for ELDU, however this unintentional beneficial consequence through the true targeted action of stress or pain reduction would justify use. Any benefits of performance and increased feed efficiency were hypothesized to be related to the stress reduction as mentioned in the chapter 3 summary. These benefits were not seen in our study, most likely due to compensatory gain. Monitoring for performance benefits at 42 days on feed consisted of ADG, DMI, and G:F. Others have reported greater carcass weights at harvest and reduced lung lesions when meloxicam was administered at BRD onset (Friton et al., 2005). Findings related to health were similar to all other findings in the trial. No evidence of reduction in 1st pulls or overall treatments. This was different from Friton et al., whom instead treated cattle with the NSAID at BRD diagnosis. Lastly the biomarkers from circulating plasma and nasal swabs were not influenced by treatment. Others have correlated increased annexins to BRD resistance (Senthilkumaran et al., 2013), and HP-MMP9 to inflammation associated with BRD (Bannikov et al., 2011). Cortisol has been measured to account for indication of stressors (Arthington et al., 2003) and substance P have also been measured to account for pain (Coetzee et al., 2008). The increases noted in the trial were in line with our hypothesis. The lack of treatment significance allowed us to reject the hypothesis of a treatment benefit to reduce these biomarkers in populations at high risk for BRD. The fifth chapter for sampling timepoints were insufficient and constrained. The primary study design objective to monitor for BRD without confounding days with data collection or stressor events. Lining up timepoints and cortisol levels to substantiate the same evidence can be
difficult. With that said, there is justification for meloxicam use regardless of the lack of significant findings on these biomarkers in chapter 5. Changes may have occurred but were missed. Simple indicators such as bodyweight, distance traveled, shrink, and origination are still the best for the practicing veterinarian when deciding risk category. Previous researchers have accounted for these results; bodyweight, shrink, and distance traveled have a high impact on BRD outcomes in the feed yard (Cernicchiaro et al., 2012).

The dissertation research confirms that meloxicam is beneficial when administered prior to transportation for reduction of the stress leukogram and potential normalization of immune function but lacks clinical efficacy in mitigation of disease outcomes or live animal performance. As the bovine industry moves forward it is critical to address the animal’s welfare and appeal to the public’s perception of industry practices. In this unavoidable life event, strategies to improve bovine welfare with the use of pharmaceutics is paramount. Meloxicam’s substantiated research as an analgesic as well as for stress reduction would still error on the side of a justifiable ELDU. Without expectation of dramatic financial gain from therapeutic use, the ability to persuade a producer into use would be difficult. Fortunately, generic meloxicam is affordable and does provide them anecdotal evidence to be beneficial. Non-reported data from a collaborative field trial with a veterinarian transporting Holstein bull calves from Texas to Iowa described treated animals as more bright, alert, and aggressive at the feed bunk. The producer was blinded to treatment. Antibiotic treatments for respiratory disease between the groups was not different in the first 45 days and weights were not different between a MEL and CONT treated groups. Data back revealed similar results to chapter
5. However with an n=90, the use of data for treatment was limited. Moving forward, further investigation is required with other novel therapeutics as well as other classes of non-pharmaceutical products that enter the market. The targeted approach of the pharmaceutical industry should be at therapeutics to modulate and enhance the immune system or pharmacologic agents to reduce anxiety without repercussions of sedation. However, the greatest impact would be a full-scale change in the beef industry. This arduous task would take a great effort from multiple parties. Based on the findings of this doctoral project, the best guided approach at combating transportation stress start before transportation. In summary, great management practices would trump any pharmaceutical intervention.

**Literature Cited:**


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…The final conclusion that I can draw from this program: Murphy’s law was proven correct time and time again: What can go wrong, will go wrong. One must be prepared to overcome.