Nutritional modulation of oxidative stress in beef steers during the feedlot receiving period: A focus on transit stress

by

Erin Lea Deters

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Program of Study Committee:
Stephanie Hansen, Major Professor
Olivia Genther-Schroeder
Elisabeth Huff-Lonergan
Anna Johnson
Daniel Loy

The student author, whose presentation of the scholarship herein was approved by the program of study committee, is solely responsible for the content of this dissertation. The Graduate College will ensure this dissertation is globally accessible and will not permit alterations after a degree is conferred.

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DEDICATION

This dissertation is dedicated to my grandparents, Garnett and Sandra Deters, for their endless love and support throughout my pursuit of this degree and life in general.

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NOMENCLATURE

ADF	Acid detergent fiber
ADG	Average daily gain
APP	Acute phase protein
ATP	Adenosine triphosphate
BRD	Bovine respiratory disease
BW	Body weight
CP	Crude protein
Cu	Copper
DM	Dry matter
DMI	Dry matter intake
DNA	Deoxyribonucleic acid
ELISA	Enzyme-linked immunosorbent assay
Fe	Iron
FRAP	Ferric reducing antioxidant potential
GC	Gas chromatography
G:F	Gain:feed
GSH	Glutathione (reduced)
GSSG	Glutathione (oxidized)
H	Hydrogen
H_2O_2	Hydrogen peroxide
HPA	Hypothalamic-pituitary-adrenal
HPLC	High-performance liquid chromatography

IU	International unit
K	Potassium
Keap1	Kelch-like ECH-associated protein 1
$LOO^{\bullet}$	Lipid peroxyl radical
MDA	Malondialdehyde
MS	Mass spectrometry
Mn	Manganese
Na	Sodium
NADPH	Nicotinamide adenine dinucleotide phosphate
NDF	Neutral detergent fiber
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
Nrf2	Nuclear factor erythroid 2-related factor
$O_2^{\bullet-}$	Superoxide radical
$OH^{\bullet}$	Hydroxyl radical
OM	Organic matter
PUFA	Polyunsaturated fatty acid
RNA	Ribonucleic acid
$ROO^{\bullet}$	Peroxyl radical
ROS	Reactive oxygen species
SAM	Sympathetic-adrenal-medullary
SCFP	*Saccharomyces cerevisiae* fermentation product
Se	Selenium
SOD	Superoxide dismutase

TBARS	Thiobarbituric acid reactive substances
TMR	Total mixed ration
VC	Vitamin C (ascorbate)
VE	Vitamin E (α-tocopherol)
Zn	Zinc

ABSTRACT

Feedlot receiving (i.e. the first 42 to 56 d after feedlot arrival) is a critical time period in the life of many beef animals. The combination of stressors (e. g. weaning, vaccination, commingling, novel pathogens and feedstuffs) experienced during feedlot receiving results in poor feed intake, decreased growth and increased susceptibility to disease. Transportation is a component of receiving period stress that is unavoidable due to the segmented nature of the beef industry. Transported cattle are subject to feed and water deprivation, psychological stress and physical exertion which may further hinder animal health and production efficiency. Therefore, investigation of strategies to mitigate the negative effects of receiving period and transit stress is warranted. The first objective of this research was to gain a more thorough understanding of how cattle biologically respond to transit stress, with an emphasis on transit-induced changes in oxidative stress biomarkers. Results from the studies presented herein have shown 1) a 10% decrease in plasma ascorbate and total antioxidant concentrations immediately post-transit, 2) a 16% increase in red blood cell lysate Mn-superoxide dismutase activity 1 d post-transit and 3) increased liver and muscle superoxide dismutase activity immediately post-transit. This research has also provided evidence that transportation of cattle stimulates tissue mobilization of energy producing substrates and elicits an inflammatory response. These biological processes can contribute to the production of reactive oxygen species and, in combination with the observed decrease in antioxidant status, result in oxidative stress and upregulation of antioxidant enzymes. Because endogenous synthesis of antioxidants requires nutrients that might otherwise be used by the animal for growth and immune function, the second objective of this research was to determine if improving antioxidant status of beef steers with nutritional supplements would increase resilience or aid in recovery from receiving period or transit stress. Increasing dietary

supplementation of vitamin E, a fat-soluble antioxidant, during the receiving period linearly increased vitamin E status, but decreased concentrations of the endogenous antioxidant glutathione, likely due to a sparing effect of vitamin E. Minimal effects of supplemental vitamin E on feedlot health and performance were noted, probably because initial vitamin E status of steers was adequate and vitamin E deficiency did not develop in this 28 d study. Steers supplemented a yeast fermentation product with indirect antioxidant properties tended to have greater liver glutathione concentrations prior to a long-distance transit event and exhibited greater average daily gain for the first 30 d post-transit. In a separate study, superoxide dismutase activity and metabolites involved in the pentose phosphate pathway were increased in muscle of transported steers, suggesting the physical exertion associated with transit resulted in oxidative stress in the muscle, which could be detrimental to post-transit growth. To mitigate this response, steers were administered injectable vitamin C, a potent antioxidant found in large quantities in the muscle, immediately prior to or after a long-distance transit event. Steers administered vitamin C pre-transit had greater post-transit plasma ascorbate concentrations and exhibited greater average daily gain for the 56 d post-transit than steers that did not receive vitamin C or steers that received vitamin C after the transit event. Collectively, these data indicate nutritional strategies to improve antioxidant status are more effective in improving post-transit performance when they are adopted proactively (pre-transit) rather than retroactively (post-transit). Future research should seek to better understand the long-term implications of oxidative stress on cattle health and performance. Additionally, further refinement of the nutritional strategies utilized herein is needed to optimize dose and timing of supplementation for oxidative stress modulation.

CHAPTER 1. GENERAL INTRODUCTION

The first 42 to 56 d after feedlot entry is termed the receiving period. Newly received calves often encounter numerous stressors prior to and during the feedlot receiving period including psychological stressors (weaning, mixing with unfamiliar animals, handling, exposure to novel environments and feedstuffs) in addition to physical stressors (vaccination, castration and dehorning). As a result of stressors encountered by cattle during this time, dry matter intake by newly received calves is typically low, averaging only 1.5% of BW for the first two weeks after arrival (Hutcheson and Cole, 1986). Decreased nutrient intake may result in nutritional deficiencies that hinder an animal's ability to maintain optimal immune function and growth. Thus, the feedlot receiving period is often characterized by poor health and performance. Indeed, bovine respiratory disease outbreaks occur frequently in highly stressed, newly received feedlot cattle (Duff and Galyean, 2007). This disease is aptly referred to as "shipping fever" because transportation often precedes disease outbreaks.

Road transportation is a component of receiving period stress that typically cannot be avoided in the U.S. beef industry due to disparity in geographical locations of cow-calf/stocker operations and feedlot operations. Similar to receiving period stress, transportation is an amalgamation of psychological and physical stressors including interaction with humans, exposure to new sights, sounds and smells as well as deprivation of feed and water (Knowles et al., 2014). Major physical stressors include muscle fatigue from standing during transit and muscle damage from harsh contact with other cattle and/or the trailer during transit, loading and unloading (Thomson et al., 2015; Lee et al., 2017). These stressors have been shown to elicit profound physiological responses such as activation of hormonal pathways that increase mobilization and metabolism of carbohydrates, fatty acids and amino acids (Warriss et al., 1995).

Additionally, biomarkers of inflammation have been shown to increase in response to transit (Marques et al., 2012). Several metabolic and inflammatory pathways activated by transit can lead to the production of reactive oxygen species (**ROS**).

Oxidative stress results when the production of prooxidants, such as ROS, overwhelms cellular antioxidant capacity (Sies, 1985). When left unchecked, ROS cause damage to cellular components including lipids, proteins and nucleic acids. Oxidative stress has negative implications for animal health and efficiency. For example, energy is often required to repair and/or degrade oxidatively damaged molecules. Additionally, endogenous synthesis of antioxidants to combat an oxidative insult requires energy and amino acids, potentially limiting resources that may otherwise be used by the animal for growth and immune function. Although cattle are equipped with a redundant antioxidant defense system comprised of endogenous nonenzymatic antioxidants, antioxidant enzymes and exogenous antioxidants, it has been observed that transportation of cattle decreases antioxidant status and increases markers of oxidative damage (Chirase et al., 2004). Therefore, cattle may benefit from nutritional intervention to improve antioxidant status prior to or post-transit.

Dissertation Organization

The following chapter (Chapter 2) will provide a detailed literature review regarding transit and oxidative stress, with emphasis on how stressors associated with transit (psychological stress, food deprivation and physical exertion) can contribute to the development of oxidative stress in cattle. Additionally, this chapter will describe some potential nutritional strategies for oxidative stress modulation (yeast fermentation products, vitamin E and vitamin C). The subsequent three chapters will present research investigating the effects of these supplements on feedlot performance and oxidative stress biomarkers in beef steers. Specifically,

this research examines various vitamin E supplementation rates during the feedlot receiving period (chapter 3), dose and timing of yeast fermentation product supplementation relative to a long-distance transit event (chapter 4) as well as administration of injectable vitamin C before or after a long-distance transit event (chapter 5). The final research chapter (chapter 6) aims to gain a more thorough understanding of how cattle biologically respond to transit. Finally, this dissertation will conclude (chapter 7) with overall findings from the research presented herein and suggestions for future research.

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CHAPTER 2. REVIEW OF THE LITERATURE

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E. L. Deters and S. L Hansen

Transit Stress

Cattle Transport Practices

Transportation of cattle has markedly evolved since the early 1600s when cattle arrived in colonial North America on ships from England (Bowling, 1942). The development of the U.S. railroad system in the 1850s substantially influenced beef cattle production. Stockmen, known as drovers, would gather, drive and concentrate cattle at railheads in the Southern Plains for transport to major feedlots and processing plants in the Midwest (Swanson and Morrow-Tesch, 2001). Due to animal welfare concerns about conditions during transport, the 28-hour law was approved in 1873 and prevented the transportation of livestock by rail for more than 28 consecutive h without unloading for a 5 h period of rest, water and feeding (USDA, 1918). With the development of refrigerated rail cars in 1867, rail transport of processed beef expanded while rail transport of live cattle decreased. In the early 1900s, transportation of live cattle by truck became increasingly common and eventually gave rise to today's commercial livestock trucking industry. The 28-hour law was amended in 1994 to include transportation of livestock by road (USDA, 1994). Although live cattle in the U.S. are predominantly transported by truck now, weaned beef calves from Hawaii may be transported to the mainland for finishing by ship or air (Cox and Bredhoff, 2003).

Transportation of cattle remains a necessary part of U.S. beef production due to the segmented nature of the beef industry and disparity in geographical locations of industry

segments. The cow-calf segment is widely dispersed across the U.S. to take advantage of forages grown on land that is typically not suitable for grain production. Alternatively, the feedlot segment is geographically more concentrated in areas where grain and grain byproduct availability are high (i.e. the Midwest) or where the climate is suitable for cattle feeding and feeds can be transported in (i.e. the Southern Plains). Therefore, a beef animal will likely be transported upwards of 3 times throughout its life: from birthplace, to an auction market, stocker/backgrounding operation, feedlot and finally to a processing facility. In addition to frequent transportation events, cattle may be transported over long distances. Data from 14,601 cohorts from 21 feedlots found the average distance traveled by feeder calves from place of origin to feedlot was 697.9 km with a maximum distance of 3086.7 km (Cernicchiaro et al., 2012). At an average driving speed of 100 km/h, the average transit duration would be 7 h with a maximum duration of 31 h.

Physiologic Responses to Transit

Transportation is an amalgamation of physical and psychological stressors including food and water deprivation, standing for long periods of time, handling as well as exposure to novel noises and environmental conditions. These stressors elicit physiological changes (**Table 1**) that may influence cattle health and performance upon arrival at their destination.

Table 1. Commonly used physiological indicators of stress during transport[1]

Stressor	Physiological variable[2]
Food deprivation	↑ BW loss ↑ NEFA ↓ glucose ↑ urea
Dehydration	↑ albumin ↑ PCV ↑ total protein
Physical exertion	↑ CK ↑ lactate
Psychological stress (e.g. fear)	↑ cortisol ↑ PCV ↑ heart rate ↑ respiration rate
Inflammation	↑ ceruloplasmin ↑ haptoglobin ↑ fibrinogen ↑ SAA

[1]Adapted from Knowles et al. (2014) and Van Engen and Coetzee (2018)
[2]NEFA = non-esterified fatty acids; PCV = packed cell volume; CK = creatine kinase; SAA = serum amyloid A

Food Deprivation and Dehydration. Commercial livestock trailers are typically not equipped to hold feed and water; thus, cattle are deprived of feed and water for at least the duration of transit resulting in loss of BW (i.e. shrink). Hereford × Friesian and continental type steers (12-18 months of age; 341 kg) transported for 5, 10 or 15 h (286, 536 or 738 km) lost 4.6, 6.5 and 7.0% of their BW, respectively (Warriss et al., 1995). Thus, the rate of shrink (% BW/h) was 0.92 from h 0-5, 0.38 from h 5-10 and 0.11 from h 10-15. Weight loss during transit is typically more accelerated and severe than cattle simply deprived of food and water but not transported. For example, total N excretion was greater in yearling crossbred steers (261 kg) transported for 13 h (trial 1) and 46 h (trial 2) compared to steers not transported but restricted from feed and water for the same duration (Cole et al., 1986). Additionally, Marques et al. (2012) observed greater shrink in steers and heifers (Angus × Hereford; 217 kg) transported for 24 h (1,200 km) compared to non-transported cattle deprived of feed and water for 24 h (9.6 vs. 8.1%), providing evidence that transportation shrink is not solely due to loss of GI tract contents. Self and Gay (1972) found less than half of the weight lost by feeder cattle (276 kg) during transit (966 km) is replaced by refilling of the GI tract upon arrival. Self and Gay (1972) also reported that cattle (267 kg) transported an average of 1,023 km experienced an average shrink of 8%; cattle purchased from a ranch and shipped directly to the feedlot shrank less than cattle purchased from a sale facility, but there was no difference in shrink between cattle classified as calves or yearlings. The length of time required to recover lost BW averaged 10.6 days (range 3-30; Self and Gay, 1972).

Food deprivation necessitates the use of body reserves to supply the animal with energy. In a fasted state, blood glucose concentrations decline due to less glucose availability from the GI tract and depletion of liver glycogen stores. The decline in blood glucose leads to decreased

insulin secretion and increased glucagon secretion from the pancreas (Gerich et al., 1974a; Gerich et al., 1974b). Glucagon stimulates the liver to increase glycogenolysis and gluconeogenesis to maintain blood glucose concentrations (Wagle and Ingebretsen, 1973; Eisenstein et al., 1974). Additionally, glucagon stimulates the hydrolysis of triglycerides into glycerol and non-esterified fatty acids to facilitate their mobilization from adipose tissue and use as metabolic fuel in other tissues including the liver and muscle (Perea et al., 1995). The most notable effects of water restriction experienced during transit are an increase in packed cell volume, a proxy for plasma volume, as well as increases in plasma total protein and albumin (Kent and Ewbank, 1983; Warriss et al., 1995). Transit induced increases in dehydration indicators returned to baseline shortly after regaining access to water (Warriss et al., 1995).

Feed and water restriction can also influence ruminal characteristics and subsequent digestive function of cattle. Total counts of rumen bacteria and protozoa were lesser in fistulated steers (477 kg) transported or restricted from feed and water for 32 h compared to control steers until 72 h post-transit/restriction (Galyean et al., 1981). Additionally, recovery of microbial numbers was slower in transported steers than in restricted steers (Galyean et al., 1981). Fluharty et al. (1994) investigated microbial populations and digestive capabilities of newly weaned steers (245 kg) transported for approximately 8.5 h to a sale barn where they were deprived of feed and water for another 15.5 h prior to transportation a feedlot. No differences due to feed and water deprivation were observed on the concentration of total bacteria or cellulolytic bacteria, but the concentration of total protozoa was lesser upon arrival at the feedlot (Fluharty et al., 1994). There were also no differences in 48 h in situ NDF disappearance between d -3 (prior to weaning) and d 0 (feedlot arrival) indicating that the stress of weaning, transport and fasting did not inhibit the ability of the ruminal microbial population to digest available substrate (Fluharty

et al., 1994). In addition to complete feed and water restriction during transit, cattle may short-term self-restrict upon arrival at their destination (Hutcheson and Cole, 1986) which may decrease ruminal short-chain fatty acid concentration and absorption as well as total tract barrier function (Zhang et al., 2013).

Feed and water restriction represent both economic and animal welfare concerns. As the percentage of shrink increases, calf value declines often in excess of $10 (Coffey et al., 2001). Additionally, Cernicchiaro et al. (2012) found that cattle originating from the central region of the US (IA, KS, MO, NE, ND, SD) transported > 250 km had a greater risk of bovine respiratory disease (**BRD**) morbidity and overall mortality compared to cattle transported < 250 km. Several methods have been investigated to mitigate the effects of transit induced feed and water restriction on cattle health and performance. Cooke et al. (2013) investigated the effects of 2 h rest stops every 430 km where steers and heifers (Angus × Hereford; 229 kg) had ad libitum access to hay and water vs. continuous transport for 1,290 km. Rest stops decreased BW shrink (5.8 vs. 10.2%) as well as cortisol and non-esterified fatty acid concentrations immediately after unloading; however, there was no benefit in ADG, DMI or G:F measured 28 d post-transit (Cooke et al., 2013). It is noteworthy that several challenges exist with regards to the adoption of rest stops including biosecurity as well as the psychological stress associated with loading and unloading. To mitigate marketing and transit induced dehydration, Tomczak et al. (2019) evaluated the effects of oral hydration therapy (0.57 L of water/45.4 kg of BW) during initial processing (day of feedlot arrival) on health and performance of high-risk crossbred beef calves. In experiment 1, heifers (197 kg) administered the hydration therapy had numerically greater DMI during the 56-d receiving period. In experiment 2, bulls and steers (189 kg) administered the hydration therapy had greater final BW and overall ADG, with a tendency for increased DMI

from d 42 to 56. However, health outcomes were not improved in either experiment (Tomczak et al., 2019).

Physical Exertion. Cattle tend not to lie down while trucks are moving (Warriss et al., 1995) but long transit durations (> 24 h) may cause more animals to lie down if adequate space is available (Knowles, 1999). Transportation may contribute to both muscle fatigue and damage as cattle are standing for long periods of time, trying to maintain their balance and may experience bumping and falls that result in bruising (Schwartzkopf-Genswein and Grandin, 2014). Muscle fatigue is defined as a decreased ability to generate appropriate amounts of contractile force and can occur shortly after onset of exercise (acute) or after exercise has been carried out for a prolonged period of time (delayed); alternatively, muscle damage is accompanied by structural damage to muscle fibers and usually takes longer to recover (Finsterer, 2012).

Lactate is a commonly measured indicator of muscle fatigue as an increase in blood lactate indicates there has been a metabolic shift in the production of ATP from oxidative phosphorylation to anaerobic glycolysis. Warriss et al. (1995) observed transit durations of 10 or 15 h increased lactate concentrations relative to pre-transit values. Creatine kinase (**CK**) is important in regeneration of cellular ATP and is found in the mitochondria and cytosol of tissues with high energy demands including cardiac muscle, skeletal muscle and the brain (Wallimann et al., 1992). This enzyme is normally confined to the muscle cell, thus the presence of CK in the blood has generally been considered to be a marker of muscle damage (Baird et al., 2012). However, it has been suggested that the appearance of CK in serum following exercise represents a disturbance to muscle energy processes rather than physical or structural damage (Baird et al., 2012). Several studies have reported an increase in blood CK activity after transport of cattle. Alam et al. (2018) observed increased serum CK activity in adult cattle (> 2 years)

immediately after a 14 h (648 km) transit event, with CK activity returning to baseline by 24 h post-transit. Plasma CK activities of Hereford × Angus calves (240 kg) transported for 63 h (1,450 km) were greatest immediately after unloading and 24 h post-transit (Werner et al., 2013).

Differentiating the extent to which skeletal muscle is fatigued vs. damaged during transit may be beneficial in developing preventative or recovery-based strategies to maximize muscle growth upon arrival. Recovery from muscle damage involves inflammatory and immune responses as well as regeneration via stimulation of satellite cells (Tiidus, 2005). Satellite cells are multipotent stem cells capable of becoming myocytes (Seale et al., 2001) and exist in a quiescent state between the basal lamina and plasma membrane of muscle fibers (Yin et al., 2013). Extracellular signals regulate satellite cell activation (hepatic growth factor; Tatsumi et al., 1998), proliferation (fibroblast growth factor; Yamada et al., 1989), differentiation (insulin like growth factor-1; Engert et al., 1996) and fusion (M-cadherin; Irintchev et al., 1994) with existing muscle fibers. Recruitment of satellite cells is the primary mechanism by which bovine skeletal muscle grows postnatally as muscle fiber number is relatively fixed at birth (Rowe and Goldspink, 1969). While satellite cell activation is necessary for skeletal muscle growth and repair, over activation may lead to depletion of the satellite cell pool (Dayanidhi and Lieber, 2014). To the authors knowledge, satellite cell proliferation has not been investigated in response to transit.

Psychological Stress. Cattle are exposed to a variety of psychological stressors during transport including handling during loading and unloading, unfamiliar noises and environments as well as mixing with unfamiliar animals. The body responds to stress by activating two major hormonal axes: the sympathetic-adrenal-medullary (**SAM**) and hypothalamic-pituitary-adrenal axis (**HPA**). In the short term, activation of the SAM-axis initiates the release of catecholamines

including epinephrine (i.e. adrenaline) and norepinephrine. These hormones support the “fight or flight” response by increasing heart rate, blood pressure and glucose availability via stimulation of hepatic glycogenolysis (Guyton et al., 1958; Chu et al., 1997). For Angus and Charolais steers (220 kg) transported for 15 h, heart rate was highest during the first 15 min of transit and gradually decreased until min 121-161 (~ 2.5 h) where it remained steady until the end of the transit event (Schwartzkopf-Genswein et al., 2007).

The long-term stress response is maintained by the HPA-axis. Briefly, corticotrophic releasing factor released from the hypothalamus stimulates the release of adrenocorticotropic hormone from the pituitary which ultimately signals the release of glucocorticoids (primarily cortisol) from the adrenal cortex (Grossman et al., 1982). Glucocorticoids stimulate the release of glycerol and fatty acids from adipose tissue as well as amino acids from muscle, directing these nutrients towards the liver for enzyme synthesis and gluconeogenesis (Baxter and Forsham, 1972). Glucocorticoids are also known to have immunosuppressive and anti-inflammatory effects (Roth and Kaeberle, 1982). An increase in cortisol is a well-documented and commonly measured indicator of psychological stress due to transport in cattle (Van Engen et al., 2018). However, longer duration transit events may result in no change or even a decrease in cortisol concentrations likely due to a combination of factors including cattle acclimation to transport (Warriss et al., 1995), negative feedback regulation of cortisol on the pituitary and hypothalamus (Reader et al., 1982) as well as the relatively short half-life of cortisol in the blood (~70-130 min; Tunn et al., 1992).

Inflammation. The inflammatory response is trigged by external stimuli (pathogen associated molecular patterns) from bacteria, fungi and viruses in addition to internal stimuli (damage associated molecular patterns) from the mitochondria, nucleus and other intracellular

compartments (Tang et al., 2012). These patterns are then recognized and presented to immune cells via pattern recognition receptors, stimulating the production and release of pro-inflammatory cytokines (e.g. interleukin-1, interleukin-6 and tumor-necrosis-factor-α). Pro-inflammatory cytokines cause fever, anorexia, leukocytosis, increased glucocorticoid hormones, muscle catabolism and changes in liver protein synthesis to support the production of acute phase proteins (**APP**; Dinarello et al., 1986; Castell et al., 1989; Ulich et al., 1989), with the ultimate goal of resolving inflammation. Major APP have low serum concentrations (<1 μg/L) in healthy animals but rise dramatically (100-1,000-fold) upon stimulation, peak at 24-48 h and then decline rapidly (Eckersall and Bell, 2010). Major bovine APP include haptoglobin and serum amyloid A. Haptoglobin has several biological functions including binding of hemoglobin as well as anti-inflammatory, bacteriostatic and chaperone activity (Ceciliani et al., 2012). Serum amyloid A also has several functions that include scavenging of cholesterol, immunomodulatory activity and opsonization of bacteria (Ceciliani et al., 2012). Moderate APP increase only 5-10-fold upon activation and peak after 48-72 h; moderate APP typically measured in cattle include ceruloplasmin and fibrinogen.

Transportation of cattle has been shown to result in systemic inflammation evidenced by increases in APP. Marques et al. (2012) observed increased plasma haptoglobin immediately post-transit in beef calves (Angus × Hereford steers and heifers; 217 kg) transported for 24 h (1,200 km). Cooke et al. (2013) observed a similar response in Angus × Hereford steers and heifers (229 kg) transported 1,290 km. Plasma haptoglobin concentrations returned to pre-transit values by 4 d post-transit in both studies (Marques et al., 2012; Cooke et al., 2013). Although Arthington et al. (2003) did not observe increased plasma haptoglobin concentrations in newly weaned steer calves (266 kg) transported 344 km, plasma fibrinogen was increased in transported

calves on d 1 and 3 post-transit. While the APP response is beneficial for animal health in the short term, persistent inflammation may negatively impact feedlot performance. Inflammation requires a significant amount of nutrients, increases maintenance requirements and decreases feed intake (Johnson, 1997). Thus, it has been hypothesized that during periods of immune challenge, proinflammatory cytokines elicit a physiological response in which nutrients are directed away from tissue growth in support of immune function (Spurlock, 1997). In support of this hypothesis, (Araujo et al., 2010) observed a negative correlation between circulating concentrations of APP (ceruloplasmin and fibrinogen) with receiving period (30 d) DMI and ADG of Braford steers (218 kg) transported 1,600 km.

Summary

Transportation of cattle is virtually unavoidable in U.S. beef production. Transit induces a series of metabolic changes including increased breakdown of adipose tissue and skeletal muscle to provide the liver with fuel to support gluconeogenesis and APP production. Although these metabolic changes are intended to re-establish homeostasis, several physiological indicators of stress discussed herein have been associated with increased feedlot morbidity and/or decreased feedlot performance:

- BW shrink (Cernicchiaro et al., 2012)
- Cortisol concentrations (Roth and Kaeberle, 1982)
- Concentrations of APP (Araujo et al., 2010)

Despite many of these indicators returning to pre-transit values in a short period of time (24-72 h), transportation may have long term implications for cattle health and performance. As it is

unlikely that transit can be avoided, emphasis should be placed on preparing cattle to respond appropriately to transit stress and/or managing the physiological outcomes.

Oxidative Stress

History and Definition

Over 3 billion years ago cells evolved to utilize oxidative metabolism to produce energy in the form of adenosine triphosphate (**ATP**); this is the primary mechanism by which present-day cells generate metabolic energy (Cooper, 2000). Oxygen is a highly reactive molecule that allows for greater ATP production (36-38 ATP/glucose) in contrast to anaerobic glycolysis (2 ATP/glucose; Zubay, 1993). However, this high reactivity contributes to the production of reactive oxygen species (**ROS**). At high concentrations, ROS negatively impact macromolecule stability and cellular function, leading to formulation of the free-radical theory of aging and the concept of oxidative stress. The free-radical theory of aging proposes that endogenous production of oxidants results in a pattern of cumulative damage and degenerative changes (Harman, 1955) while oxidative stress was first defined as "a disturbance in the prooxidant-antioxidant balance in favor of the former" (Sies, 1985). This simplified definition was later expounded to "an imbalance between oxidants and antioxidants in favor of the oxidants, leading to a disruption of redox signaling and control and/or molecular damage" (Sies, 2007).

Oxidants

Reactive oxygen species can come from exogenous sources or be produced within cells. Exogenous sources of ROS include cigarette smoke, ultraviolet light, radiation and pollutants (Schröder and Krutmann, 2005) while endogenous sources of ROS include mitochondria, peroxisomes and various enzymatic systems. Mitochondria are the major intracellular source of

ROS due to the leaking of electrons from coenzyme Q onto oxygen in the electron transport chain to form superoxide radicals (**$O_2^{\bullet-}$**; Turrens et al., 1985). It was originally estimated that 2-5% of oxygen consumed by mitochondria was converted to $O_2^{\bullet-}$ (Boveris and Chance, 1973). However, more recent evidence has suggested that this number may be much lower (~0.15%; St-Pierre et al., 2002). Intracellular $O_2^{\bullet-}$ is also produced by cytochrome P-450 enzymes during hydroxylation reactions in the endoplasmic reticulum (Sligar et al., 1974), by nicotinamide adenine dinucleotide phosphate (**NADPH**) oxidase during immune cell phagocytosis (Hohn and Lehrer, 1975) and by xanthine oxidase during purine metabolism (Nilsson et al., 1969). Although $O_2^{\bullet-}$ is relatively unreactive and membrane impermeable, it is the precursor of most other ROS and propagates oxidative chain reactions (Sawyer and Valentine, 1981).

Spontaneous or enzymatic dismutation of $O_2^{\bullet-}$ produces hydrogen peroxide (**H_2O_2**) which can transverse membranes and has a relatively long half-life (1 ms) compared to other ROS, making it suitable as a signaling molecule (Reth, 2002). Approximately 35% of endogenously produced H_2O_2 is generated by peroxisomes (Boveris et al., 1972), small organelles involved in metabolism of D-amino acids as well as β-oxidation of branched and very long chain fatty acids (Schrader and Fahimi, 2006). In the presence of transition metals, such as Cu and Fe, H_2O_2 can participate in Fenton and Haber-Weiss reactions, leading to production of the hydroxyl radical (**$OH^{\bullet}$**; Fenton, 1894; Haber and Weiss, 1932). The hydroxyl radical is highly reactive with a short-half life (10^{-9} s); thus, $OH^{\bullet}$ reacts closely to its site of formation and can oxidize most classes of biomolecules (Halliwell and Gutteridge, 1992). For example, interaction of $OH^{\bullet}$ with polyunsaturated fatty acids results in formation of lipid peroxyl radicals (**$LOO^{\bullet}$**; (Gutteridge et al., 1979). Peroxyl radicals (**$ROO^{\bullet}$**) can also be produced by lipoxygenase and cyclooxygenase

enzymes during synthesis of arachidonic acid derived cell signaling molecules (Hamburg and Samuelsson, 1967; Tang et al., 1997).

Antioxidants

Endogenous Nonenzymatic Antioxidants. Endogenously produced nonenzymatic antioxidants include glutathione, bilirubin and uric acid. Glutathione is a tripeptide composed of glutamine, cysteine and lysine that is synthesized in a two-step ATP dependent process in the liver and transported to tissues in the blood (Johnston and Bloch, 1951; Griffith, 1999). The thiol group of glutathione has several functions including recycling of other cellular antioxidants, neutralization of ROS and regulation of protein function via interaction with cysteine residues (Niki et al., 1982; Stein et al., 1991; Velu et al., 2007). Upon H donation, oxidized glutathione can be regenerated to its reduced form by glutathione reductase utilizing NADPH (Racker, 1955). Bilirubin is the end product of heme degradation and has strong antioxidant potential against $ROO^{\bullet}$ (Stocker et al., 1987) but is present at much lower concentrations in tissues than glutathione (20-50 nM vs. 5-10 mM; Sedlak et al., 2009). Uric acid is a byproduct of purine metabolism and, like bilirubin, can scavenge $ROO^{\bullet}$ (Ames et al., 1981). Additionally, uric acid can chelate prooxidant metals to limit the production of $OH^{\bullet}$ via Fenton and Haber-Weiss reactions (Davies et al., 1986). Other metal binding proteins that limit the availability of Cu and Fe include ceruloplasmin and albumin (Orena et al., 1986; Halliwell, 1988).

Vitamin C (**VC**; ascorbic acid) is the major water-soluble antioxidant present in cells. Several species (e.g. humans, guinea pigs, bats) have lost the ability to endogenously synthesize VC due to mutations in the L-gulonolactone oxidase gene which codes for the enzyme that catalyzes the last step of VC biosynthesis (Drouin et al., 2011). This enzyme is functional in the liver of cattle which allows for the synthesis of VC from glucose (Chatterjee, 1973) and, in most cases, eliminates the need for exogenous supplementation. Ascorbic acid can reduce ROS

through two successive electron donations; the first donation results in the formation of the ascorbyl free radical which can undergo a second electron donation to form dehydroascorbic acid (Bendich et al., 1986). Mechanisms of VC recycling include reduction by intracellular dehydroascorbic acid reductases like thioredoxin reductase (May et al., 1997) as well as other endogenous antioxidants like glutathione (Winkler et al., 1994).

Antioxidant Enzymes. The primary antioxidant enzymes within cells include superoxide dismutase (**SOD**), glutathione peroxidase (**GPX**) and catalase (**CAT**). Superoxide dismutase catalyzes the dismutation of $O_2^{\bullet-}$ to form H_2O_2 and water (McCord and Fridovich, 1969). Three SOD isoforms have been characterized in mammalian cells based on the metal cofactor required for activity and cellular localization. Isoforms that require Cu and Zn cofactors include SOD1, located in the cytosol and intermembrane space of mitochondria (Weisiger and Fridovich, 1973a; Okado-Matsumoto and Fridovich, 2001), and SOD3, located in the extracellular space (Marklund et al., 1982). The Mn dependent SOD2 is located within the mitochondrial matrix (Weisiger and Fridovich, 1973b). In rat liver the majority (84%) of SOD activity was present in the soluble fraction of the cell, with the rest being associated with mitochondria; half of the mitochondrial associated activity was localized in the intermembrane space and the other half within the matrix (Peeters-Joris et al., 1975). As mentioned previously, mitochondria are the primary endogenous source of $O_2^{\bullet-}$ which gives rise to other ROS. Thus, Mn-SOD represents an important first line of antioxidant defense, demonstrated by embryonic or early postnatal lethality in mice with complete loss of Mn-SOD (Li et al., 1995; Lebovitz et al., 1996). Homozygous SOD2 knockout mice that survived up to 3 weeks of age exhibited lesser growth rates as well as lesser adipose tissue and skeletal muscle mass compared to control mice while

the growth rates and size of heterozygous SOD2 knockout mice were similar to control mice (Lebovitz et al., 1996).

Hydrogen peroxide can be reduced to water by the action of GPX or CAT. Major mammalian GPX isoforms include cytosolic GPX 1 (Mills, 1957) and membrane associated GPX3 (Ursini et al., 1982), both of which require Se as a cofactor and utilize the reducing power of glutathione (Rotruck et al., 1973). Catalase is an Fe dependent enzyme distributed throughout mammalian cells but is found mostly in peroxisomes where high concentrations of H_2O_2 are produced (Christian and Baudhuin, 1966). Phagocytic immune cells also express high levels of CAT to protect cells from high concentrations of H_2O_2 produced to kill pathogens during the respiratory burst (Gee et al., 1970). Although GPX and CAT have similar functions, CAT has a lower affinity for H_2O_2 and thus activity is dependent on higher concentrations of H_2O_2 (Cohen and Hochstein, 1963). Therefore, it has been suggested that GPX is more active than CAT in removing H_2O_2 from cells (Hochstein and Utley, 1968).

Additional enzymes that contribute to antioxidant defense include peroxiredoxins (**PRX**), thioredoxins (**TRX**) and glutaredoxins (**GRX**). Similar to GPX and CAT, PRX reduces H_2O_2 to water, but does so via catalytic cysteine residues (Kim et al., 1988; Chae et al., 1994). Six PRX isoforms have been characterized in mammalian cells with similar catalytic mechanisms in which the active site cysteine is oxidized to sulfenic acid (Ellis and Poole, 1997; Rhee et al., 2001). However, PRX isoforms differ in cellular location and mechanism by which the cysteine sulfenic acid is reduced (Poole et al., 2000; Seo et al., 2000; Peshenko and Shichi, 2001). The inactivation of PRX by phosphorylation or hyperoxidation may serve as a cell signaling mechanism by allowing for accumulation of H_2O_2 (Woo et al., 2010). Thioredoxins (cytosolic and nuclear TRX1; mitochondrial TRX2) reduce oxidized cysteine groups on proteins via two

catalytic cysteine residues which are then reduced by NADPH dependent TRX reductase via a catalytic selenocysteine residue (Holmgren, 1989; Gasdaska et al., 1999; Miranda-Vizuete et al., 1999). Like TRX, GRX (cytosolic GRX1; mitochondrial GRX2) reduces protein disulfides but are themselves reduced nonenzymatically by glutathione (Gladyshev et al., 2001).

Exogenous Antioxidants. Antioxidant compounds that must be consumed or delivered parenterally include vitamin E (**VE**; tocopherols) and provitamin A compounds found in plant pigments (carotenoids). Vitamin E is the major lipid-soluble antioxidant present in cells and thus plays a vital role in maintenance of cell membrane integrity (Burton et al., 1982). The antioxidant function of VE is due to its ability to donate a phenolic hydrogen to ROS, most commonly $LOO^{\bullet}$ within the membrane (Burton and Ingold, 1981). This donation results in formation of the relatively unreactive tocopheroxyl radical which can be reduced by VC or irreversibly oxidized to tocopherolquinone (Van Acker et al., 1993). Several lipid soluble carotenoids (e.g. β-carotene, lycopene) have been shown to exhibit antioxidant activity (Foote and Denny, 1968; Di Mascio et al., 1989). However, these compounds are present at much lower concentrations in tissues than VE and thus thought to be less significant in cellular antioxidant defense (Burton and Ingold, 1984).

Transcriptional Regulation of Antioxidants. Cells respond to changes in redox state through several transcription-related pathways including the nuclear factor erythroid 2-related factor (**Nrf2**)/Kelch-like ECH-associated protein 1 (**Keap1**) pathway and the nuclear factor kappa-light-chain-enhancer of activated B cells (**NF-κB**) pathway. The Nrf2/Keap1 pathway is considered a key regulator of oxidative stress responses (Kaspar et al., 2009). Under normal cellular conditions Keap1 is bound to Nrf2 in the cytosol which promotes ubiquination and proteasomal degradation of Nrf2 (Itoh et al., 1999; Nguyen et al., 2003). In the presence of ROS,

cysteine residues of Keap1 become oxidized, preventing binding to Nrf2 and subsequent degradation (Zhang and Hannink, 2003). This allows Nrf2 to translocate to the nucleus and bind to regulatory gene regions on DNA known as antioxidant response elements (Rushmore et al., 1991). Target genes of Nrf2 are involved in ROS catabolism (SOD3, GPX2&3, PRX1; Rangasamy et al., 2004), antioxidant recycling (TRX reductase, glutathione reductase; Rundlöf et al., 2001; Harvey et al., 2009) and metal binding proteins (metallothionein, ferritin; Dalton et al., 1994; Pietsch et al., 2003). Antioxidant responses are also regulated by the NFκB signaling pathway. Hydrogen peroxide promotes dissociation of inhibitor proteins from NF-κB leading to its activation, nuclear translocation and DNA binding (Schmidt et al., 1995). The NF-κB pathway can also be activated in response to proinflammatory stimuli like cytokines (DiDonato et al., 1997). Activation of NF-κB can increase the transcription of both antioxidant (MnSOD, TRX; Das et al., 1995; Djavaheri-Mergny et al., 2004) and prooxidant genes (cyclooxygenase, NADPH oxidase; Hwang et al., 1997; Anrather et al., 2006).

Oxidative Stress Outcomes

Under normal cellular conditions, the level of oxidants is balanced by the cell's antioxidant defense system and thus fluctuates within a certain range referred to as basal oxidative stress or oxidative eustress (Niki, 2016). An increase in the production of oxidants, depletion of antioxidants or a combination of the two may increase oxidants above steady-state concentrations and result in different physiological outcomes depending on the intensity (low, intermediate, high) and duration (acute, chronic, repetitive) of the increase (Lushchak, 2014). Low intensity oxidative stress produces an adaptive response via redox regulation of transcription factors (Nrf2 and NF-κB) that upregulate antioxidant enzymes. The most well-defined mechanism of redox signaling is H_2O_2 oxidation of cysteine residues within proteins

(Rhee et al., 2000). First-degree oxidation of cysteine residues to form sulfenic acid can lead to the formation of disulfide bonds with other proteins or glutathione, which serves as a reversible signal transduction mechanism by regulating the enzymatic and transcriptional activity of proteins, while further oxidation of cysteine residues to form sulfinic and sulfonic acid is often irreversible (Poole and Nelson, 2008). In addition to antioxidant defense systems, redox signaling has been implicated in the activation of pathways involved in cell growth, proliferation and survival (Sundaresan et al., 1995; Bae et al., 1997); the innate and adaptive immune response (Chandel et al., 2000; Wheeler and Defranco, 2012); as well as stem cell self-renewal and differentiation (Ito et al., 2006; Malinska et al., 2012).

Intermediate intensity oxidative stress also produces an adaptive response but may involve damage to cellular components including proteins, lipids and nucleic acids. Proteins can be oxidized directly by ROS or indirectly by other oxidatively damaged molecules, such as lipid peroxidation products, that interact with the polypeptide backbone or amino acid side chains of a protein (Berlett and Stadtman, 1997). These oxidative modifications may be reversible (e.g. first-degree oxidation of cysteine and methionine residues) or irreversible (e.g. carbonylation) and can alter how the protein functions as a receptor, transporter or enzyme (Meng et al., 2002). Moderate oxidative damage to proteins enhances their susceptibility to proteolysis, predominantly by the ATP-dependent proteasomal system (Grune et al., 1997). However, extremely oxidized proteins can form crosslinks and aggregates that are not as susceptible to proteolysis (Okada et al., 1999; Bence et al., 2001). Accumulation of these damaged proteins can result in cellular dysregulation and initiation of cell death (Demasi and Davies, 2003).

Lipids, particularly polyunsaturated fatty acids that make up cell membranes, are susceptible to peroxidation. Lipid peroxidation is initiated by prooxidant abstraction of a

hydrogen to form a lipid radical which then reacts with oxygen to form LOO• (Buege and Aust, 1978). The LOO• propagates the reaction by abstracting H from other lipid molecules and will continue until antioxidants, like VE, terminate the reaction by donating a H atom (Burton and Ingold, 1981). Nucleic acids of DNA and RNA are also vulnerable to oxidation. Oxidative modification of DNA can result in base alterations, single and double stranded breaks as well as DNA-protein crosslinks (Dizdaroglu, 1992). Several repair mechanisms exist to counteract DNA damage including base excision repair, nucleotide excision repair and mismatch repair; base excision repair is the main pathway used to repair oxidative DNA damage (Demple and Harrison, 1994). Several steps of the base excision repair pathway require ATP including chromatin remodeling, DNA polymerase and DNA ligase (Lehman, 1974; Menoni et al., 2007). Due to its single stranded nature, RNA may be more susceptible to oxidation (Nunomura et al., 1999) and can ultimately result in loss of normal protein abundance and protein functionality (Shan et al., 2007). Damaged RNA can be degraded by ribonucleases or repaired (Hayakawa et al., 2001; Aas et al., 2003).

When oxidative stress reaches a high intensity, referred to as oxidative distress, cell death via necrosis and/or apoptosis may result (Saito et al., 2006). Necrosis is a relatively uncontrolled process characterized by cell swelling, cytoplasmic blebbing, membrane rupture and expulsion of cellular contents into the extracellular space, often inducing an inflammatory response (Proskuryakov et al., 2003). Alternatively, apoptosis is a highly coordinated mechanism of cell replacement, tissue remodeling and removal of damaged cells (Hengartner, 2000). This pathway of cell death is characterized by cell shrinkage, DNA fragmentation and formation of apoptotic bodies which are then phagocytosed by macrophages or nearby cells (Kerr et al., 1972).

Measuring Oxidative Stress

Because oxidative stress is defined as an imbalance between oxidants and antioxidants in favor of the oxidants (Sies, 2007), it is important to consider both sides of this equation when assessing oxidative status in biological samples (**Table 2**).

Table 2. Commonly measured biomarkers of oxidative stress

Category	Examples[1]
Reactive oxygen species	Electron spin resonance, ROMs
Oxidative modifications	Proteins: carbonyls, other oxidized amino acid products Lipids: MDA/TBARS, F_2-isoprostanes Nucleic acids: 8-OHdG, other oxidized base products
Nonenzymatic antioxidants	Glutathione, bilirubin, uric acid, α-tocopherol, ascorbic acid
Antioxidant enzymes	Superoxide dismutase, glutathione peroxidase, catalase
Antioxidant capacity	Total antioxidant capacity, FRAP

[1]ROMs = reactive oxygen metabolites; MDA = malondialdehyde; TBARS = thiobarbituric acid reactive substances; 8-OHdG = 8-hydroxy-2'-deoxyguanosine; FRAP = ferric reducing antioxidant potential

Oxidants. Oxidants may be measured via quantification of ROS as well as oxidative modifications to lipids, proteins and nucleic acids. Electron spin resonance is considered the "gold standard" for direct measurement of ROS, but the method is complex and requires specific equipment not readily available in most laboratories (Kohno, 2010). The reactive oxygen metabolites assay was developed, and validated using electron spin resonance, to more efficiently measure oxygen centered free radicals (e.g. $O_2^{\bullet-}$ and $OH^{\bullet}$) as well as nonradical ROS (e.g. H_2O_2; Iamele et al., 2002). Oxidative modifications of amino acid side chains result in numerous oxidation products (Berlett and Stadtman, 1997), but the most frequently used biomarker of protein oxidation is carbonyls which are relatively stable compounds that can be assessed by spectrophotometry or immunoassay (Dalle-Donne et al., 2005). Protein carbonylation occurs when ROS interact with amino acid (proline, lysine, arginine or threonine)

side chains of proteins in the presence of transition metals (Fe, Cu), resulting in the formation of ketones or aldehydes that can react with 2,4-dinitrophenylhydrazine; secondary protein carbonylation can result from the interaction of amino acid side chains with carbonyl groups on carbohydrates and lipids (Stadtman and Oliver, 1991; Yuan et al., 2007).

Malondialdehyde (**MDA**) is the most commonly measured indicator of polyunsaturated fatty acid peroxidation and can be measured using spectrophotometry or via high-performance liquid chromatography (**HPLC**; Moselhy et al., 2013). The spectrophotometric method quantifies MDA based on the pigment produced from the reaction of MDA with thiobarbituric acid substances (**TBARS**; Sinnhuber et al., 1958). Despite being the most widely used method of MDA analysis, the spectrophotometric method has been criticized for its lack of specificity as thiobarbituric acid may react with several other compounds including pyrimidine, unsaturated aldehydes and biliverdin (Knight et al., 1988). Alternatively, F_2-isoprostanes are prostaglandin like compounds formed from peroxidation of arachidonic acid and are extremely reliable markers of oxidative stress *in vivo* (Morrow and Roberts, 1997). Isoprostanes are typically assessed in plasma and urine using mass spectrometry (Morrow and Roberts, 1999). Similar to proteins, oxidation of nucleic acids results in several oxidized products (Dizdaroglu, 1992). However, 8-hydroxy-2'-deoxyguanosine is the predominant biomarker used to assess oxidative damage to nuclear and mitochondrial DNA (Valavanidis et al., 2009). Upon DNA repair, 8-hydroxy-2'-deoxyguanosine is excreted in the urine and can be quantified using several methods including HPLC with electrochemical detection (De Martinis et al., 2002), gas chromatography with mass spectrometry (Lin et al., 2004) and liquid chromatography with tandem mass spectrometry (Hu et al., 2004). Commercially available ELISA kits are also available for analysis of 8-hydroxy-2'-deoxyguanosine, but Hu et al. (2004) reported liquid chromatography with

tandem mass spectrometry was more accurate and exhibited no correlation with values obtained by ELISA.

Antioxidants. The antioxidant capacity of a sample may be evaluated based on concentrations of nonenzymatic antioxidants and activities of antioxidant enzymes. Both the oxidized and reduced forms of glutathione can be quantified and the ratio of the two is a useful indicator of the redox state of a sample. Under normal cellular conditions, reduced glutathione constitutes up to 98% of total glutathione (Kosower and Kosower, 1978) while oxidative stress conditions may decrease this contribution to less than 90% (Ithayaraja, 2011). Similarly, vitamins E and C can be measured in their oxidized (tocopherol quinone, dehydroascorbic acid) and reduced (tocopherol, ascorbic acid) forms (Washko et al., 1992; Yamauchi et al., 2002). Activity of antioxidant enzymes such as SOD, GPX and CAT have shown differential responses to oxidizing stimuli. As mentioned previously, antioxidant enzyme gene expression can be transcriptionally upregulated by ROS. Thus, oxidative conditions may increase protein abundance of antioxidant enzymes, but ROS are also capable of deactivating antioxidant enzymes (Asahi et al., 1995; Yamakura and Kawasaki, 2010). Therefore, caution must be exercised when interpreting changes in antioxidant enzyme activity. In addition to measuring individual antioxidants within a sample, several assays have been developed to measure the overall antioxidant potential of a sample including the total antioxidant capacity assay (Kampa et al., 2002) and ferric reducing antioxidant potential (Benzie and Strain, 1996).

Summary

Although ROS are produced under normal cellular conditions, increased production of ROS coupled with a decrease in antioxidant capacity can result in oxidative distress

characterized by damage to biomolecules, decreased cellular function and increased cell death. Several biomarkers of oxidative stress discussed herein have been associated with disease and production stressors in livestock species:

- Respiratory infection in cattle (Ledwozyw and Stolarczyk, 1992; Wessely-Szponder et al., 2004)
- Exercise in horses (Chiaradia et al., 1998; White et al., 2001)
- Heat stress in poultry and pigs (Lin et al., 2006; Montilla et al., 2014)
- Transit stress in cattle, horses and sheep (see next section)

Despite acknowledgement of the role oxidative stress may play in livestock health and production, a panel of reliable oxidative stress biomarkers and normal reference ranges for these markers have yet to be established.

Transit and Oxidative Stress

Transit-Induced ROS Production

This section will focus on how the physiologic responses to transit described previously (psychological stress, food deprivation and physical exertion) contribute to the production of ROS (**Figure 1**). Transit-induced food deprivation and psychological stress stimulate hormone mediated release of energy producing substrates, like fatty acids and glucose, from adipose tissue and skeletal muscle. The uptake and aerobic metabolism of these substrates by other tissues, such as the liver, increases mitochondrial derived ROS simply as a result of greater electron flow through the electron transport chain and subsequent electron leakage to oxygen. In support of the free radical theory of aging, caloric restriction has been shown to decrease mitochondrial ROS production and DNA damage (Gredilla et al., 2001) as well as increase longevity (Liang et al.,

2018). However, severe or complete caloric restriction (fasting) has been shown to have detrimental effects. Prior to feedlot arrival, cattle may experience periods of fasting lasting up to 72 h. This includes time in transit as well as time spent at auction markets where feed may not be provided. Furthermore, cattle may self-restrict feed intake upon arrival which could further contribute to oxidative stress conditions depending on the severity of the restriction.

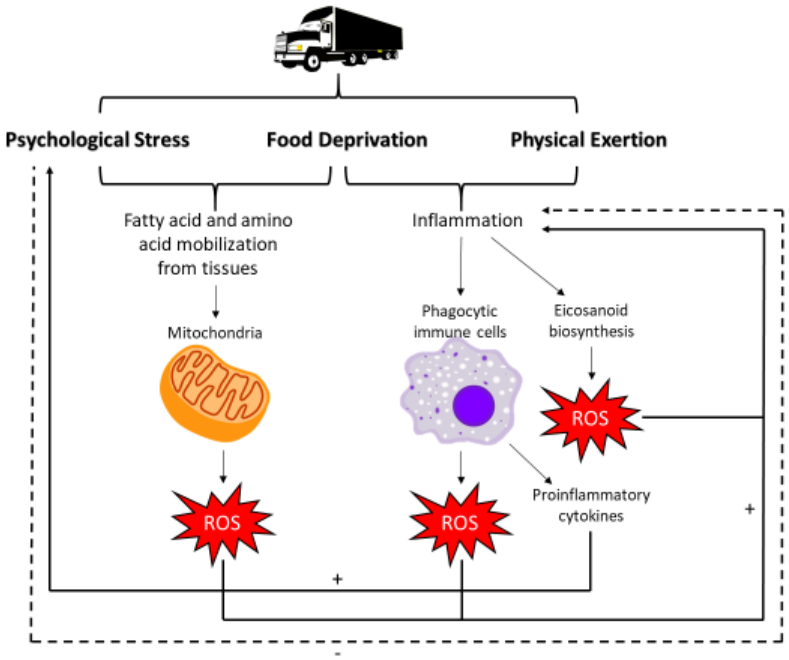

Figure 1. Potential sources of transit-induced reactive oxygen species

For example, Stankovic et al. (2013) compared the effects of different degrees of chronic restriction (80-90, 60-70, 40-50 or 20-30% of daily caloric needs for 5 weeks) and acute fasting (1 week without food intake) on oxidative stress parameters in rat liver. Rats exposed to severe caloric restriction (< 50% of daily caloric needs) or acute fasting displayed increased concentrations of MDA and nitric oxide as well as decreased SOD activity and glutathione concentrations. Alternatively, moderate caloric restriction (60-70% of daily caloric needs) did not cause hepatocyte damage and increased antioxidant capacity based on greater Mn-SOD activity and glutathione concentrations (Stankovic et al., 2013). Sorensen et al. (2006) also

observed detrimental effects of fasting where liver mitochondria from rats fasted for 72 h exhibited increased electron leak and free radical generation at complex III of the electron transport chain, which resulted in a greater degree of lipid and protein oxidative damage.

Feed restriction alone, as well as transit, have been shown to increase inflammatory biomarkers in cattle (Marques et al., 2012). Inflammation is initiated by macrophage recognition of endogenous danger signals and/or intracellular pathogens (Zhang and Mosser, 2008). Macrophages then recruit other phagocytic immune cells via secretion of chemokines and cytokines (Vieira et al., 2009; Griffin et al., 2012). Upon phagocyte activation, assembly of the NADPH oxidase complex at the cell membrane is stimulated (Nauseef, 2004). This enzyme uses an electron from intracellular NADPH to reduce extracellular O_2 to $O_2^{\bullet-}$ (Babior et al., 1973). The production of ROS by phagocytes to kill pathogens has received much attention in the literature. More recently, the signaling role of phagocyte derived ROS has also been investigated, as $O_2^{\bullet-}$ is rapidly dismutated to H_2O_2 either nonenzymatically or enzymatically by SOD (Salin and McCord, 1974). The pro-inflammatory NFκβ signaling pathway is a potential target of ROS produced by the NADPH oxidase system (Schreck et al., 1991). Because ROS are both products and stimulators of inflammatory processes, a deleterious feedback loop can exacerbate conditions of inflammation and oxidative stress. Pro-inflammatory cytokines, like interleukin-1, produced by activated macrophages and neutrophils increase the production of glucocorticoids (Besedovsky et al., 1986) which occurs via ROS generating reactions by cytochrome P-450 enzymes (Zangar et al., 2004). In contrast, glucocorticoids elicit anti-inflammatory and immunosuppressive effects by repressing NFκβ signaling and subsequent proliferation of immune cells as well as cytokine and antibody secretion (Scheinman et al., 1995). Due to their anti-inflammatory properties, synthetic glucocorticoids like dexamethasone are frequently

administered to cattle as an ancillary therapy for BRD, despite their negative effects on bovine immune cell function (Roth and Kaeberle, 1982).

Additional inflammatory mediators include eicosanoids which are molecules derived from arachidonic acid or other polyunsaturated fatty acids released from membrane phospholipids by phospholipase enzymes, especially phospholipase A_2 (Funk, 2001). These molecules exert a diverse array of biological functions including regulation of blood pressure (Williams, 1979) and clotting (Hamberg et al., 1975) as well as pro- and anti-inflammatory effects (Node et al., 1999; Levy et al., 2001). The enzymatic oxidation of polyunsaturated fatty acids by cyclooxygenase, lipoxygenase and cytochrome P-450 monooxygenase enzymes can produce $O_2^{\bullet-}$ and intermediate compounds (e.g. 15-hydroperoxyeicosatetraenoic acid) that act as potent hydroperoxides which can cause oxidative damage to cells (Weaver et al., 2001; Sordillo et al., 2005). Reactive oxygen species also contribute to nonenzymatic eicosanoid biosynthesis. Phospholipase A_2 and subsequent synthesis of eicosanoids has been shown to be positively regulated by epinephrine (Singh et al., 1986) and negatively regulated by glucocorticoids (Croxtall et al., 2000). The eicosanoid biosynthetic pathway can also be targeted with nonsteroidal anti-inflammatory drugs which inhibit cyclooxygenase pathways (Meade et al., 1993). According to the USDA (2013), 55.9% of large feedlots (≥ 1,000 head) administered a nonsteroidal anti-inflammatory drug such as flunixin or aspirin to cattle as part of an initial course of treatment for BRD.

As skeletal muscle represents 25-30% of total body energy expenditure in cattle (Lobley, 2003), it is likely that muscle derived ROS make a large contribution to whole body oxidative status. Like other cells, muscle cells generate endogenous ROS via mitochondria (Davies et al., 1982), NADPH oxidase enzymes (Javesghani et al., 2002); Xia et al., 2003), phospholipase A_2

dependent processes (Gong et al., 2006) and nitric oxide synthases (Kobzik et al., 1994). Since the 1950s the field of exercise redox biology has worked to characterize exercise-induced oxidative stress and determine the impact of ROS on muscle function. This research may provide valuable insight into the effects of transit on muscle physiology as cattle may be standing for long durations depending on the length and method of transit. Exercise has been shown to increase whole body indicators of oxidation in the blood (Sen et al., 1994; Wierzba et al., 2006), with the general consensus that exercised-induced ROS production occurs predominantly by contracting heart and skeletal muscle (Powers and Jackson, 2008). Mitochondria were originally thought to be the primary source of ROS produced in skeletal muscle during exercise. However, mitochondria produce more ROS during state 4 (basal) respiration than during state 3 (maximal ADP-stimulated) respiration (Cortassa et al., 2014), which is the predominant state of mitochondria during aerobic contractions. Similar to the effects of caloric restriction, regular/moderate aerobic exercise is beneficial while unaccustomed or exhaustive exercise can be detrimental. For example, rats trained on a treadmill for 1 h/day, 3 days/week for 14 weeks displayed increased fiber diameter in oxidative (soleus) muscle and increased fiber number in glycolytic (tibialis anterior) muscle (Abruzzo et al., 2014). The authors proposed this was a result of ROS activation of redox-sensitive transcription factors (NFκβ, activator protein-1 and mitogen-activated protein kinase) that regulate muscle regulatory factors, which in turn regulate satellite cell proliferation and differentiation. Alternatively, endurance exercise promotes muscle damage which stimulates an inflammatory response (Suzuki et al., 1999). The increased ROS production by macrophages and other phagocytic cells within the muscle tissue could explain the activation of the Nrf2/Keap1 pathway in mice subjected to 6 h of treadmill running compared to mice exercised for 1 h or non-exercised control mice (Li et al., 2015).

Evidence of Transit-Induced Oxidative Stress in Livestock Species

Approximately 15 years after Helmut Sies defined the concept of oxidative stress, scientists began to investigate transit-induced oxidative stress in livestock. A summary of research studies that have been conducted in this field over the past 20 years is presented in **Table 3**. The effects of transit on ROS production has been assessed in various species transported up to 51 h. Urban-Chmiel et al. (2006) reported an increase in ROS production of isolated bovine leukocytes post-transit and Piccione et al. (2012) reported that ROS production was increased at 12, 24 and 48 h post-transit in sheep serum. Interestingly, both studies also report increases in antioxidant capacity, suggesting that cellular antioxidants may be upregulated to combat increased ROS production. Total antioxidant status was also increased post-transit in horses (Niedźwiedź et al., 2013; Padalino et al., 2017). Alternatively, Chirase et al. (2004) observed a decrease in total antioxidant capacity of transported beef steers that was sustained through 28 d post-transit. This coincides with several studies that report transit-induced decreases in concentrations of nonenzymatic antioxidants like Se and VE (Chirase et al., 2001; Nazifi et al., 2009; Burke et al., 2009). Because these are nutrients that must be delivered exogenously, endogenous antioxidants may be upregulated to overcome this depletion. However, reduced glutathione concentrations have also been shown to decrease post-transit (EL-Deeb and El-Bahr, 2014; Polycarp et al., 2016). Additionally, numerous studies have reported a decrease in antioxidant enzyme (glutathione reductase, GPX, SOD) activity post-transit (Burke et al., 2009; Onmaz et al., 2011; El-Deeb and El-Bahr, 2014; Polycarp et al., 2016; Niedźwiedź et al., 2012). Most authors conclude that the observed decrease in enzyme activity is a result of increased ROS production and subsequent consumption of the enzyme for antioxidant reactions. However, Nazifi et al. (2009) observed an increase in GPX activity 24 h post-transit and concluded that this was a compensatory response of cells to mitigate increased oxidant concentrations. As positive

or negative changes in antioxidant enzyme activity can result in the same conclusion, it is important to pair enzyme activity with more direct markers of oxidative stress. For example, MDA is a commonly measured indicator of oxidatively damaged lipids. Numerous (12 of 17) studies presented here assessed MDA concentrations via HPLC or TBARS methodology; 11 of those 12 studies reported increased MDA concentrations due to transit.

Summary

As a result of psychological stress, food deprivation and physical exertion experienced during transit, ROS may be produced via:

- Mitochondrial electron leak
- NADPH oxidase enzymes of phagocytic immune cells
- Cyclooxygenase, lipoxygenase and cytochrome P-450 enzymes during eicosanoid biosynthesis

In general, the literature reviewed herein suggests transit increases ROS production and markers of oxidative damage resulting in decreased concentrations of nonenzymatic antioxidants and activity of antioxidant enzymes. Additionally, food deprivation alone can contribute to ROS producing pathways (oxidative metabolism and inflammation) also induced by psychological stress and physical exertion. Therefore, further research is needed to determine how these transit-induced physiological responses contribute to oxidative stress.

Table 3. Summary of the scientific literature examining changes in oxidative stress biomarkers due to transportation of livestock

Reference	Animal Description	Transit duration/distance	Tissue	Biomarker[1]	Effect of Transit[2]
Chirase et al. (2001)	120 crossbred beef steers (185 kg)	18 h/1890 km	serum	α-tocopherol	decrease
Chirase et al. (2004)	105 crossbred beef steers (207 ± 21.2 kg)	19 h 40 min/1930 km	serum	Total antioxidant capacity (TACA)	decrease
			serum	MDA (HPLC)	increase
Urban-Chmiel (2006)	9 heifer calves (150 kg)	1.5 h/80 km	leukocytes	MDA (TBARS)	increase
			leukocytes	FRAP	increase
			leukocytes	ROS production	increase
Wernicki et al. (2006)	9 heifer calves (100 kg)	2 h/120-140 km	plasma	MDA (TBARS)	increase
Nazifi et al. (2009)	10 Iranian dromedary camels (300 kg)	5 h/300 km	plasma	MDA	increase
			plasma	α-tocopherol	numerical decrease
			erythrocytes	SOD activity	none
			whole blood	GPX activity	increase
Burke et al. (2009)	36 crossbred beef steers (243 ± 20.8 kg)	2 h/172 km	plasma	MDA	decrease
			leukocytes	GPX activity	none
			leukocytes	GR activity	decrease
			plasma	Se	decrease
Zhong et al. (2011)	24 Ujumqin sheep (23-29 kg)	8 h	serum	MDA (TBARS)	increase
Aktas et al. (2011)	30 Holstein cows (400-600 kg)	22 h/1250 km (1 h break every 8 h)	serum	MDA (TBARS)	increase
Onmaz et al. (2011)	10 horses (480 ± 33 kg)	12 h/538 km	plasma	MDA (TBARS)	increase
			plasma	SOD activity	decrease

Table 3. (continued)

Piccione et al. (2012)	20 Comisana ewes (55 ± 4 kg)	6 h/490 km	serum	reactive oxygen species (dROMs)	increase
			serum	antioxidant barrier (Oxy-adsorbent)	increase
			serum	thiol antioxidant barrier (SHp)	increase
Niedźwiedź et al. (2012)	60 horses (680 ± 50 kg)	8 h	erythrocytes	GR activity	decrease
			erythrocytes	GPX activity	decrease
			erythrocytes	GST activity	none
El-Deeb and El-Bahr (2014)	50 water buffalo calves (165 ± 13 kg)	4 h/250 km	plasma	MDA	increase
			plasma	nitric oxide	decrease
			plasma	SOD activity	decrease
			plasma	reduced GSH	decrease
Niedźwiedź et al. (2013)	60 horses (680 ± 50 kg)	8 h/560 km	plasma	FRAP	increase
Wernicki et al. (2014)	60 beef bull calves (110 kg)	120 km	serum	MDA (TBARS)	increase
Adenkola and Ayo (2013)	7 rams	8 h/300 km	serum	MDA (TBARS)	increase
Polycarp et al. (2016)	35 Boer goats (23-25 kg)	7 h/350 km	plasma	MDA (TBARS)	increase
			erythrocytes	SOD activity	decrease
			plasma	reduced GSH	decrease
Padalino et al. (2017)	16 horses	94 h (51 h in transit)	plasma	total antioxidant status (TAS)	increase
			plasma	reactive oxygen metabolites (ROMs)	none

[1]FRAP = ferric reducing antioxidant potential; GSH = glutathione; GPX = glutathione peroxidase; GR = glutathione reductase; HPLC = high-performance liquid chromatography; MDA = malondialdehyde; ROS = reactive oxygen species; SOD = superoxide dismutase; TBARS = thiobarbituric acid reactive substances

[2]In reference to baseline (pre-transit) values or a non-transported control group

Nutritional Oxidative Stress Modulation

As discussed in the previous section, transit stress has been shown to decrease antioxidant status and increase biomarkers of oxidative stress in various livestock species. The nutritional supplements discussed herein have direct antioxidant activity and/or indirect antioxidant effects by modulating other physiological responses to transit, like inflammation.

Yeast Fermentation Products

History and Definition. Not all yeast fermentation products are produced using the same strain of yeast and fermentation technology, resulting in products that likely differ in the biological effects they elicit. Therefore, this review will focus specifically on *Saccharomyces cerevisiae* fermentation products (**SCFP**) manufactured by the animal health and nutrition company, Diamond V (Cedar Rapids, IA), and its daughter company which focuses on human health and nutrition, Embria Health Sciences (Ankeny, IA). These SCFP are produced by a unique anaerobic fermentation process that causes the live yeast to produce metabolites. The resulting product contains yeast cell walls (β-glucans and mannan-oligosaccharides), cell solubles, vitamins, proteins, peptides, amino acids, nucleotides, lipids and organic acids (Jensen et al., 2008). Over 20 years ago, employees working in a fermentation facility in Cedar Rapids were found to have unusually low sick leave rates and health care claims compared to other companies of similar size in the same region. This observation led to a pilot study that reported several improvements in immune function of factory workers compared to office workers that were not in contact with the yeast product (Schauss and Vojdani, 2006). Since this discovery, several antioxidant and immunomodulating properties of SCFP have been elucidated.

Oxidative Stress and Inflammation. *Saccharomyces cerevisiae* fermentation products have been found to exhibit high ROS scavenging activity (Schauss and Vojdani, 2006) and

increase antioxidant capacity *in vitro* and *in vivo*. *In vitro* antioxidant capacity of SCFP was evaluated by adding a reagent that becomes fluorescent upon exposure to free radicals to red blood cells and neutrophils either untreated or incubated for 90 min with SCFP (XP, Diamond V) prior to 45 min of H_2O_2 exposure (Jensen et al., 2008). Mean fluorescence intensity was decreased by pre-treatment of both cell types with SCFP. In red blood cells the most significant decrease was observed with the highest dose tested (10 mg/mL) while in neutrophils the most significant decrease was observed with the lowest dose tested (0.0001 mg/mL; Jensen et al., 2008). The authors hypothesized that the inverse dose response observed in neutrophils was due to the presence of both pro-inflammatory (β-glucans) and antioxidant compounds (Jensen et al., 2008). In support of this hypothesis, Deters et al. (2018a) observed a quadratic response of SCFP (XPC, Diamond V) on antioxidant defense of newly weaned beef steers (278 kg). Steers supplemented SCFP at 14 g·steer^{-1}·d^{-1} tended to have greater concentrations of reduced glutathione on d 27 and 56 of supplementation compared to steers supplemented SCFP at 0 or 28 g·steer^{-1}·d^{-1} (Deters et al., 2018a).

In addition to antioxidant capacity, SCFP have demonstrated anti-inflammatory activity in humans (EpiCor, Jensen et al., 2007; XP, Jensen et al., 2008). Evidence of this anti-inflammatory effect has also been reported in cattle. Newly weaned beef steers (323 kg) were supplemented 0, 14 or 28 g SCFP·steer^{-1}·d^{-1} for 56 d to determine the effect of SCFP (XPC, Diamond V) on the APP response induced by a *Mannheimia haemolytica* vaccination given 34 d after the start of supplementation (Deters et al., 2018b). Ceruloplasmin concentrations were lesser on d 14 post-vaccination for steers supplemented SCFP at 14 g·steer^{-1}·d^{-1} compared to steers supplemented SCFP at 0 or 28 g·steer^{-1}·d^{-1} (Deters et al., 2018b). Additionally, serum IL-8 and haptoglobin concentrations tended to be lesser post-vaccination for steers supplemented

SCFP at 14 g·steer^{-1}·d^{-1} compared to 28 g·steer^{-1}·d^{-1}, suggesting that the anti-inflammatory properties of SCFP are dose dependent (Deters et al., 2018b). In contrast, Zaworski et al. (2014) did not observed a differential dose response on APP in transition dairy cows supplemented SCFP (XP, Diamond V) at 0, 56 or 112 g daily; cows fed SCFP had lower haptoglobin concentrations prepartum and higher SAA concentrations during the first week post-calving. Dairy cows supplemented SCFP (NutriTek, Diamond V) for 3 weeks after calving had lesser serum haptoglobin concentrations on d 7 post-calving (Knoblock et al., 2019). However, feeding SCFP increased plasma MDA concentrations in primiparous cows fed high starch diets and decreased plasma total antioxidant capacity in multiparous cows fed low starch diets (Knoblock et al., 2019).

Vitamin E

Functions. Vitamin E is a term used to define eight structurally similar compounds that include four tocopherol and four tocotrienol derivatives (Pennock et al., 1964). The tocopherols have greater biological activity than the tocotrienols due to their lack of side chain double bonds, with α-tocopherol being the most active VE derivative (Burton and Ingold, 1981). As mentioned previously, the major biological function of VE is as a lipid soluble antioxidant and therefore plays a vital role in protecting cell membranes from oxidative damage. The antioxidant activity of VE is thought to be the underlying mechanism by which VE supports overall health, particularly in protecting immune cells from ROS produced during phagocytosis. Several non-antioxidant functions of VE have also been identified. For example, VE has been shown to transcriptionally regulate genes involved in glutathione synthesis (γ-glutamylcysteine synthetase; Fischer et al., 2001) and inflammation (interleukin-1β; Akeson et al., 1991). Additionally, VE has been shown to post-transcriptionally inhibit phospholipase A_2 (Chandra et al., 2002) and 5-

lipoxygenase (Reddanna et al., 1985). Phospholipase A_2 catalyzes the hydrolysis and subsequent release of arachidonic acid from the plasma membrane (Murakami and Kudo, 2002) while 5-lipoxygenase catalyzes the synthesis of proinflammatory leukotrienes from arachidonic acid (Dixon et al., 1990). The inhibition of this signaling pathway indicates an anti-inflammatory role of VE.

Cattle Requirements. Vitamin E requirements have not been clearly established for beef cattle due to lack of knowledge of dietary Se and VE concentrations as well as overall oxidative stress and antioxidant capacity of individual animals (NASEM, 2016). Other antioxidant nutrients like Se, as part of GPX, and VC can perform some of the biochemical functions of VE and act as sparing factors (Hamilton et al., 2000). Vitamin E and Se also elicit similar deficiency symptoms (e.g. exudative diatheses, liver necrosis and muscle necrosis), making it difficult to establish requirements. The previous VE requirement for beef cattle was estimated to be between 15 and 60 IU/kg DMI (NRC 2000) where an IU is defined as 1 mg of all racemic α-tocopherol acetate. Once cattle have been received and adapted to feedlot conditions, the current recommendation is 25 to 30 IU VE/kg DMI or 0.52 to 0.73 IU VE/kg BW (NASEM, 2016). This recommendation may not be met by naturally occurring VE in feedstuffs as it is rapidly destroyed by oxidation during drying, grinding and storing (Adams, 1982). An increased recommendation (400 to 500 IU/d or 1.6 to 2.0 IU/kg BW) was recently established for newly received calves based on previous evidence that elevated levels of VE during periods of high stress may promote animal health (NASEM, 2016). Many dietary and physiological factors may influence an animal's VE requirement and response to supplementation including concentrations of grain, polyunsaturated fatty acids and other antioxidants in the diet as well as rapid growth, stress and exercise (Nockels, 1991).

Absorption, Transport and Storage. Dietary VE is absorbed by nonsaturable passive diffusion in the small intestine via mixed micelles (Hollander et al., 1975) and thus absorption of VE is dependent on the presence of fat in the diet, bile salts and pancreatic secretions (Gallo-Torres, 1970). Absorption rates of VE vary widely (20 to 80%; Rigotti, 2007) and the efficiency of VE absorption has been found to decrease with increasing doses (Traber et al., 1986). Upon absorption, VE is transported to tissues via the lymph as part of chylomicrons and/or plasma attached to lipoproteins (Bjørneboe et al., 1986). Alpha-tocopherol represents the majority of VE activity within plasma and tissues due to selective incorporation of this derivative into lipoproteins (Traber and Kayden, 1989). Cellular uptake of VE can occur by several different mechanisms including lipoprotein lipase and phospholipid transfer protein mediated exchange (Traber et al., 1985; Kostner et al., 1995), receptor mediated endocytosis of VE carrying lipoproteins (Traber and Kayden, 1984) as well as selective lipid uptake (Goti et al., 1998). Intracellular VE is localized in the hydrophobic regions of the cell including the membrane fractions and bulk lipid fractions (Traber and Kayden, 1987; Buttriss and Diplock, 1988). Vitamin E stored in adipose tissue, which can account for up to 90% of VE in the body (Blatt et al. 2001), is mobilized slowly while plasma and liver concentrations respond more rapidly to changes in VE intake (Machlin et al., 1979). Circulating VE concentrations have been found to rise during intensive exercise (Aguiló et al., 2003), suggesting that VE may be mobilized from fixed pools, like adipose tissue, in response to lipolytic signals.

Vitamin C

Functions. Vitamin C (ascorbic acid) is the major water-soluble antioxidant in plasma and tissues (Frei et al., 1989). In addition to scavenging free radicals, the reducing ability of VC is required by several enzymes to maintain metal cofactors in the reduced state. Of particular

interest to this review are VC dependent enzymes involved in catecholamine and carnitine synthesis. Ascorbate serves as an electron donor for dopamine β-monooxygenase, a Cu dependent enzyme located in the adrenal gland that hydroxylates dopamine to the neurotransmitter norepinephrine (Friedman and Kaufman, 1966). Norepinephrine is released upon activation of the SAM-axis in response to stress. This reaction results in formation of the ascorbyl radical which is reduced to ascorbate by semidehydroascorbate reductase (Diliberto and Allen, 1980). Ascorbate is also a cofactor in two Fe containing hydroxylases (γ-butyrobetaine 2-oxoglutarate 4-dioxygenase and trimethyllysine 2-oxoglutarate dioxygenase) involved in the synthesis of carnitine (Lindstedt, 1967; Hulse et al., 1978). Carnitine is required for transport of fatty acids into the mitochondria for β-oxidation (Fritz, 1959) and thus, high concentrations are found in tissues dependent on energy generated by β-oxidation including heart, skeletal muscle, liver and kidney (Vaz and Wanders, 2002).

Cattle Requirements. There is no established VC requirement for cattle (NASEM, 2016) as cattle can synthesize VC from glucose in the liver (Bánhegyi et al., 1997). However, exogenous supplementation of VC to species without a requirement has been shown to decrease endogenous VC production (Tsao and Young, 1989) and thus, may spare glucose for other biological processes such as growth and immune function. Additionally, stress has been shown to decrease tissue VC concentrations and stimulate biosynthesis in capable species (Nakano and Suzuki, 1984; Chan et al., 2005), suggesting that exogenous supplementation may be beneficial under certain circumstances. In response to stress, particularly adrenocorticotropic hormone, VC will be released from the adrenal gland along with newly synthesized glucocorticoids (Kipp and Rivers, 1987). Glucocorticoids are known to have immunosuppressive effects in cattle (Roth and Kaeberle, 1982), which is likely why stress often precedes disease outbreaks like BRD. Roth and

Kaeberle (1985) demonstrated that neutrophil function in cattle was impaired by administration of an exogenous glucocorticoid (dexamethasone) and a subcutaneous injection of VC was able to mitigate this effect in a dose dependent manner, with the greatest effect observed in cattle administered the highest VC dose (40 mg/kg BW) and no effect observed in cattle administered the lowest VC dose (10 mg/kg BW). The interaction between glucocorticoids, immune function and VC is likely the basis for the adoption of VC as an ancillary therapy in cattle at the time of antibiotic treatment for BRD (USDA, 2013).

Vitamin C must be supplemented to ruminant animals in ways that avoid destruction of VC in the rumen (Knight et al., 1941; Zinn et al., 1987) and thus allow for absorption of VC into the blood stream; supplementation methods include encapsulation of dietary VC as well as parenteral (intramuscular, subcutaneous or intravenous) administration. Padilla et al. (2007) supplemented 0, 10, 20, 40 or 60 mg of VC coated with hydrogenated soybean oil to five Japanese Black × Holstein cows in a Latin square design to determine the effect of VC supplementation on plasma concentrations and urinary excretion of VC. Both plasma and urinary excretion increased quadratically as supplemental VC increased with the lowest dose having no effect. Based on excretion rates, the authors proposed that at least half of the supplemented VC escaped ruminal degradation and was absorbed but not utilized and excreted in the urine (Padilla et al., 2007). Knight et al. (1941) investigated the effects of intravenous or subcutaneous administration of 24 g of ascorbic acid for 3 consecutive days to Holstein dairy cows. Both injection methods increased VC concentrations in the blood, milk and urine. However, the effects of subcutaneous injection were more gradual and less pronounced (Knight et al., 1941).

Absorption, Transport and Storage. Due to its polar nature, VC must be actively transported across cell membranes to enter tissues. Sodium dependent VC transporters (SVCT1

and SVCT2) are the primary mechanism of cellular VC uptake; SVCT1 is expressed predominantly in epithelial cells of the intestine, liver and kidney while SVCT2 is highly expressed in the brain, retina, endocrine and neuroendocrine tissues (Wang et al., 2000). These transporters exchange one ascorbate molecule for two Na molecules (Malo and Wilson, 2000); the Na concentration gradient is maintained by Na-K ATPases on the cell membrane. The oxidized form of VC (dehydroascorbic acid), which represents 10-20% of plasma VC, can be taken up by cells via glucose transporters (GLUT1, GLUT3 and GLUT4) and rapidly reduced to ascorbate by intracellular dehydroascorbic acid reductases (Rumsey et al., 1997; Rumsey et al., 2000). These transporters are differentially expressed in tissues: GLUT1 in the brain and placenta, GLUT2 in the brain and nerve cells, GLUT4 in skeletal muscle, heart and adipose tissue (Gould and Holmant, 1993). Tissues with the highest concentrations of VC include the adrenals, pituitary and lens of the eye while the greatest quantity of VC is found in the liver and muscle (Combs, 2008). The total ascorbic acid pool of newborn Holstein calves was estimated to be 23.1 ± 6.8 mg/kg with muscle and liver accounting for 40 and 33% of this pool, respectively (Toutain et al., 1997). Uptake of VC by skeletal muscle via GLUT1 and GLUT3 has been shown to be competitively inhibited by pretreatment of cells with glucose (Korcok et al., 2003). Skeletal muscle cells also express SVCT2, which is preferentially expressed in oxidative fibers (Low et al., 2009). Gene expression of SVCT2 has been shown to be redox sensitive in C2C12 myotubes, where expression was increased when cells were treated with H_2O_2 and decreased when cells were treated with an antioxidant (Savini et al., 2007).

Summary

Based on functions of the aforementioned nutritional supplements, the experiments detailed in upcoming chapters sought to investigate the effects of pre- or post-transit nutritional

strategies on antioxidant defense as well as receiving period health and performance of beef steers.

- Chapter 3: dietary VE
- Chapter 4: dietary SCFP
- Chapter 5: intramuscular VC injection

The final research chapter (Chapter 6) aims to provide novel insights into mechanisms of transit induced oxidative stress in various tissues of beef steers, with the goal of aiding in the development of nutritional strategies for oxidative stress modulation.

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CHAPTER 3. VITAMIN E SUPPLEMENTATION STRATEGIES DURING FEEDLOT RECEIVING: EFFECTS ON BEEF STEER PERFORMANCE, ANTIBODY RESPONSE TO VACCINATION AND ANTIOXIDANT DEFENSE

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E. L. Deters and S. L. Hansen

Abstract

This study utilized 204 Angus-based beef steers (249 ± 23 kg SD) from a single ranch with initial serum α-tocopherol concentrations of 3.9 ± 1.0 mg/L to determine the effect of varying doses of vitamin E (**VE**) on feedlot performance, antibody response to vaccination and antioxidant defense. Seven days after arrival, steers were blocked by body weight and weaning protocol (pre-weaned, unweaned heavy and unweaned light) and randomly assigned to pens within blocks (12 pens/block). Pre-weaned steers had been weaned for approximately 35 d prior to arrival, and unweaned steers were weaned when leaving the origin ranch. Pens within block were randomly assigned to supplemental VE (ROVIMIX E-50 Adsorbate, DSM Nutritional Products, Heerlen, Netherlands) treatments (n = 9 pens/treatment): no supplemental VE (**CON**), 25 IU/kg dry matter (**DM**; **LOW**), 500 IU/steer daily (**MED**) or 1,000 IU/steer daily (**HIGH**). Back calculated supplemental VE intake was 0, 151 (24.8 IU/kg DM), 484 and 995 IU/d for CON, LOW, MED and HIGH, respectively. On d 6, all steers received a booster vaccine against bovine viral diarrhea virus (**BVDV**; Bovi-Shield Gold, One Shot, Zoetis). Steers were weighed on d -1, 0, 14, 26 and 27. One steer per pen representative of the average body weight of the pen was chosen as a sampling animal for blood (d -1, 6, 14, 26 and 28) and liver (d -3 and 24). Data were analyzed as a randomized complete block design using Proc Mixed of SAS with pen as the experimental unit and the fixed effects of treatment and block. Linear, quadratic and cubic

contrast statements were constructed using Proc IML; morbidity data were analyzed using Proc Glimmix. Day 24 liver and d 26 serum α-tocopherol concentrations were linearly increased by supplemental VE ($P < 0.01$). Supplemental VE did not affect DM intake, average daily gain or gain:feed from d 0 to 27 ($P \geq 0.37$), or the percentage of steers treated for respiratory disease ($P \geq 0.44$). Day 24 liver glutathione concentrations decreased linearly due to supplemental VE ($P \leq 0.02$). Total and Mn-superoxide dismutase activities were quadratically affected by supplemental VE ($P \leq 0.07$), with MED steers exhibiting the greatest activity. Over time, BVDV type 1 and 2 antibody titers numerically decreased, whereas the decrease in BVDV type 1 titers was lesser for HIGH steers (linear $P = 0.04$). Increasing doses of VE improved VE status but did not affect overall receiving period performance in steers with minimal to adequate VE status upon arrival.

Introduction

Beef cattle experience numerous stressors prior to and upon feedlot receiving including recent weaning, commingling, transportation and vaccination. Stressors associated with feedlot receiving have been shown to stimulate an acute inflammatory response (Arthington et al., 2003; Marques et al., 2012) and increase markers of oxidative stress (Chirase et al., 2004), both of which have been negatively associated with animal health and performance (Iqbal et al., 2005; Lykkesfeldt and Svendsen, 2007; Cooke, 2017). Transit stress decreases circulating concentrations of vitamin E (**VE**; Han et al., 1999), a fat-soluble antioxidant that is required for optimal humoral and cell-mediated immunity (Pekmezci, 2011).

The current recommended dose of VE during feedlot receiving is 1.6 to 2.0 IU/kg body weight (**BW**; or 400 to 500 IU/animal daily), and then 25 to 35 IU/kg dry matter (**DM**; or 0.52 to 0.73 IU/kg of BW) for the remainder of the feeding period (NASEM, 2016). However, supplementing VE at ≥ 400 IU/animal daily tended to increase weight gain and tended to

decrease bovine respiratory disease morbidity in feedlot cattle (Secrist et al., 1997). Stressed cattle may benefit from supplementation of VE at greater doses than what is currently recommended due to a possible increased need for antioxidants to combat oxidative stress during the receiving period. Therefore, the objective of this study was to determine the effects of increasing doses of VE in the diets of newly received beef cattle on feedlot performance, antibody response to vaccination and antioxidant defense. The hypothesis was that increasing doses of supplemental VE would improve VE and antioxidant status and result in subsequent improvements in humoral immune function and performance.

Materials and Methods

Animals and Experimental Design

All experimental procedures were approved by the Iowa State University Animal Care and Use Committee (#9-17-8609-B).

In October 2017, 220 Angus-based beef steers were transported approximately 7.5 h (685 km) from a single ranch in Nebraska to the Iowa State University Beef Nutrition Farm (Ames, IA). Upon arrival (d -7) steers were offered long stem grass hay top dressed with a corn silage based receiving diet (**Table 1**). No additional hay was fed after the second full day. On d -4, steers were weighed and received visual and electronic identification tags. Steers that did not meet weight criteria and/or displayed signs of illness were excluded from the study; 204 steers (249 ± 23 kg; 197 ± 15 d of age) were utilized. On d 0, steers were blocked by BW and weaning protocol (3 blocks; n = 12 pens/block) and randomly assigned to partially covered concrete pens within blocks. Blocks consisted of: pre-weaned (5 steers/pen, initial BW = 228 kg), unweaned heavy (6 steers/pen, initial BW = 273 kg) and unweaned light (6 steers/pen, initial BW = 243 kg). Steers in the pre-weaned block were weaned approximately 35 d prior to arrival while steers

in the unweaned blocks were not separated from their dams until transportation to IA. Pens within block were then randomly assigned to 1 of 4 supplemental VE (ROVIMIX E-50 Adsorbate, DSM Nutritional Products, Heerlen, Netherlands) treatments (n = 9 pens/treatment in total): no supplemental VE (**CON**), VE at 25 IU/kg DM (**LOW**), VE at 500 IU/steer daily (**MED**) or VE at 1,000 IU/steer daily (**HIGH**). Treatments were selected to represent current recommendations for feedlot cattle (LOW; NASEM, 2016), for stressed feedlot cattle (MED; NASEM, 2016) or at a perceived pharmacological dose (HIGH) that may impact aspects of immune function. Individual premixes were made for supplemental VE treatments (LOW, MED and HIGH) using dried distillers grains as a carrier and premixes were delivered as part of the total mixed ration (**TMR**). Diets were mixed and delivered in the order of CON, LOW, MED and HIGH and the mixer was flushed with long stem grass hay between treatments. The LOW premix was delivered at 5% of the diet (DM basis) throughout the trial. Percent inclusions of MED and HIGH treatment premixes were adjusted weekly based on projected treatment group DM intake (**DMI**) to ensure target intake of VE was maintained. Back calculated supplemental VE intake was 0, 151 ± 28, 484 ± 28 and 995 ± 69 IU/d for CON, LOW, MED and HIGH, respectively. On a BW basis, back calculated supplemental VE intake was 0, 0.55, 1.79 and 3.67 IU/kg BW for CON, LOW, MED and HIGH, respectively.

On d 6, a pour-on doramectin solution (Dectomax, Zoetis, Parsippany, NJ) was administered and steers received a booster vaccine against bovine viral diarrhea virus (**BVDV**) type 1 and 2 (Bovi-Shield Gold, One Shot, Zoetis). Steers were weighed prior to feeding on two consecutive days at the beginning (d -1 and 0 = initial BW) and end (d 26 and 27 = final BW) of the trial, as well as on d 6 and 14. Pen DMI, average daily gain (**ADG**) and gain:feed (**G:F**) were calculated from d 0 to 14, 14 to 27 and 0 to 27. Morbidity was assessed daily throughout the

course of the study and steers were treated (Draxxin, Zoetis) by farm personnel if visual symptoms (nasal discharge, labored breathing, lethargy and/or gauntness) were observed and rectal temperature ≥ 39ºC. Due to an outbreak of coccidiosis, Corid (Merial Inc., Duluth, GA) was included in the diet of all steers from d 8 through 12. All steers were subjected to 24 h feed restriction beginning on d 27 and ending on d 28 to determine the effect of supplemental VE on the cortisol response elicited by feed restriction (Marques et al., 2012).

Sample Collection and Analytical Procedures

Total feed offered and bunk scores were recorded daily and TMR samples were collected weekly for DM determination by drying in a forced air oven at 70°C for 48 h. Weekly TMR samples of the CON diet were dried, ground and composited for analysis of nitrogen (crude protein; AOAC, 1999b; method 990.03), neutral detergent fiber (AOAC, 2005; method 2002.04) and ether extract (AOAC, 1999a; method 920.39) by a commercial laboratory (Dairyland Laboratories, Inc., Arcadia, WI); analyzed compositions are presented in **Table 1**.

One steer per pen that represented the average weight of the pen was chosen as a sampling animal for liver and blood; the same 36 steers were sampled each time. Blood was collected prior to feeding via jugular venipuncture into vacuum tubes (serum, #366430; sodium heparin, #367874, Becton Dickinson, Franklin Lakes, NJ) on d -1, 6 (prior to vaccination), 14, 26 (prior to feed restriction) and 28 and was transported to the laboratory on ice. Serum tubes remained at room temperature for at least 90 min to allow for coagulation prior to centrifugation at 1,000 × *g* for 10 min at 4ºC; serum was then aliquoted and frozen at -80ºC until further analysis. Serum collected on d -1 and 26 was sent to the Iowa State University Veterinary Diagnostic Laboratory (**ISUVDL**) for analysis of Se concentrations via inductively coupled plasma mass spectrometry (Analytik Jena Inc. Woburn, MA) and α-tocopherol concentrations

via high-performance liquid chromatography (**HPLC**). Parameters for HPLC included a mobile phase of 95 (90 methanol/10 chloroform)/5 water, a flow rate of 1 mL/min, a C18 column (Pecosphere, 3µM, 4.6 x 33mm; Perkin Elmer, Waltham, MA) and a detection wavelength of 292 nm. Serum collected on d 6, 14 and 26 was sent to the ISUVDL for analysis of BVDV type 1 and 2 antibody titers via virus neutralization (method 9.104; Kalkwarf, 2014). Briefly, serum samples were serially diluted (10^{-1} to 10^{-4}) and incubated with the virus in 96 well culture plates for 2 h at 25ºC. The highest dilution that neutralized 100% of the challenge virus was considered the endpoint. Heparin tubes were centrifuged at 1,000 × *g* for 10 min at 4ºC; plasma was aliquoted and frozen at -80ºC until further analysis. Plasma collected on d -1, 26 and 28 was analyzed for malondialdehyde (**MDA**) concentrations using a commercially available kit (#700870, Cayman Chemical); intra- and inter-assay CV were 7.3% and 2.0%, respectively. Plasma collected on d -1, 26 and 28 was also analyzed for cortisol concentrations using a commercially available ELISA kit (#K003-H1/H5, Arbor Assays, Ann Arbor, MI); intra- and inter-assay CV were 4.3% and 10.1%, respectively.

Liver biopsies were performed on d -3 and 24 using a modified method described by Engle and Spears (2000). Briefly, lidocaine was injected, and a small incision made with a scalpel blade between the 11^{th} and 12^{th} ribs on a line from the point of the hip to the point of the shoulder. A modified bone marrow biopsy probe was then inserted into the liver and negative pressure applied with a 10-cc syringe to draw the sample into the probe. Liver samples were snap frozen in liquid nitrogen and transported to the laboratory where they were stored at -80°C. Liver samples were ground in liquid nitrogen prior to analysis of α-tocopherol concentrations, superoxide dismutase (**SOD**) activity and glutathione concentrations. Liver samples were sent to the ISUVDL for α-tocopherol analysis via HPLC using the same parameters as described for

serum α-tocopherol. Liver samples for total and Mn-SOD activity (0.15 g tissue; wet basis) were homogenized in 0.75 mL of 20 mM 4-(2-Hydroxyethyl)piperazine-1-ethanesulfonic acid buffer and centrifuged at 1500 × *g* for 5 min at 4ºC. The supernatant was then removed, aliquoted and stored at -80ºC until further analysis (#706002, Cayman Chemical, Ann Arbor, MI). Intra- and inter-assay CV for Mn-SOD were 5.3% and 8.9%, respectively; intra- and inter-assay CV for total-SOD were 8.8% and 10.1%, respectively. Copper/Zn-SOD activity was calculated by subtracting Mn-SOD from total SOD activity. Activity is reported as units/mg protein where one unit is defined as the amount of enzyme required to dismutate 50% of the superoxide radical. Protein concentrations of the samples analyzed for SOD activity were determined using a commercially available kit (#23200, Thermo Scientific, Rockford, IL); intra- and inter- assay CV were 1.5% and 6.4%, respectively. Liver samples for total (**tGSH**) and oxidized (**GSSG**) glutathione concentrations (0.15 g tissue; wet basis) were homogenized in 0.75 mL of 50 mM 2-(N-Morpholino) ethanesulfonic acid buffer and prepared for analysis (#703002, Cayman Chemical) as previously described (Hartman et al., 2017). Reduced glutathione (**GSH**) concentrations were calculated by subtracting GSSG from tGSH. Glutathione concentrations are reported as µM/g wet tissue. Intra-assay CV for tGSH and GSSG were 4.9 and 0.5, respectively; inter-assay CV for tGSH and GSSG were 1.8 and 1.1, respectively.

Statistical Analysis

Two LOW steers were removed from the study (one on d 6, one on d 14) due to severe respiratory illness unrelated to treatment; performance data for the subsequent periods were adjusted accordingly. Performance data for one MED pen were removed due to overall negative ADG by one steer. Data were analyzed using Proc MIXED of SAS 9.4 with pen as the experimental unit (n = 9/treatment). The model included the fixed effect of treatment and block,

where block consisted of weaned, unweaned heavy and unweaned light as described earlier. Orthogonal contrast statements (linear, quadratic and cubic) were constructed to determine the effects of supplemental VE; contrast coefficients were determined using Proc IML of SAS 9.4 based on back calculated supplemental VE intakes for individual treatment groups. To account for animal variation at the start of the trial, initial values for serum, plasma and liver analytes were used as covariates in analysis of subsequent sampling dates. Data were tested for normality and homogeneity of variance using the Shapiro-Wilks test; antibody titers were natural log transformed to meet the assumption of normality and log transformed means and SEM are presented. Outliers were determined on a pen basis using Cook's D statistic and removed if Cook's D > 0.05; one pen from LOW was removed from all performance analyses and one pen (one sampler steer) from HIGH was removed from all glutathione and SOD analyses. Morbidity data were analyzed using the GLIMMIX procedure of SAS 9.4 with pen as the experimental unit, the fixed effects of treatment and block, a logit link function and binomial distribution. Pearson correlations between serum and liver α-tocopherol concentrations were determined using Proc CORR of SAS 9.4. Data are reported as least square means ± SEM. Significance is declared at $P \leq 0.05$ and tendencies from $0.05 < P \leq 0.10$.

Results

Feedlot Performance and Morbidity

Receiving period performance data are presented in **Table 2**. Initial and final BW were not affected by supplemental VE ($P \geq 0.75$). From d 0 to 14 there was a cubic effect of VE on DMI ($P = 0.01$) driven by greater DMI by LOW and HIGH pens. There was also a tendency for a cubic effect of VE on DMI from d 14 to 27 ($P = 0.10$). A tendency for a quadratic effect of VE on ADG from d 0 to 14 ($P = 0.08$) was observed driven by greater ADG by CON and HIGH

pens. From d 14 to 27 there was a quadratic effect of VE on ADG and G:F ($P \leq 0.02$) driven by MED pens exhibiting greater ADG and G:F. There were no effects of VE on DMI, ADG or G:F from d 0 to 27 ($P \geq 0.37$). All treatments for respiratory disease occurred prior to d 10 of the study and supplemental VE did not affect the percentage of steers treated for respiratory disease ($P \geq 0.44$; **Table 2**). However, there was a numerical trend for a block effect ($P = 0.11$) where treatment percentages were 0, 2.7 and 13.6% for pre-weaned, unweaned heavy and unweaned light, respectively.

Se and Vitamin E (α-tocopherol) Status

Serum Se, serum α-tocopherol and liver α-tocopherol concentrations are reported in **Table 3**. Serum Se on d 26 was not affected by supplemental VE ($P \geq 0.56$). Day 26 serum and d 24 liver α-tocopherol concentrations were increased linearly due to supplemental VE ($P < 0.01$). Initial liver (d -3) and serum (d -1) α-tocopherol concentrations were positively correlated (r = 0.44; $P = 0.01$) as were final liver (d 24) and serum (d 26) concentrations (r = 0.69; $P < 0.01$).

Vaccination and Feed Restriction Response

There was no effect of VE on d 14 (8 d after booster vaccine) BVDV type 1 or 2 antibody titers (**Table 4**; $P \geq 0.17$). Day 26 (20 d after booster vaccine) BVDV type 1 antibody titers were linearly increased due to supplemental VE ($P = 0.04$); however, there was no effect of VE on d 26 BVDV type 2 antibody titers ($P \geq 0.32$). There was no effect of VE on d 26 (pre-restriction) or d 28 (post-restriction) plasma cortisol concentrations ($P \geq 0.11$; **Table 5**). A tendency for a quadratic effect of VE on d 26 plasma MDA concentrations ($P = 0.10$) was observed with MED steers having the greatest MDA concentrations. Additionally, there was a quadratic effect of VE on d 28 plasma MDA concentrations ($P = 0.01$) driven by lesser MDA concentrations for CON.

Liver Antioxidants

Liver glutathione and SOD data are presented in **Table 6**. Day 24 total, oxidized and reduced glutathione concentrations decreased linearly with increasing supplemental VE ($P \leq 0.02$). There was no effect of VE on the ratio of oxidized to reduced glutathione on d 24 ($P \geq 0.39$). Supplemental VE had a quadratic effect on d 24 total-SOD activity ($P = 0.03$) and tended to have a quadratic effect on d 24 Mn-SOD activity ($P = 0.07$), driven by MED steers exhibiting the greatest activity. Copper/Zn-SOD activity and the ratio of Mn to total-SOD activity were not affected by supplemental VE on d 24 ($P \geq 0.38$).

Discussion

Beef cattle experience various stressors during the feedlot receiving period that can result in inflammation (Arthington et al., 2003; Marques et al., 2012) and oxidative stress (Chirase et al., 2004) which may hinder cattle health and performance after arrival at the feedlot. Additionally, stress has been shown to negatively impact VE status of cattle (Nockels et al., 1996; Han et al., 1999). Vitamin E is required for oxidative stress protection and optimal immune function, suggesting the feedlot receiving period may be a time when increased dietary supplementation of VE is warranted. The VE supplementation strategies utilized in the present study represent the current recommendation for feedlot cattle (25 to 35 IU/kg DMI; back calculated intake = 24.8 IU/kg DM; NASEM, 2016), the recently established VE recommendation for stressed cattle (400 to 500 IU/animal daily; back calculated intake = 484 IU/steer daily; NASEM, 2016) and a pharmacological dose (1,000 IU/animal daily; back calculated intake = 995 IU/steer daily). These dietary treatments likely represent the majority of VE supplementation strategies utilized in feedlot receiving diets (Samuelson et al., 2016).

Vitamin E is stored in both a fixed pool (adipose tissue) that is mobilized slowly as well as labile pools (plasma and liver) which are depleted rapidly when dietary VE is limiting (Machlin et al, 1979). Serum and liver α-tocopherol concentrations were positively correlated ($r \geq 0.44$) in the present study and were reflective of dietary VE treatments. Serum α-tocopherol concentrations < 2.0, 2.0 to 3.0, 3.0 to 4.0, and > 4.0 mg/L are considered deficient, marginal, minimal but adequate, and adequate, respectively (Adams, 1982) and steers began the study with minimal to adequate VE status (3.9 ± 1.0 mg/L) regardless of assigned treatment. By the end of the 27-d trial CON steers had marginal serum α-tocopherol concentrations (2.7 mg/L), LOW steers maintained their minimal status (3.4 mg/L), while MED and HIGH steers had increased serum α-tocopherol concentrations (4.6 and 5.8 mg/L, respectively) above the threshold for adequacy. These data suggest even cattle with adequate VE status may experience a rapid decline in VE status upon arrival due to the stress of feedlot receiving and subsequent increased demand for VE. In agreeance, Carter et al. (2005) observed a decrease in serum α-tocopherol concentrations of receiving cattle from 5.7 mg/L on d 0 to 1.1 mg/L on d 28 when VE was not supplemented in the diet. In the present study, VE supplemented at current recommendations for feedlot cattle (NASEM, 2016) was adequate to prevent a decline in VE status.

Despite changes in VE status, overall receiving period performance did not differ between treatments. Previous studies have observed inconsistent performance responses to VE supplementation during the receiving period. Secrist et al. (1997) reviewed 5 studies regarding the impact of supplemental VE on performance of calves during the first month after transport and found ADG and feed efficiency tended to improve when supplemental VE (450 to 1,400 IU/animal daily) was provided. More recently, Elam (2007) analyzed the results of 7 receiving studies with supplemental VE ranging from 0 to 2,000 IU/animal daily and found no predictive

relationship for VE supplementation on DMI, ADG or G:F. Responses to VE supplementation observed among individual trials were highly variable likely due to many factors including stress experienced by calves, previous diet, duration of VE supplementation and initial VE status. Steers in the current study were not deficient in VE to start or end the trial, even those that received no supplemental VE, which may have contributed to the lack of performance response. However, it is unclear how prior status affects cattle response to supplemental VE as previous studies seldom report indicators of VE status, possibly due to the assumption that calves with previous exposure to green pastures have adequate VE status upon arrival at the feedlot (Secrist et al., 1997).

The inconsistency of performance responses to VE supplementation indicates the primary objective for greater VE supplementation for receiving cattle may be to support immune system function, primarily via antioxidant protection of immune cells from free radicals released during phagocytosis of pathogens (Babior, 1984). Vitamin E deficiency has been shown to compromise phagocytic, bactericidal and chemotactic responses of immune cells as well as suppress lymphocyte production, impair T-cell function and decreased antibody production (Pekmezci, 2011). Alternatively, animals consuming diets that contain more than 5 times the recommended daily allowance of VE have exhibited increased humoral and cell-mediated immune responses (Tengerdy et al., 1973; Tanaka et al., 1979). Rivera et al. (2002) observed a linear increase in ovalbumin antibody titers 21 d after calves received an ovalbumin vaccine, with the greatest antibody response observed in calves receiving supplemental VE at 1,140 IU/d. In the current study, a booster vaccine against BVDV (administered on d 6) was used to elicit an antibody response and determine the effects of varying levels of supplemental VE on antibody concentrations. However, regardless of treatment, BVDV type 1 and 2 antibody titers

numerically decreased over time (d 14 and 26). This lack of antibody response could be a result of increased initial antibody titers from a previous vaccination that occurred at the ranch of origin or the duration of sampling (20 d post-vaccination) was insufficient to capture an increase in antibody titers that may have occurred later. The decrease in BVDV type 1 antibody concentrations over time was numerically less for steers receiving supplemental VE at 1,000 IU/d.

The meta-analysis conducted by Elam (2007) suggests for every 100 IU increase in VE intake/d, a 0.35% decrease in morbidity would be expected. However, the percentage of steers treated for respiratory disease was not affected by supplemental VE in the current study. Since all respiratory treatments occurred prior to d 10 of the study, supplemental VE may not have been fed long enough by that time to influence morbidity rates. Additionally, though the present study had in excess of 200 steers, illness rates were not high. Carter et al. (2005) studied the effect of supplementing VE at 2,000 IU/animal daily for 0, 7, 14 or 28 d and observed a numeric decrease in morbidity for calves supplemented for 14 or 28 d, suggesting the positive effects of VE on health might be time dependent. Although VE did not affect morbidity in the present study, there was a trend for a block effect where treatment percentages were least for pre-weaned calves and greatest for lightweight unweaned calves. These data confirm that weaning cattle prior to feedlot entry and heavier BW are critical factors in decreasing morbidity upon arrival. Age is also an important factor as yearlings may be expected to have lower morbidity rates during the receiving period than cattle that enter the feedlot as calves.

The current study also sought to determine the effects of supplementing an exogenous antioxidant (i.e. VE) on endogenous components of the antioxidant defense system including glutathione and SOD. It is important to note that Se and VE both have antioxidant functions and

can therefore spare each other. However, serum Se concentrations were not affected by supplemental VE and steers had adequate Se status to start and end the trial (Herdt and Hoff, 2011) indicating Se status had minimal influence on the antioxidant measures discussed herein. Glutathione concentrations were linearly decreased due to supplemental VE, possibly due to a sparing effect where supplementation of an exogenous antioxidant resulted in less signaled need for endogenous antioxidant production. The synthesis of glutathione has also been shown to be upregulated in response to VE deficiency (Morante et al., 2005). Thus, it is possible that supplementing VE at high concentrations could have had the opposite effect and directly inhibited glutathione synthesis. Steers supplemented VE at 500 IU/daily had the greatest activity of the antioxidant enzyme SOD, driven primarily by changes in the Mn-dependent isoform. A previous study in rats revealed an increase in Mn-SOD mRNA expression and activity after 4 weeks of twice weekly intraperitoneal injections of VE at greater doses (30 or 100 mg/kg BW) vs. lesser doses (0 or 10 mg/kg BW; Hajiani et al., 2013). However, the greatest VE dose in the current study (1,000 IU/steer daily) resulted in Mn-SOD activity similar to CON-steers. This could be due to the prooxidant capabilities of VE when there is a lack of other antioxidants, such as vitamin C and glutathione, to reduce the α-tocopherol radical (Rietjens et al., 2002). The decreased glutathione concentrations discussed previously suggest that the pharmacological VE dose may have contributed to prooxidant conditions and subsequent inactivation of Mn-SOD (Yamakura and Kawasaki, 2010).

Steers in the current study were subjected to 24 h of feed restriction to elicit a cortisol response and determine if supplemental VE affected this response. However, cortisol concentrations were not increased post-restriction. There was also no effect of supplemental VE on pre or post-restriction cortisol concentrations while Reddy et al. (1987) observed lesser serum

cortisol concentrations in dairy calves supplemented with 125, 250 or 500 IU of VE/calf daily compared to non-supplemented calves. In addition to stimulating a cortisol response, it was hypothesized that feed restriction would increase concentrations of MDA, a marker of lipid peroxidation, and VE would mitigate this increase due to its role as a lipid soluble antioxidant. However, MDA concentrations were numerically lesser for all treatment groups post-restriction and MED steers exhibited the greatest plasma MDA concentrations. Cusack et al. (2005) also observed greater plasma MDA concentrations in heifer calves supplemented VE at 822 IU/d and suggested that this response may be partially due to MDA being a normal intermediate formed during biosynthesis of eicosanoids, lipid signaling molecules involved in immunity and inflammation (Harizi et al., 2008). It has also been suggested that the method used to analyze MDA concentrations in the current study (thiobarbituric acid reactive substances) may contribute to sample oxidation during analysis and may not be specific enough due to the ability of thiobarbituric acid to react with a variety of compounds (Del Rio et al., 2005).

Despite linear increases in circulating VE and VE stores, supplemental VE had no effect on steer morbidity and minimal effects on growth performance in the current study. However, other variables of interest were differentially affected by VE supplementation. For example, supplementation of VE at the recommended rate for feedlot cattle (25 IU/kg DMI; NASEM, 2016) prevented the decline in VE status observed in control steers while supplementation of VE at the recommended rate for stressed cattle (500 IU/steer daily; NASEM, 2016) increased activity of the antioxidant enzyme SOD. Additionally, supplementation of VE at a pharmacological dose (1,000 IU/steer daily) lessened the decrease in BVDV antibody titers over time but decreased concentrations of the endogenous antioxidant glutathione, indicating VE supplementation rate may differentially affect variables of interest to feedlot producers. The

responses to VE supplementation observed herein were likely impacted by initial VE status (minimal to adequate) and lack of VE deficiency, even in CON steers. Therefore, the physiological needs of the animal (e.g. growth, immune function, antioxidant defense) and VE status upon arrival at the feedlot should be taken into consideration when defining recommended VE supplementation rates for receiving cattle.

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Table 1. Ingredient composition of the control diet

Dry matter (DM), % as fed basis	61
Ingredient, % DM basis	
Corn silage	40
Cracked corn	30
DDGS[1]	28.25
Limestone	1.4
Salt	0.31
Rumensin[2]	0.0135
Trace mineral premix[3]	0.025
Analyzed composition[4], %	
Crude protein	15.3
Neutral detergent fiber	25.6
Ether extract	4.8
Calculated composition	
α-tocopherol[5], IU/kg DM	11.7
Net energy for gain[6], Mcal/kg	1.23

[1]Dried distillers grains with solubles; carrier for micro-ingredients and vitamin E (ROVIMIX E-50 Adsorbate, DSM Nutritional Products, Heerlen, Netherlands) treatments
[2]Provided 150 mg monensin/steer daily (Elanco Animal Health, Greenfield, IN)
[3]Provided per kg of diet DM: 10 mg of Cu, 30 mg of Zn, 20 mg of Mn, 0.5 mg of I, 0.1 mg of Se, and 0.1 mg of Co all from inorganic sources and 2,200 IU vitamin A (NASEM, 2016)
[4]Based on total mixed ration analysis from Dairyland, Inc., Arcadia, WI
[5]Alpha-tocopherol content of corn and corn silage based on values reported by Hidiroglou et al. (1992); α-tocopherol content of DDGS based on values reported by Jung et al. (2013)
[6]Net energy for gain based on NASEM (2016) reported NEg values of feedstuffs

Table 2. Effect of supplemental vitamin E (VE) on receiving period performance of beef steers

	Supplemental VE Treatment[1]					Contrast *P*-value		
	CON n = 9 pens	LOW n = 8 pens	MED n = 8 pens	HIGH n = 9 pens	SEM[2]	Linear	Quadratic	Cubic
VE intake[3], IU/d	0	151	484	995	-	-	-	-
Initial BW[4], kg	248	250	245	247	8.2	0.88	0.84	0.75
Final BW[5], kg	300	301	301	300	4.8	0.96	0.81	0.99
Dry matter intake, kg/d								
d 0 to 14	5.0	5.2	4.9	5.1	0.08	0.57	0.28	0.01
d 14 to 27	7.2	7.0	7.1	7.1	0.09	0.98	0.48	0.10
d 0 to 27	6.1	6.1	6.0	6.1	0.08	0.79	0.37	0.71
Average daily gain, kg/d								
d 0 to 14	1.92	1.83	1.81	1.92	0.055	0.70	0.08	0.65
d 14 to 27	1.95	2.01	2.16	2.02	0.061	0.39	0.02	0.53
d 0 to 27	1.94	1.92	1.98	1.97	0.042	0.39	0.67	0.46
Gain:feed								
d 0 to 14	0.389	0.356	0.369	0.377	0.013	0.99	0.24	0.13
d 14 to 27	0.272	0.288	0.304	0.283	0.008	0.39	0.01	0.94
d 0 to 27	0.319	0.316	0.330	0.322	0.007	0.54	0.38	0.38
Treated[6], %	4.3	9.4	6.7	6.7	15.6	0.84	0.68	0.44

[1]VE = vitamin E (ROVIMIX E-50 Adsorbate, DSM Nutritional Products, Heerlen, Netherlands); CON = control (no supplemental VE); LOW = VE at 25 IU/kg DM; MED = VE at 500 IU/steer daily; HIGH = VE at 1,000 IU/steer daily

[2]Highest SEM of any treatment reported

[3]Back calculated supplemental VE intake/steer daily

[4]Initial body weight (BW) = average of BW collected on d -1 and 0

[5]Final BW = average of BW collected on d 26 and 27

[6]Percentage of steers treated for respiratory illness

Table 3. Effect of supplemental vitamin E (VE) on serum Se and α-tocopherol and liver α-tocopherol concentrations of beef steers

	Supplemental VE Treatment[1]					*P*-value		
	CON	LOW	MED	HIGH	SEM	Linear	Quadratic	Cubic
Serum Se, µg/L								
d -1[2]	75.2	75.8	76.2	70.4	-	-	-	-
d 26	96.9	98.0	94.0	97.1	3.65	0.90	0.56	0.58
Serum α-tocopherol, mg/L								
d -1[2]	4.0	3.5	4.2	3.9	-	-	-	-
d 26	2.7	3.4	4.6	5.8	0.26	<0.01	0.20	0.81
Liver α-tocopherol, mg/kg wet tissue								
d -3[2]	11.0	8.5	10.3	11.1	-	-	-	-
d 24	6.4	8.7	10.1	15.2	0.86	<0.01	0.70	0.26

[1]VE = vitamin E (ROVIMIX E-50 Adsorbate, DSM Nutritional Products, Heerlen, Netherlands); CON = control (no supplemental VE); LOW = VE at 25 IU/kg DM; MED = VE at 500 IU/steer daily; HIGH = VE at 1,000 IU/steer daily

[2]Values from d -1 and -3 (prior to treatment initiation) were utilized as a covariate in analysis

Table 4. Effect of supplemental vitamin E (VE) on bovine viral diarrhea virus (BVDV) type 1 and 2 antibody titers of beef steers

	Supplemental VE Treatment[1]					Contrast *P*-value		
	CON	LOW	MED	HIGH	SEM	Linear	Quadratic	Cubic
BVDV Type 1[2]								
d 6[3] (0)[4]	2.3	3.0	3.6	2.4	-	-	-	-
d 14 (8)	1.7	1.7	1.4	2.1	0.24	0.32	0.17	0.47
d 26 (20)	1.5	2.1	2.0	2.5	0.33	0.04	0.77	0.29
BVDV Type 2[2]								
d 6[3] (0)[4]	4.6	5.3	5.2	5.0	-	-	-	-
d 14 (8)	3.7	3.9	4.1	3.9	0.34	0.61	0.47	0.91
d 26 (20)	4.2	4.5	4.2	4.6	0.31	0.42	0.72	0.32

[1]VE = vitamin E (ROVIMIX E-50 Adsorbate, DSM Nutritional Products, Heerlen, Netherlands); CON = control (no supplemental VE); LOW = VE at 25 IU/kg DM; MED = VE at 500 IU/steer daily; HIGH = VE at 1,000 IU/steer daily

[2]Natural log transformed; transformed means and SEM presented

[3]Blood was collected prior to administration of a booster vaccine (Bovi-Shield Gold, One Shot, Zoetis) on d 6 of the study; Values from d 6 were utilized as a covariate in analysis

[4]Day relative to vaccination indicated in parentheses

Table 5. Effect of supplemental vitamin E (VE) on plasma cortisol and malondialdehyde (MDA) concentrations of beef steers

	Supplemental VE Treatment[1]					Contrast *P*-value		
	CON	LOW	MED	HIGH	SEM	Linear	Quadratic	Cubic
Cortisol, ng/mL								
d -1[2]	28.9	29.4	25.0	26.2	-	-	-	-
d 26	20.0	18.2	19.0	21.1	3.32	0.68	0.67	0.78
d 28[3]	25.5	19.6	24.0	20.6	2.73	0.49	0.98	0.11
MDA, µM								
d -1[2]	7.7	7.1	6.8	6.6	-	-	-	-
d 26	7.1	7.6	8.5	7.5	0.61	0.65	0.10	0.86
d 28[3]	5.0	6.7	7.4	7.2	0.45	0.01	0.01	0.18

[1]VE = vitamin E (ROVIMIX E-50 Adsorbate, DSM Nutritional Products, Heerlen, Netherlands); CON = control (no supplemental VE); LOW = VE at 25 IU/kg DM; MED = VE at 500 IU/steer daily; HIGH = VE at 1,000 IU/steer daily

[2]Values from d -1 (prior to treatment initiation) were utilized as a covariate in analysis

[3]Blood collected after steers were restricted from feed for 24 h

Table 6. Effect of supplemental vitamin E (VE) on liver glutathione concentrations (µM per gram of wet tissue) and superoxide dismutase activity (units/mg protein; one unit = the amount of enzyme required to dismutate 50% of the superoxide radical) of beef steers

	Supplemental VE Treatment[1]					Contrast *P*-value		
	CON	LOW	MED	HIGH	SEM[2]	Linear	Quadratic	Cubic
Glutathione								
d -3[3]								
Total	2.04	1.84	2.05	2.02	-	-	-	-
Oxidized	0.37	0.35	0.35	0.34	-	-	-	-
Reduced	1.68	1.49	1.70	1.68	-	-	-	-
Ratio[4]	0.232	0.251	0.223	0.215	-	-	-	-
d 24								
Total	2.24	2.11	1.84	1.77	0.126	0.01	0.25	0.85
Oxidized	0.40	0.37	0.34	0.32	0.021	<0.01	0.32	0.80
Reduced	1.83	1.74	1.50	1.46	0.116	0.02	0.28	0.79
Ratio[4]	0.223	0.217	0.234	0.214	0.0135	0.76	0.39	0.45
Superoxide Dismutase								
d -3[3]								
Total	162	159	141	148	-	-	-	-
Mn	124	118	105	119	-	-	-	-
Cu/Zn	38.3	40.4	35.2	28.3	-	-	-	-
Ratio[5]	0.78	0.75	0.75	0.81	-	-	-	-
d 24								
Total	135.2	150.2	162.3	139.7	9.4	0.90	0.03	0.90
Mn	106.5	120.4	122.0	105.7	7.5	0.61	0.07	0.53
Cu/Zn	29.3	29.2	37.0	37.1	6.43	0.63	0.63	0.68
Ratio[5]	0.77	0.80	0.79	0.75	0.037	0.38	0.46	0.70

[1]VE = vitamin E (ROVIMIX E-50 Adsorbate, DSM Nutritional Products, Heerlen, Netherlands); CON = control (no supplemental VE); LOW = VE at 25 IU/kg DM; MED = VE at 500 IU/steer daily; HIGH = VE at 1,000 IU/steer daily

[2]Highest SEM of any treatment reported

[3]Values from d -3 (prior to treatment initiation) utilized as a covariate in analysis

[4]Ratio = oxidized/reduced glutathione

[5]Ratio = Mn-SOD/Total-SOD

CHAPTER 4. EFFECT OF SUPPLEMENTING A SACCHAROMYCES CEREVISIAE FERMENTATION PRODUCT DURING A PRECONDITIONING PERIOD PRIOR TO TRANSIT ON RECEIVING PERIOD PERFORMANCE, NUTRIENT DIGESTIBILITY AND ANTIOXIDANT DEFENSE BY BEEF STEERS

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E. L. Deters and S. L. Hansen

Abstract

Forty-eight newly weaned crossbred beef steers from a single-source were used to determine the effects of feeding a *Saccharomyces cerevisiae* fermentation product (**SCFP**; NaturSafe, Diamond V) on receiving period performance, nutrient digestibility and antioxidant defense. Seven days after arrival, steers were stratified by BW (257 ± 18 kg), sorted into pens (n = 1 pen/treatment) and pens assigned to dietary treatments: SCFP at 0 (**CON**), 12 (**SCFP12**), 18 (**SCFP18**), or 0 g·steer^{-1}·day^{-1} during preconditioning (**PRE**; d -19 to 0), then 18 g·steer^{-1}·day^{-1} during receiving (**REC**; d 0 to 58; **CON18**). On d -1 BW and blood were collected, steers were loaded onto a semi-truck and transported 1,748 km over 19 h. Upon return, steers were weighed, stratified by BW within treatment and sorted into pens with GrowSafe bunks (n = 12 steers/treatment). Steers were weighed on d -1, 0, 29, 30, 57 and 58. Blood was collected from all steers on d -1, 1 and 8 and liver biopsies were performed on all steers on d -20, -3 and 59. Titanium dioxide was included as an indigestible marker in the diet of all steers from d 14 through 29 to determine total tract nutrient digestibility. Data were analyzed as a completely randomized design using ProcMixed of SAS with the fixed effect of treatment. Steer was the experimental unit for REC period variables. Contrast statements compared the linear and quadratic effects of feeding SCFP throughout the trial (CON, SCFP12, SCFP18) and the effect of supplementation at 18 g·steer^{-1}·day^{-1} for the entire trial or starting in REC (SCFP18 vs. CON18).

Steers fed SCFP12 exhibited the greatest ADG and G:F from d 0 to 30 (quadratic $P \leq 0.04$). Total tract digestibility of NDF and ADF were linearly decreased by SCFP (linear $P \leq 0.03$). On d -3, SCFP12-fed steers tended to have the greatest liver concentrations of total, oxidized and reduced glutathione (quadratic $P = 0.06$). Red blood cell lysate Mn:total-superoxide dismutase activity was 16% greater 1 d post-transit compared to pre-transit values (day $P \leq 0.01$). Timing of SCFP supplementation (SCFP18 vs. CON18) did not affect any of the variables assessed herein ($P \geq 0.19$). Supplementing SCFP at 12 $g \cdot steer^{-1} \cdot day^{-1}$ tended to affect antioxidant capacity prior to transit and improved early receiving period performance; however, overall receiving period performance was not affected by SCFP supplementation. Further research is necessary to determine the optimal dose and timing of SCFP supplementation for beef cattle.

Introduction

Beef cattle experience various physical and psychological stressors during the feedlot receiving period. These stressors include recent weaning, vaccination, commingling and transportation. The combination of stress and exposure to pathogens increases disease susceptibility and decreases feedlot performance (Galyean et al., 1999; Loerch and Fluharty, 1999). Additionally, transit has been shown to increase markers of oxidative stress in cattle that were associated with increased incidence of bovine respiratory disease (Chirase et al., 2004). *Saccharomyces cerevisiae* fermentation products (**SCFP**) have decreased the number of first pulls and repulls as well as antibiotic usage in two retrospective analyses (NaturSafe; Diamond V, 2017a,b). Feed efficiency was also improved for cattle fed SCFP throughout the entire feeding period compared to cattle that were not fed SCFP but received a metaphylactic antibiotic upon arrival (Diamond V, 2017a). The positive influence of SCFP on health and performance suggest that SCFP may be a beneficial addition to receiving cattle diets.

The first objective of this study was to determine the effects of varying doses of SCFP on receiving period performance, total tract nutrient digestibility and oxidative stress biomarkers in beef steers. Due to the segmented nature of the beef industry, calves often change ownership prior to arrival at the feedlot and calf nutrition prior to feedlot receiving likely influences how they perform upon arrival (Duff and Galyean, 2007). Therefore, the second objective was to determine the effects of supplementing SCFP during a preconditioning phase prior to a 19-h transit event on subsequent receiving period performance. The final objective was to examine changes in markers of oxidative stress relative to transit with the hypothesis that transit would increase markers of oxidative stress and that supplementing SCFP during a preconditioning period prior to transit would have positive implications on the oxidative stress response and receiving period performance of beef steers.

Materials and Methods

Animals and Experimental Design

All experimental procedures were approved by the Iowa State University Animal Care and Use Committee (#8376-B). Sixty newly weaned (bawling) crossbred beef steers (253 ± 23 kg) from a single-source were transported approximately 265 km to the Iowa State University Beef Nutrition Farm (Ames, IA) where they were received into open dirt lots (23.6 × 33.5 m; 15 steers/pen) with concrete bunks (12.2 m of linear bunk space) and one automatic waterer/pen. Steers were offered long-stem hay top dressed with the preconditioning (**PRE**) TMR on the first day. Bunks were scored the morning after arrival and if bunks were clean an additional 0.45 kg of DM per steer was offered. The 48 steers most uniform in weight (257 ± 18 kg), disposition and health status were utilized in this trial which consisted of two phases, PRE (d -19 to -1) followed by receiving (**REC**; d 0 to 58), separated by a 19-h transit event. Diet composition and

nutrient analysis is shown in **Table 1**. Weekly control TMR samples were dried, ground and composited within PRE and REC periods for analysis of N (AOAC, 1195b; method 990.03), NDF (AOAC, 2005; method 2002.04) and ether extract (AOAC, 1995a; method 920.39) by a commercial laboratory (Dairyland Laboratories, Inc., Arcadia, WI) as well as Cu, Fe, Mn and Zn using inductively coupled optical emission spectroscopy as described by Richter et al. (2012).

Preconditioning. This period served to mimic the group feeding style of preconditioning periods common on farms and to address the second objective of this study: determining the effect of supplementing SCFP during a preconditioning period prior to a transit event on subsequent receiving period performance. Seven days after arrival (d -19) steers were weighed, vaccinated against viral infections (Pyramid 5, Presponse SQ; Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO) and treated for parasites (Ivomec Eprinomex, Boehringer Ingelheim Vetmedica, Inc.). Steers were stratified by BW and sorted into open dirt lots as described previously (n = 1 pen/treatment; 12 steers/pen). Pens were then randomly assigned to 1 of 4 dietary treatments: SCFP (NaturSafe, Diamond V, Cedar Rapids, IA) at 0 (**CON**), 12 (**SCFP12**), 18 (**SCFP18**) $g \cdot steer^{-1} \cdot day^{-1}$ during both PRE and REC, or 0 $g \cdot steer^{-1} \cdot day^{-1}$ during PRE then 18 $g \cdot steer^{-1} \cdot day^{-1}$ during REC (**CON18**). The current manufacturer's recommended dose for receiving cattle is 12 $g \cdot steer^{-1} \cdot d^{-1}$ or 2 kg/metric ton DM in the ration. Treatments were delivered as part of a premix using dried distillers grains as a carrier and premix inclusions were adjusted weekly based on treatment group DMI to ensure target intakes of SCFP were maintained. Intake of SCFP was back calculated based on pen DMI and premix inclusion rates; SCFP intakes for PRE were 12.0 and 18.9 $g \cdot steer^{-1} \cdot day^{-1}$ for SCFP12 and SCFP18, respectively. On d -1, steers were weighed, blood samples collected, loaded onto a single commercial livestock trailer (Silverstar PSDCL-402; Wilson Trailer Company, Sioux City, IA) and transported 1,748 km

over 19 h. Steers were stratified by dietary treatment across truck compartments to account for compartment variability.

Receiving. Upon return (d 0), steers were weighed, stratified by BW, and sorted into partially covered concrete pens (3.7 × 12.2 m) with one GrowSafe (GrowSafe Systems Ltd., Airdrie, Alberta, Canada) bunk and one automatic waterer/pen (experimental unit = steer; n = 12 steers/treatment; 6 steers/pen). Steers were fed the same dose of SCFP during REC as PRE, except CON18-fed steers switched from 0 to 18 $g \cdot steer^{-1} \cdot day^{-1}$. Intake of SCFP was back calculated based on steer DMI and premix inclusion rates; SCFP intakes for REC were 11.4, 18.1, and 18.6 $g \cdot steer^{-1} \cdot day^{-1}$ for SCFP12, SCFP18, and CON18, respectively. The shrunk BW collected when steers arrived back at the Iowa State University Beef Nutrition Farm after transit (d 0) was used as the final BW for PRE and the initial BW for REC. Weights were collected prior to feeding on d 0, 29, 30, 57 and 58. Feed efficiency (G:F) was calculated from d 0 to 30 (average BW collected on d 29 and 30), 30 to 58 (average BW collected on d 57 and 58) and 0 to 58 from steer DMI and weight gain. On d 1 steers were implanted with 200 mg progesterone and 20 mg estradiol (Component E-S with Tylan, Elanco, Indianapolis, IN). Morbidity was assessed daily throughout the course of the study and steers were treated with tulathromycin (Draxxin, Zoetis, Parsippany, NJ) by farm personnel if visual symptoms were observed and rectal temperature was ≥ 39ºC.

Sample Collection and Analytical Procedures

Digestibility. To determine the effect of SCFP on total tract nutrient digestibility, titanium dioxide was included as an indigestible marker in the diet of all steers (10 $g \cdot steer^{-1} \cdot day^{-1}$) from d 14 through 29. Back calculated daily intakes of titanium dioxide were 10.4, 9.7, 10.2 and 10.3 g/steer for CON, SCFP12, SCFP18, and CON18, respectively. Fecal samples were collected

prior to feeding on d 29 and 30 for digestibility analyses. Treatment TMR samples from the digestibility period as well as fecal samples collected on d 29 and 30 were dried and then ground to pass through a 2 mm screen in a Retsch ZM 100 grinding mill (Retsch GmbH, Haan, Germany) and analyzed for DM, OM, NDF, ADF and N using methods described by Russell et al. (2016a). Titanium dioxide was analyzed using methods outlined by Myers et al. (2004). Nutrient and titanium dioxide concentrations were analyzed separately for consecutive day fecal samples and the average was used for final digestibility calculations as previously described (Russell et al., 2016a).

Blood and Liver. Blood was collected from the jugular vein of all steers on d -1, 1 and 8 into vacuum tubes (sodium heparin, No. 367874, Becton Dickinson, Franklin Lakes, NJ), transported to the laboratory on ice, and centrifuged at 1,000 × g for 10 min at 4ºC. Plasma was removed, aliquoted and stored at -80ºC until analysis of malondialdehyde (**MDA**) concentrations (#700870, Cayman Chemical, Ann Arbor, MI); inter- and intra-assay CV were 7.7 and 6.4%, respectively. Once the remaining plasma was removed and the white buffy layer discarded, 2 mL of the red blood cell fraction was transferred into a 30 mL Teflon tube, lysed with 8 mL of ice-cold ultrapure water and centrifuged at 10,000 × g for 15 min at 4ºC. The supernatant (red blood cell lysate; **RBCL**) was removed, aliquoted and stored at -80ºC until analysis of total and manganese superoxide dismutase (**SOD**) activity (#706002, Cayman Chemical). Activity is reported as units (U)/g hemoglobin where one U is defined as the amount of enzyme required to dismutate 50% of the superoxide radical. Inter- and intra-assay CV for total-SOD activity were 10.7 and 9.0%, respectively; inter- and intra-assay CV for Mn-SOD activity were 7.8 and 8.6, respectively. Copper/Zn-SOD activity was calculated by subtracting Mn-SOD from total SOD activity. Hemoglobin was determined using methods described by Hansen et al. (2010).

Liver biopsies were performed as described by Engle and Spears (2000) on all steers on one of two days prior to the start of PRE (d -21 and -20) as well as prior to shipping (d -3) and the end of REC (d 59). Liver samples were snap frozen in liquid nitrogen and transported to the laboratory where they were stored at -80°C. Samples were ground in liquid nitrogen prior to homogenization. Liver for SOD activity (0.15 g tissue; wet basis) was homogenized in 0.75 mL of 20 mM HEPES buffer, centrifuged at 1500 × g for 5 min at 4ºC, and the supernatant was removed, aliquoted, and stored at -80ºC until further analysis (#706002, Cayman Chemical). Liver SOD activity is reported as U/mg protein. Inter- and intra-assay CV for total-SOD activity were 10.2 and 6.5%, respectively; inter- and intra-assay CV for Mn-SOD activity were 11.4 and 9.9%, respectively. Protein concentration of the sample analyzed for SOD activity was determined using a commercially available kit (#23200, Thermo Scientific, Rockford, IL). Liver for total (**tGSH**) and oxidized (**GSSG**) glutathione concentrations (0.15 g tissue; wet basis) was homogenized in 0.75 mL of 50 mM MES buffer and centrifuged at 10,000 × g for 15 min at 4ºC. Samples were then deproteinated by removing 0.5 mL of supernatant, adding 0.5 mL of MPA reagent, vortexing and allowing to sit at room temperature for 5 min prior to centrifugation at 3000 × g for 3 min at 4ºC. The supernatant was removed, aliquoted and stored at -80ºC until further analysis (#703002, Cayman Chemical). Inter- and intra-assay CV for tGSH were 2.8 and 1.1%, respectively; inter- and intra-assay CV for GSSG were 5.1 and 2.1%, respectively. Reduced glutathione (**GSH**) concentrations were calculated by subtracting GSSG from tGSH. Glutathione concentrations are reported as µM/g wet tissue. Remaining liver was dried, prepared and analyzed for Cu, Fe, Mn, and Zn concentrations using inductively coupled optical emission spectroscopy (ICP-OES) as described by Richter et al. (2012).

Statistical Analysis

Data were analyzed as a completely randomized design using the Mixed procedures of SAS 9.4 (SAS Inst., Inc., Cary, NC) with the fixed effect of treatment. Steer was the experimental unit for blood and liver analyses, digestibility, as well as REC performance (n = 12 steers/treatment). One steer from SCFP12 died during REC from illness unrelated to treatment and was therefore removed from the analysis of all data excluding PRE performance means. Orthogonal polynomial (linear and quadratic) contrast statements were constructed to compare the effects of SCFP inclusion throughout the trial (CON, SCFP12 and SCFP18). Contrast coefficients were determined using the IML procedure of SAS based on back calculated SCFP intake (0, 11.4 and 18.1 $g \cdot steer^{-1} \cdot day^{-1}$ for CON, SCFP12 and SCFP18, respectively). An additional contrast statement (SCFP18 vs. CON18) was used to determine the effect of supplementing SCFP throughout the entire trial or just during REC. Dry matter intake from titanium dioxide feeding period (d 14 through 29) was utilized as a covariate in analysis of all nutrient digestibility data. Values from d -20 (prior to treatment initiation) were utilized as covariates in analyses of liver SOD, glutathione and trace mineral data. Plasma MDA and RBCL SOD were analyzed as repeated measures using the repeated effect of day without a covariate in the model. The autoregressive (AR1) covariance structure was used for all repeated measures analyses based on lowest Akaike's information criterion. Data were tested for normality and homogeneity of variance using the Shapiro-Wilks test; RBCL total and Mn-SOD activity were log transformed to meet the assumption of normality and back transformed means and SEM are presented. Outliers were determined using Cook's D statistic and removed if Cook's D > 0.5; one steer from SCFP12 was removed from liver glutathione analyses. Pearson correlations between liver mineral concentrations and liver SOD activity were determined using Proc CORR

of SAS. Data are reported as least square means ± SEM. Significance was declared at $P \leq 0.05$ and tendencies from $0.05 < P \leq 0.10$.

Results and Discussion

Feedlot Performance

The feedlot receiving period is often characterized by poor performance and increased incidence of disease. Preconditioning programs have been shown to improve the subsequent health of calves in the feedlot resulting in improved ADG and feed efficiency (Hilton, 2015). As SCFP have also been shown to positively influence cattle health (Diamond V, 2017a,b), supplementing SCFP during preconditioning programs may have positive implications for subsequent feedlot health and performance. Average DMI, ADG and G:F for the PRE period were 6.1 kg/d, 1.60 kg/d and 0.261 kg/kg, respectively. Regardless of treatment, the 19-h transit event resulted in an average BW shrink of 7.1% (SD = 1.4%).

Receiving period performance data are presented in **Table 2**. Supplementing SCFP throughout the trial did not affect final BW or DMI ($P \geq 0.32$). There was a quadratic effect of SCFP on ADG and G:F from d 0 to 30 ($P \leq 0.04$) driven by greatest performance by SCFP12-fed steers; however, there were no effects of treatment on ADG or G:F from d 30 to 58 or overall (d 0 to 58; $P \geq 0.22$). A retrospective study utilizing data from beef steers and heifers at a large commercial feedlot observed that cattle fed SCFP (NaturSafe, Diamond V) at 1.56 kg/metric ton DM in the starter ration and 1.62 kg/metric ton DM in the finisher ration vs. those that were not fed SCFP but received an antibiotic treatment upon arrival had numerically greater ADG (1.45 vs. 1.32 kg/d) and numerically lesser feed:gain (3.01 vs. 3.08; Diamond V, 2017a). It is possible that the performance benefits noted in the retrospective study were a result of improved performance early in the receiving period, however results are only presented for the entire

feeding period. Other sources of discrepancy between the retrospective analysis and the current study include diet type (steam flaked corn vs. corn silage), dose and duration of supplementation (entire feeding period vs. receiving period only), as well as nutritional and environmental background of the cattle (commingled vs. single-source). More work is needed to determine the influence of diet type and environment on the way cattle respond to SCFP supplementation.

Timing of SCFP supplementation may also be vital to how cattle respond. This study sought to address this question by supplementing SCFP at 18 $g \cdot steer^{-1} \cdot d^{-1}$ during both PRE and REC (SCFP18) or supplementing SCFP at 18 $g \cdot steer^{-1} \cdot d^{-1}$ only during REC (CON18). No effects of supplementation timing were observed for receiving period performance ($P \geq 0.25$). However, improved performance early in the receiving period for cattle supplemented SCFP at 12 $g \cdot steer^{-1} \cdot d^{-1}$ during both PRE and REC suggests that these cattle were better equipped to handle the stress of transit as well as a novel diet and environment. This suggests that timing of supplementation may have influenced receiving period performance if a lower dose had been utilized to address this objective.

Total Tract Nutrient Digestibility

Cattle performance is influenced by diet digestibility and SCFP have been shown to affect gastrointestinal tract microflora. Because SCFP contain no live yeast, the effects of this product on gastrointestinal tract microbial communities are likely due to the unique metabolites, including vitamins, amino acids, organic acids and oligosaccharides, that are produced during the fermentation process. Feye et al. (2016) reported that concentrations of Salmonella and E. coli in the feces of heifers fed SCFP (NaturSafe, Diamond V) were decreased by 74 and 58%, respectively, compared to heifers fed monensin, tylosin and a direct-fed microbial. *Saccharomyces cerevisiae* fermentation products have also shown variable effects on nutrient

digestibility. In the current study, there were no effects of treatment on total tract digestibility of DM, OM or CP ($P \geq 0.19$; **Table 3**); however, SCFP linearly decreased NDF and ADF digestibility ($P \leq 0.03$). This was unexpected because several studies have reported an increase in ruminal cellulolytic bacteria due to SCFP (Wiedmeier et al., 1987; Callaway and Martin, 1997) which would aid in ruminal fiber digestion. In support of this, Shen et al. (2018) observed improved ruminal and total NDF digestibility when SCFP (NaturSafe, Diamond V) was delivered directly to the rumen of cannulated beef heifers fed high-grain diets in a Latin square design with 28 d periods (21 d for adaptation and 7 d for data collection). Although NDF content of the barley grain-based diet fed by Shen et al. (2018) and the corn-silage based receiving diet fed in the current study were similar (29.7 vs. 24.5%), the physical nature of this fiber would be very different and likely contribute to differences in fiber digestibility by rumen microbes. Additionally, total tract digestibility analysis via titanium dioxide may be more susceptible to individual animal variation as opposed to a Latin square design in which each animal is exposed to both control and SCFP treatments.

Antioxidant Measures

Oxidative stress occurs when cellular oxidants exceed antioxidants (Sies, 2007) and can result in damage to cell components including lipids, proteins and nucleic acids. *Saccharomyces cerevisiae* fermentation products manufactured in a similar manner as the SCFP used in the current study (NaturSafe, Diamond V) have been shown to exhibit high ROS scavenging activity (Schauss and Vojdani, 2006), have demonstrated antioxidant properties *in vitro* (Original XP, Diamond V; Jensen et al., 2008) and have increased antioxidant capacity *in vivo* (EpiCor, Embria Health Sciences; Jensen et al., 2011). Additionally, anti-inflammatory properties demonstrated by SCFP (EpiCor, Embria Health Sciences; Jensen et al., 2007) would likely decrease the

production of free radicals by phagocytic immune cells. Therefore, it was expected that SCFP supplementation would positively influence antioxidant status in the current study.

The liver plays a pivotal role in systemic homeostasis of the endogenous antioxidant glutathione by exporting much of the glutathione it synthesizes into the plasma for utilization by other tissues (Lu, 2012). The oxidized to reduced glutathione ratio is an indicator of cellular redox state and a ratio greater than 0.1 is indicative of oxidative stress (Ithayaraja, 2011). Based on this threshold steers in the current study were experiencing some degree of oxidative stress regardless of sampling day or treatment (**Table 4**). At the end of PRE (d -3), there was a tendency for a quadratic effect of SCFP on total, oxidized and reduced glutathione concentrations ($P = 0.06$) driven by greatest concentrations observed in SCFP12-fed steers. Additionally, there was a tendency for a linear decrease in the oxidized to reduced glutathione ratio due to SCFP ($P = 0.07$). Greater concentrations of reduced glutathione, the form in which glutathione can function as an antioxidant, suggests these steers had greater antioxidant capacity prior to the transit event. This greater antioxidant capacity may have contributed to the greater ADG and G:F for SCFP12-fed steers early in the REC period as oxidative damage is energetically expensive and has been associated with decreased production efficiency (Iqbal et al., 2004, 2005). No treatment effects on d 59 glutathione concentrations were observed ($P \geq 0.29$). Deters et al. (2018) measured RBCL glutathione concentrations in newly weaned beef steers receiving SCFP (Original XPC, Diamond V) and observed that steers supplemented SCFP at 14 $g \cdot steer^{-1} \cdot d^{-1}$ had greater concentrations of reduced glutathione vs. steers supplemented SCFP at 0 or 28 $g \cdot steer^{-1} \cdot d^{-1}$. The similar dose response observed in these two studies may be due to a proinflammatory state stimulated by the presence of more cell wall components (β-glucans and mannan-oligosaccharides) in the greater SCFP dose, though inflammation was not

measured in the present study. Inflammation can increase the production of prooxidant species due to an increase in neutrophil oxidative burst (Babior, 1984) and contribute to depletion of antioxidant status.

Although activity of the antioxidant enzyme SOD was not affected by SCFP supplementation in the current study ($P \geq 0.24$), liver Cu/Zn-SOD activity increased throughout the trial (**Table 5**) and liver Cu concentrations followed a similar trend (**Table 6**). Indeed, liver Cu/Zn-SOD activity and liver Cu were positively correlated (r = 0.51, $P \leq 0.01$) while liver concentrations of Zn and Mn were not correlated with their respective liver SOD isoforms ($P \geq 0.22$). Similarly, Russell et al. (2016b) did not observe correlations between RBCL mineral dependent antioxidant enzyme activities and liver mineral concentrations. This could be a result of RBCL and liver differing in both the magnitude and timing of an oxidative stress response or due to animals being adequate in trace minerals. Steers in the current study were adequate in Cu, Fe, Mn and Zn (Kincaid, 2000).

Transit appears to affect oxidative stress biomarkers in various livestock species including cattle, horses and sheep (Chirase et al., 2004; Onmaz et al., 2011; Piccione et al., 2013). Therefore, it was expected that the 19-h transit event in the current study would elicit changes in plasma MDA, a product of lipid peroxidation, and RBCL SOD activity. Regardless of treatment, plasma concentrations of MDA were greatest immediately prior to and 1 d post-transit vs. 8 d post-transit (day $P \leq 0.01$; **Fig. 1**). In contrast, Chirase et al. (2004) observed a 3-fold increase in serum MDA concentrations of crossbred beef steers immediately after an approximately 20-h transit event vs. 3 d prior to transit. Blood was not collected immediately post-transit in the current study to avoid possible effects of decreased blood volume from

dehydration on markers of oxidative stress. Therefore, it is possible that MDA concentrations had already returned to pre-transit values when blood was collected 1 d post-transit.

Although RBCL total and Cu/Zn-SOD activity were decreased 1 d post-transit, Mn-SOD activity was increased (day $P \leq 0.01$; **Fig. 2**), resulting in a greater Mn:total-SOD activity ratio on d 1 (day $P \leq 0.01$; **Fig. 3**). Several studies have reported a decrease in SOD activity due to transportation of livestock (Onmaz et al., 2011; El-Deeb and El-Bahr, 2014; Polycarp et al., 2016), possibly due to increased ROS production and subsequent consumption of the enzyme for antioxidant reactions. However, these studies only reported total SOD activity rather than specific SOD isoforms. Expression of Mn-SOD mRNA has been shown to be induced by adrenocorticotropic hormone (Chinn et al., 2002) which is secreted in response to a perceived psychological stressor and stimulates the release of cortisol, a potent glucocorticoid that acts on various tissues to increase cellular metabolism (Brockman and Laarveld, 1986). Although cortisol was not measured in the current study, it has been well established that transit increases concentrations of circulating cortisol in cattle (Crookshank et al., 1979; Marques et al., 2012; Cooke et al., 2013). It is possible that increased secretion of adrenocorticotropic hormone in response to transit stress resulted in upregulation of the Mn-SOD gene and contributed to the greater RBCL Mn-SOD activity observed 1 d post-transit, as a strong relationship exists between Mn-SOD gene expression, protein expression and enzyme activity (Tiedge et al., 1997).

Implications

In summary, supplementing SCFP at 12 $g \cdot steer^{-1} \cdot d^{-1}$ was optimal compared to SCFP at 0 or 18 $g \cdot steer^{-1} \cdot d^{-1}$ as evidenced by a tendency for greater antioxidant capacity prior to transit and improved performance early in the receiving period (d 0 to 30). Although no effects of supplementation timing were observed utilizing the dose of 18 $g \cdot steer^{-1} \cdot d^{-1}$, given the positive

effects noted in steers receiving 12 g·steer^{-1}·d^{-1} throughout PRE and REC, further research should evaluate supplementation prior to entering the feedlot utilizing lesser doses. Additionally, activity of the powerful antioxidant enzyme Mn-SOD was increased post-transit and due to the detrimental effects of oxidative stress on animal health and performance, further investigation of the oxidative stress response in beef cattle post-transit is warranted.

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Table 1. Composition of diets fed during preconditioning (PRE) and receiving (REC)

	PRE[1]	REC[2]
DM, %	56	61
Ingredient, % DM basis		
Corn silage	50	40
Dry-rolled corn	20	30
Dried distillers grains[3]	28.15	28.15
Limestone	1.4	1.4
Salt	0.31	0.31
Rumensin[4]	0.0135	0.0135
Vitamin A and E premix[5]	0.1	0.1
Trace mineral premix[6]	0.024	0.024
Analyzed composition[7], % DM		
Crude protein	13.2	13.7
Neutral detergent fiber	27.9	24.5
Ether extract	5.1	5.1
Analyzed composition[8], mg/kg DM		
Cu	15.8	13.8
Fe	69	69
Mn	38	35
Zn	68	62

[1]d -19 to -1
[2]d 0 to 58
[3]Carrier for micro-ingredients and *Saccharomyces cerevisiae* fermentation product (NaturSafe, Diamond V, Cedar Rapids, IA)
[4]Provided 200 mg monensin·steer^{-1}·d^{-1} (Rumensin, Elanco Animal Health, Greenfield, IN)
[5]Premix provided 2,200 IU vitamin A and 25 IU vitamin E/kg diet DM
[6]Provided per kilogram of diet DM: 10 mg of Cu, 30 mg of Zn, 20 mg of Mn, 0.5 mg of I, 0.1 mg of Se, and 0.1 mg of Co all from inorganic sources
[7]Based on analysis of TMR from Dairyland Laboratories, Inc., Arcadia, WI
[8]Analyzed mineral values reflect control diet total, which includes supplemental mineral

Table 2. Effect of *Saccharomyces cerevisiae* fermentation product on performance of beef steers during a 58-d receiving period

	Treatment[1]					Contrast *P*-value[2]		
	CON *n* = 12 steers	SCFP12 *n* = 11 steers[3]	SCFP18 *n* = 12 steers	CON18 *n* = 12 steers	SEM[4]	Linear	Quadratic	Timing
SCFP intake[5]	0.0	11.4	18.1	18.9	-	-	-	-
Initial BW[6], kg	289	287	284	290	5.7	0.49	0.85	0.42
Final BW, kg	382	386	375	377	6.9	0.58	0.32	0.83
DMI, kg/d								
d 0 to 30	6.7	7.0	6.9	6.9	0.25	0.55	0.51	0.79
d 30 to 58	8.3	8.3	8.0	8.3	0.28	0.41	0.63	0.38
d 0 to 58	7.5	7.6	7.4	7.6	0.24	0.87	0.54	0.53
ADG, kg/d								
d 0 to 30	1.37	1.65	1.36	1.32	0.099	0.77	0.02	0.74
d 30 to 58	1.84	1.77	1.81	1.71	0.109	0.81	0.72	0.49
d 0 to 58	1.60	1.71	1.58	1.51	0.081	0.98	0.24	0.53
G:F								
d 0 to 30	0.206	0.237	0.198	0.189	0.014	0.90	0.04	0.65
d 30 to 58	0.222	0.218	0.224	0.205	0.012	0.94	0.70	0.25
d 0 to 58	0.213	0.226	0.211	0.197	0.009	0.97	0.22	0.25

[1]*Saccharomyces cerevisiae* fermentation product (SCFP; NaturSafe, Diamond V, Cedar Rapids, IA) at 0 g·steer^{-1}·day^{-1} (CON), 12 g·steer^{-1}·day^{-1} (SCFP12), 18 g·steer^{-1}·day^{-1} (SCFP18), or 0 g·steer^{-1}·day^{-1} during preconditioning (d -19 to -1) then 18 g·steer^{-1}·day^{-1} during receiving (d 0 to 58; CON18)

[2]Linear and quadratic contrast statements compare CON, SCFP12 and SCFP18; Timing contrast statement compares SCFP18 vs. CON18

[3]One steer from NS12 died during the course of the study from illness unrelated to treatment

[4]Highest SEM of any treatment reported

[5]Back calculated SCFP intake; g·steer^{-1}·day^{-1}

[6]Initial BW = shrunk BW after 19-hr transit event

Table 3. Effect of *Saccharomyces cerevisiae* fermentation product on total tract nutrient digestibility by beef steers on d 29 and 30

	Treatment[1]					Contrast *P*-value[2]		
	CON	SCFP12	SCFP18	CON18	SEM[3]	Linear	Quadratic	Timing
DMI[4], kg	7.7	7.9	7.8	7.9	0.28	0.91	0.65	0.72
Nutrient, %								
DM	70.2	69.4	71.2	71.5	1.17	0.61	0.33	0.85
OM	71.3	70.7	72.6	72.9	1.23	0.53	0.35	0.85
NDF	57.6	52.8	53.5	54.9	1.56	0.03	0.24	0.49
ADF	55.3	49.2	50.3	53.1	1.85	0.02	0.19	0.24
CP	68.8	69.2	69.4	67.9	0.91	0.58	0.99	0.19

[1]*Saccharomyces cerevisiae* fermentation product (SCFP; NaturSafe, Diamond V, Cedar Rapids, IA) at 0 g·steer^{-1}·day^{-1} (CON), 12 g·steer^{-1}·day^{-1} (SCFP12), 18 g·steer^{-1}·day^{-1} (SCFP18), or 0 g·steer^{-1}·day^{-1} during preconditioning (d -19 to -1) then 18 g·steer^{-1}·day^{-1} during receiving (d 0 to 58; CON18)

[2]Linear and quadratic contrast statements compare CON, SCFP12 and SCFP18; Timing contrast statement compares SCFP18 vs. CON18

[3]Highest SEM of any treatment reported

[4]Dry matter intake (DMI) during titanium dioxide feeding period (d 14 through 29) was utilized as a covariate in analysis of all nutrients

Table 4. Effect of *Saccharomyces cerevisiae* fermentation product on liver glutathione concentrations of beef steers

	Treatment[1]					Contrast *P*-value[2]		
	CON	SCFP12	SCFP18	CON18	SEM[3]	Linear	Quadratic	Timing
Liver glutathione[4], µM								
d -20[5]								
Total	1.54	1.91	1.69	1.86	-	-	-	-
Oxidized	0.17	0.22	0.19	0.21	-	-	-	-
Reduced	1.37	1.67	1.52	1.65	-	-	-	-
Ratio[6]	0.114	0.130	0.122	0.124	-	-	-	-
d -3								
Total	2.14	2.26	1.96	1.97	0.103	0.27	0.06	0.92
Oxidized	0.27	0.28	0.23	0.24	0.017	0.11	0.06	0.54
Reduced	1.87	1.99	1.73	1.73	0.090	0.34	0.06	0.94
Ratio	0.143	0.139	0.131	0.138	0.006	0.07	0.65	0.29
d 59								
Total	2.14	1.93	2.02	2.08	0.101	0.29	0.31	0.63
Oxidized	0.25	0.22	0.23	0.24	0.015	0.35	0.31	0.76
Reduced	1.89	1.72	1.78	1.84	0.088	0.28	0.34	0.59
Ratio	0.132	0.128	0.131	0.129	0.004	0.77	0.55	0.80

[1]*Saccharomyces cerevisiae* fermentation product (SCFP; NaturSafe, Diamond V, Cedar Rapids, IA) at 0 g·steer^{-1}·day^{-1} (CON), 12 g·steer^{-1}·day^{-1} (SCFP12), 18 g·steer^{-1}·day^{-1} (SCFP18), or 0 g·steer^{-1}·day^{-1} during preconditioning (d -19 to -1) then 18 g·steer^{-1}·day^{-1} during receiving (d 0 to 58; CON18)

[2]Linear and quadratic contrast statements compare CON, SCFP12 and SCFP18; Timing contrast statement compares SCFP18 vs. CON18

[3]Highest SEM of any treatment reported

[4]Gluathione concentrations reported as µM per gram of wet tissue

[5]Values from d -20 (prior to treatment initiation) utilized as a covariate in analysis

[6]Ratio calculated by dividing oxidized by reduced glutathione concentrations

Table 5. Effect of *Saccharomyces cerevisiae* fermentation product on liver superoxide dismutase (SOD) activity of beef steers

	Treatment[1]					Contrast *P*-value[2]		
	CON	SCFP12	SCFP18	CON18	SEM[3]	Linear	Quadratic	Timing
Liver SOD activity[4]								
d -20[5]								
Total	215	212	221	180	-	-	-	-
Mn	121	123	137	108	-	-	-	-
Cu/Zn	94	89	85	72	-	-	-	-
Ratio[6]	0.56	0.60	0.62	0.62	-	-	-	-
d -3								
Total	289	292	287	311	19.6	0.98	0.87	0.41
Mn	152	168	153	163	10.4	0.80	0.24	0.48
Cu/Zn	142	127	139	135	19.5	0.85	0.58	0.88
Ratio	0.52	0.58	0.55	0.56	0.043	0.51	0.48	0.86
d 59								
Total	336	360	350	303	30.4	0.64	0.67	0.26
Mn	137	146	143	127	12.8	0.68	0.71	0.38
Cu/Zn	202	214	206	173	28.6	0.87	0.78	0.40
Ratio	0.43	0.41	0.43	0.43	0.046	0.92	0.76	0.90

[1]*Saccharomyces cerevisiae* fermentation product (SCFP; NaturSafe, Diamond V, Cedar Rapids, IA) at 0 g·steer^{-1}·day^{-1} (CON), 12 g·steer^{-1}·day^{-1} (SCFP12), 18 g·steer^{-1}·day^{-1} (SCFP18), or 0 g·steer^{-1}·day^{-1} during preconditioning (d -19 to -1) then 18 g·steer^{-1}·day^{-1} during receiving (d 0 to 58; CON18)

[2]Linear and quadratic contrast statements compare CON, SCFP12 and SCFP18; Timing contrast statement compares SCFP18 vs. CON18

[3]Highest SEM of any treatment reported

[4]SOD activity is reported as units (U)/mg protein where one U is defined as the amount of enzyme required to dismutate 50% of the superoxide radical.

[5]Values from d -20 (prior to treatment initiation) utilized as a covariate in analysis

[6]Ratio calculated by dividing Mn-SOD activity by total SOD activity

Table 6. Effect of *Saccharomyces cerevisiae* fermentation product on liver mineral concentrations of beef steers

	Treatment[1]					Contrast *P*-value[2]		
	CON	SCFP12	SCFP18	CON18	SEM[3]	Linear	Quadratic	Timing
Liver mineral, mg/kg DM								
d -20[4]								
Cu	119	142	171	143	-	-	-	-
Fe	172	157	171	165	-	-	-	-
Mn	8.5	8.0	8.6	8.6	-	-	-	-
Zn	120	124	117	110	-	-	-	-
d -3								
Cu	203	219	202	215	11.0	0.92	0.20	0.34
Fe	163	170	171	167	7.5	0.30	0.85	0.61
Mn	9.0	7.9	8.5	8.2	0.47	0.19	0.14	0.50
Zn	137	130	138	145	9.3	0.99	0.48	0.48
d 59								
Cu	332	351	327	303	20.9	0.98	0.38	0.42
Fe	157	158	162	154	7.1	0.58	0.78	0.41
Mn	10.5	9.5	10.0	9.7	0.48	0.25	0.23	0.67
Zn	124	146	139	123	11.9	0.22	0.32	0.31

[1]*Saccharomyces cerevisiae* fermentation product (SCFP; NaturSafe, Diamond V, Cedar Rapids, IA) at 0 g·steer^{-1}·day^{-1} (CON), 12 g·steer^{-1}·day^{-1} (SCFP12), 18 g·steer^{-1}·day^{-1} (SCFP18), or 0 g·steer^{-1}·day^{-1} during preconditioning (d -19 to -1) then 18 g·steer^{-1}·day^{-1} during receiving (d 0 to 58; CON18)

[2]Linear and quadratic contrast statements compare CON, SCFP12 and SCFP18; Timing contrast statement compares SCFP18 vs. CON18

[3]Highest SEM of any treatment reported

[4]Values from d -20 (prior to treatment initiation) utilized as a covariate in analysis

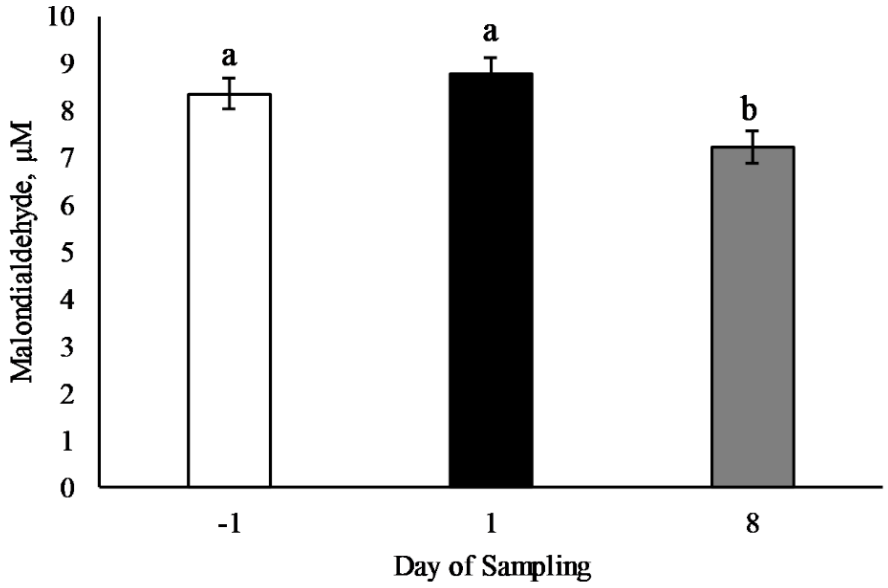

Figure 1. Effect of day on plasma malondialdehyde concentrations of beef steers in relation to a 19-h transit event; initial blood samples were collected immediately prior to transit on d -1. Based on repeated measures analysis, bars with unlike superscripts indicate a difference ($P \leq 0.05$) between days (day $P < 0.01$; treatment × day $P \geq 0.94$).

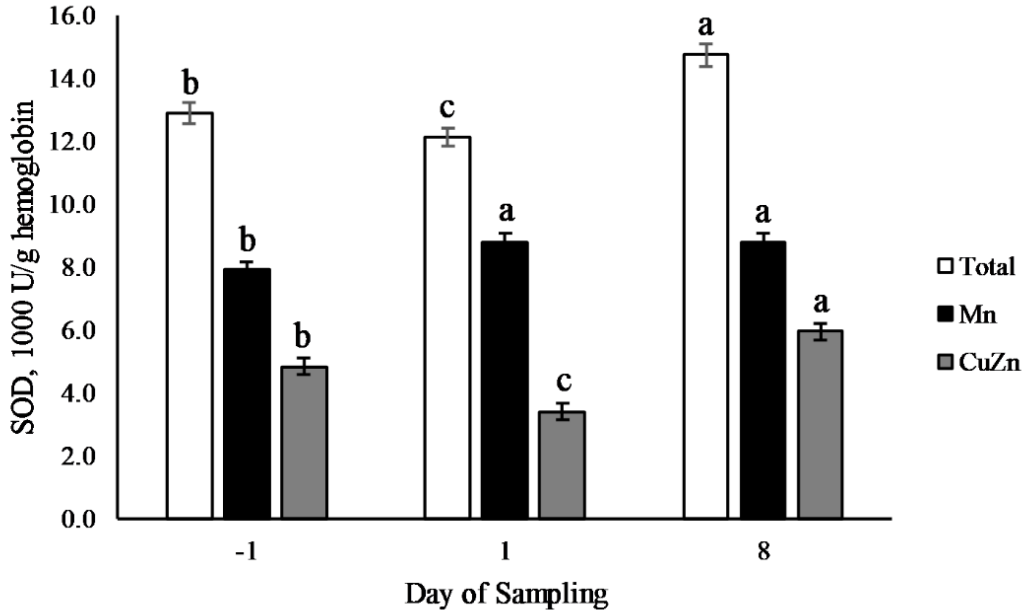

Figure 2. Effect of day on red blood cell lysate total, Mn, and Cu/Zn-superoxide dismutase (SOD) activity of beef steers in relation to a 19-h transit event; initial blood samples were collected immediately prior to transit on d -1. One unit of SOD activity (U) is defined as the enzyme required to dismutate 50% of the superoxide radical. Activity is reported as 1,000 U/g hemoglobin. Values for total and Mn-SOD were log transformed prior to statistical analysis; back transformed means and SEM are presented. Within SOD type, bars with unlike superscripts indicate a difference ($P \leq 0.05$) between days (day $P < 0.01$; treatment × day $P \geq 0.51$).

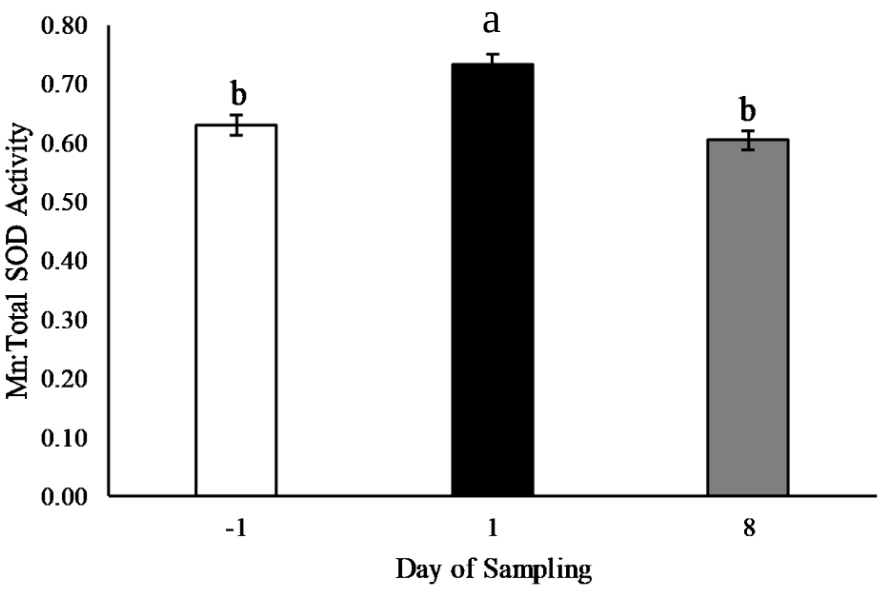

Figure 3. Effect of day on red blood cell lysate ratio of Mn to total superoxide dismutase (SOD) activity of beef steers in relation to a 19-h transit event; initial blood samples were collected immediately prior to transit on d -1. Based on repeated measures analysis, bars with unlike superscripts indicate a difference ($P \leq 0.05$) between days (day $P < 0.01$; treatment × day $P = 0.71$).

CHAPTER 5. PRE-TRANSIT VITAMIN C INJECTION IMPROVES POST-TRANSIT PERFORMANCE OF BEEF STEERS

Modified from a manuscript accepted for publication in *Animal*

E. L. Deters and S. L. Hansen

Abstract

This study investigated the effects of a vitamin C (**VC**) injection delivered before or after a long-distance transit event on blood parameters and feedlot performance of beef steers. Fifty-two days prior to trial initiation, 90 newly weaned, Angus-based steers from a single source were transported to Ames, IA. On day 0, 72 steers (356 ± 17 kg) were blocked by BW and randomly assigned to intramuscular injection treatments (24 steers/treatment): saline injection pre- and post-transit (**CON**), VC (Vet One, Boise, ID; 5 g sodium ascorbate/steer) injection pre-transit and saline injection post-transit (**PRE**) or saline injection pre-transit and VC injection post-transit (**POST**). Following pre-transit treatment injections, steers were transported on a commercial livestock trailer for approximately 18 h (1,675 km). Post-transit (day 1), steers were sorted into pens with one GrowSafe bunk/pen (4 pens/treatment; 6 steers/pen). Steers were weighed on day 0, 1, 7, 30, 31, 56 and 57. Blood was collected from 3 steers/pen on day 0, 1, 2 and 7; liver biopsies were performed on the same 3 steers/pen on day 2. Data were analyzed as a randomized complete block design (experimental unit = steer; fixed effects = treatment and block) and blood parameters were analyzed as repeated measures. A pre-transit VC injection improved steer average daily gain from day 7 to 31 ($P = 0.05$) and overall (day 1 to 57; $P = 0.02$) resulting in greater BW for PRE-steers on day 30/31 ($P = 0.03$) and a tendency for greater final BW (day 56/57; $P = 0.07$). Steers that received VC pre- or post-transit had greater DM intake from day 31 to 57 ($P = 0.01$) and overall ($P = 0.02$) vs. CON-steers. Plasma ascorbate

concentrations were greatest for PRE-steers on day 1 and POST-steers on day 2 (treatment × day; $P < 0.01$). Liver ascorbate concentrations on day 2 followed a similar trend where concentrations were numerically greatest for POST-steers ($P = 0.11$). No interaction or treatment effects were observed for other blood parameters ($P \geq 0.21$). Plasma ferric reducing antioxidant potential and malondialdehyde concentrations were decreased post-transit (day; $P < 0.01$) while serum non-esterified fatty acids and haptoglobin concentrations were increased post-transit (day; $P < 0.01$). In general, blood parameters returned to pre-transit values by day 7. Based on differential responses observed for pre- vs. post-transit VC injection, timing of administration is important for optimal effectiveness.

Introduction

Due to the segmented nature of the U.S. beef industry, cattle will often be transported multiple times between birth and harvest. Transportation involves numerous stressors including feed and water deprivation, physical exertion and psychological stress that predispose cattle to poor health and performance upon arrival at the feedlot (Van Engen and Coetzee, 2018). Transit has been shown to decrease antioxidant status as well as increase biomarkers of oxidative stress and inflammation in cattle (Deters and Hansen, 2020; Marques et al., 2012). Oxidative damage and inflammation are energetically expensive processes that have been associated with increased feedlot morbidity and decreased feedlot performance (Chirase et al., 2004; Cooke, 2017). Ascorbate (vitamin C; **VC**) is the major water-soluble antioxidant present in plasma and tissues (Combs, 2008). Cattle are capable of synthesizing VC from glucose in the liver and thus VC is not considered an essential dietary nutrient. Nevertheless, tissue and circulating concentrations of this nutrient have been shown to decrease in response to stress (Nakano and Suzuki, 1984; Padilla et al., 2006). This suggests cattle may be unable to produce enough VC to meet their

requirement during periods of stress, such as long-distance transit events. Injectable VC is a relatively easy and cost-effective method to rapidly increase VC status. However, due to its water-soluble nature, excess VC is quickly excreted, suggesting timing of VC administration is vital for its effectiveness. This research sought to determine the effects of injectable VC before or after an 18 h transit event on blood parameters and feedlot performance of beef steers. It was hypothesized that an injection of VC prior to transit would mitigate the decline in antioxidant status and dampen the inflammatory response, subsequently improving feedlot performance over steers receiving no VC, while an injection of VC post-transit would simply replete antioxidant stores that were lost due to transit.

Materials and Methods

Animals and Experimental Design

Ninety bawling, Angus-based steer calves (265 ± 14 kg) from a single-source were purchased and transported (~7 h/645 km) to the Iowa State Beef Nutrition Farm (Ames, IA) in October, 2018. Three days after arrival (day -49), steers received visual and electronic identification tags. Additionally, steers were vaccinated against clostridial (Vision 7, Merck Animal Health, Madison, NJ) and respiratory (Vista Once SQ, Merck Animal Health) diseases and injected with doramectin (Dectomax, Zoetis, Parsippany, NJ) for control of internal and external parasites. Steers were fed a common corn silage-based diet and housed in open dirt lots until day -17 when 80 steers were moved to partially covered concrete pens equipped with GrowSafe bunks (GrowSafe Systems Ltd., Airdrie, Alberta, Canada) for a pre-trial acclimation period. On day 0, the 72 steers most uniform in weight (356 ± 17 kg), disposition and health status were blocked by BW and randomly assigned to 1 of 3 treatments (24 steers/treatment): saline injection pre- and post-transit (**CON**), VC (Vet One, Boise, ID; 250 mg sodium

ascorbate/mL; 5 g/steer; 14.1 ± 0.7 mg/kg BW) injection pre-transit and saline injection post-transit (**PRE**) or saline injection pre-transit and VC injection post-transit (**POST**). Saline and VC treatments were delivered intramuscularly at a dose rate of 20 mL/steer (10 mL/injection site on opposite sides of the neck). Following pre-transit treatment injections (~1430 h) steers were loaded onto a commercial livestock trailer (Silverstar PSDCL-402; Wilson Trailer Company, Sioux City, IA) and transported for approximately 18 h (1,675 km), during which time steers did not have access to feed or water. The average loading density was 1.35 m^2/steer which is in accordance with the minimum area allowances outlined in the Guide for the Care and Use of Agricultural Animals in Research and Teaching (Federation of Animal Science Societies, 2010). Steers were stratified by treatment to truck compartments to account for compartment variability.

On day 1, steers returned to the Iowa State University Beef Nutrition Farm (~0830 h), immediately received post-transit treatment injections and were sorted into pens equipped with one GrowSafe bunk/pen (4 pens/treatment; 6 steers/pen). From day 1 to 57, all steers were fed a common total mixed ration (**TMR**; **Table 1**) that was delivered once daily at approximately 0800 h and allowed *ad libitum* access to feed and water. Steers were weighed on day 0 (pre-transit) and 1 (post-transit) as well as prior to feeding on day 7, 30, 31, 56 and 57; based on average BW shrink due to transit, a 6% pencil shrink was applied to all other BW. Steer dry matter intake (**DMI**), average daily gain (**ADG**) and feed efficiency (gain:feed; **G:F**) were calculated from day 1 to 7, 7 to 31, 31 to 57 and 1 to 57. On day 7, steers were implanted with 80 mg trenbolone acetate, 16 mg estradiol USP and 29 mg tylosin tartrate (Component TE-IS, Elanco Animal Health, Indianapolis, IN). Morbidity was assessed daily throughout the study and steers were treated (1^{st} treatment = florfenicol and flunixin meglumine, Resflor Gold, Merck Animal Health;

2nd treatment = tulathromycin, Draxxin, Zoetis) by farm personnel if cattle displayed visual symptoms (nasal discharge, cloudy eyes, drooping heads, etc.) and rectal temperature ≥ 40ºC.

Sample Collection and Analytical Procedures

Weekly TMR samples were collected for DM determination and subsamples dried in a forced air oven at 70°C for 48 h. These DM values were used to calculate daily steer DMI based on individual as-fed intakes recorded by the GrowSafe system. Dried TMR samples were ground to pass through a 2 mm screen in a Retsch ZM 100 grinding mill (Retsch GmbH, Haan, Germany) and composited for analysis of nitrogen (CP; Association of Official Analytical Chemists [AOAC], 1999a; method 990.03), NDF (AOAC, 2005; method 2002.04) and ether extract (AOAC, 1999b; method 920.39) by a commercial laboratory (Dairyland Laboratories, Inc., Arcadia, WI); analyzed compositions are presented in **Table 1**.

Three steers per pen (12 steers/treatment) were selected as sampling animals for blood and liver collection; the same steers were sampled for all time points. Blood was collected via jugular venipuncture into vacuum tubes (serum, #366430; sodium heparin, #367874, Becton Dickinson, Franklin Lakes, NJ) on day 0 (prior to pre-transit treatment injections), 1 (prior to post-transit treatment injections), 2 and 7. Serum samples were allowed to clot at room temperature for 90 min prior to centrifugation at 1,500 × g for 20 min at 4ºC; serum was aliquoted into microcentrifuge tubes and stored at -80ºC for future analysis of haptoglobin (**HP**) or -20ºC for non-esterified fatty acids (**NEFA**). Serum HP concentrations were analyzed using a bovine specific ELISA kit (Hapt-11, Life Diagnostics, Inc., West Chester, PA; intra-assay CV = 4.8%, inter-assay CV = 12.4%) and NEFA concentrations were analyzed using a commercially available colorimetric kit (Wako Diagnostics, Mountain View, CA; intra-assay CV = 5.4%, inter-assay CV = 6.0%). Sodium heparin tubes were centrifuged at 1,000 × g for 20 min at 4ºC;

plasma was then removed, aliquoted into microcentrifuge tubes and stored at -80ºC for future analysis of ascorbate, ferric reducing antioxidant potential (**FRAP**) and malondialdehyde (**MDA**). To prevent degradation of ascorbate, plasma for ascorbate analysis was stabilized with diethylenetriaminepentaacetic acid prior to freezing and all samples were assayed for ascorbate within 45 days of sample collection. Commercially available kits were used for analysis of plasma ascorbate (#700420, Cayman Chemical, Ann Arbor, MI; intra-assay CV = 3.5%, inter-assay CV = 9.0%), FRAP (#K043-H1, Arbor Assays, Ann Arbor, MI; intra-assay CV = 1.4%, inter-assay CV = 10.6%) and MDA (#700870, Cayman Chemical; intra-assay CV = 10.3%, inter-assay CV = 12.0%) concentrations.

Liver biopsies were performed on day 2 on the 36 steers selected as sampling animals using a method previously described by Engle and Spears (2000). Liver samples were snap frozen in liquid nitrogen and transported to the laboratory on dry ice, then stored at -80°C. Samples were ground in liquid nitrogen, 50 mg (wet weight) of ground liver was weighed (in duplicate) into microcentrifuge tubes for ascorbate analysis via gas chromatography-mass spectrometry (GC-MS; Agilent Technologies Model 6890 GC coupled to Model 5975 MS) at the W.M. Keck Metabolomics Research Laboratory (Ames, IA). Briefly, internal standards (ribitol and nonadecanoic acid) and cold extraction solvent (8:2 methanol:water) were added to microcentrifuge tubes prior to homogenization on a bead mill, followed by sonication for 5 min and centrifugation for 7 min at 13,000 × g. The supernatant was put into an amber GC-MS vial and dried on a speed vacuum overnight prior to derivatization via sialylation for 30 min at 60ºC. Samples were protected from light as much as possible throughout preparation and analysis. Sample separation was obtained on an Agilent-HP5MSI (30 m long, 0.250 mm ID, 0.25 μm film thickness) column. The oven program was as follows: initial temperature of 70ºC for 1 min

followed by, 25ºC/min ramp to 150ºC and 15ºC/min ramp to 220ºC followed by 25ºC/min ramp to 320ºC with a final hold for 6 min. The inlet and the interface temperatures were maintained at 280ºC. Detection mass range was set from 40–800 m/z and the GC-MS was controlled by the Agilent ChemStation software. Ascorbate was identified using total ion mass spectrum and comparison to the National Institute of Standards and Technology (NIST) mass spectral library (NIST, 2014). Ascorbate was quantified by comparing the ascorbate response observed in the samples to an ascorbate standard line generated using the same method as described previously. Liver samples were prepared by block and analyzed on two different GC-MS runs within one week.

Statistical Analysis

Cattle growth performance and liver ascorbate concentrations were analyzed as a randomized complete block design using the Mixed Procedure of SAS 9.4 (SAS Institute Inc., Cary, NC) with steer as the experimental unit and the fixed effects of treatment and block. One steer from PRE was removed from all performance analyses due to possible electronic identification failure and subsequent invalidity of DMI data. Day 0 BW was utilized as a covariate in analysis of mid and final BW and average steer DMI from day -7 through -1 was utilized as a covariate in analysis of subsequent DMI data. Blood measures from day 0, 1, 2 and 7 were analyzed as repeated measures with the fixed effects of treatment and block and the repeated effect of day. The treatment by day interaction term was removed from the model if $P > 0.30$. The compound symmetry covariance structure was utilized for analysis of FRAP and plasma ascorbate data while the heterogeneous autoregressive covariance structure was utilized for analysis of MDA, NEFA and haptoglobin data based on lowest Akaike's information criterion (Littell et al., 1998). Day 0 NEFA concentrations were used as a covariate in analysis of

NEFA concentrations on subsequent sampling days. Data were tested for normality using the Shapiro-Wilk's test; serum NEFA and haptoglobin concentrations were natural log transformed to meet the assumption of normality and back transformed means and SEM are presented. Data were tested for outliers using Cook's D statistic and removed if Cook's D ≥ 0.50; data from one steer was removed from analysis of FRAP on all sampling dates. Significance was declared at P ≤ 0.05 and tendencies from 0.05 < P ≤ 0.10.

Results

Feedlot Performance

Pre-transit (day 0) and post-transit (day 1) BW as well as percent BW shrink after the 18 h transit event were not affected by injectable VC treatment (**Table 2**; $P \geq 0.28$). Steers that received injectable VC pre-transit had the greatest BW at the midpoint of the trial (day 30/31; $P = 0.03$) and tended to have the greatest final BW (day 56/57; $P = 0.07$). Steer ADG was not affected by treatment from day 1 to 7 or 31 to 57 ($P = 0.25$). However, PRE-steers exhibited the greatest ADG from day 7 to 31 ($P = 0.05$), resulting in greater ADG by PRE-steers overall (day 1 to 57; $P = 0.02$). Steer DMI was not affected by injectable VC from day 1 to 7 ($P = 0.13$). However, there was a tendency for greater DMI by PRE or POST-steers compared to CON-steers from day 7 to 31 ($P = 0.08$). From day 31 to 57 PRE or POST-steers exhibited greater DMI compared to CON-steers ($P = 0.01$) resulting in greater DMI by these steers overall ($P = 0.02$). Injectable VC did not affect G:F throughout the trial ($P \geq 0.18$).

Liver and Blood Metabolites

Treatment means for liver and blood metabolites are presented in **Table 3**. Liver ascorbate concentrations on day 2 were numerically greater for POST-steers compared to CON-

steers ($P = 0.11$). Based on repeated measures analysis of blood samples collected on day 0, 1, 2 and 7, there were no overall treatment effects for the blood metabolites measured ($P \geq 0.21$). However, a treatment × day effect was observed for plasma ascorbate ($P < 0.01$) and day effects were observed for the remaining metabolites ($P < 0.01$). Pre-transit (day 0) plasma ascorbate concentrations were similar among treatments, but steers that received injectable VC pre-transit had the greatest plasma ascorbate concentrations post-transit (day 1) while steers that received injectable VC post-transit had the greatest concentrations on day 2 (**Figure 1**). Plasma ascorbate concentrations were similar to pre-transit values on day 7 for all treatments. Plasma FRAP concentrations were decreased post-transit (day 1) before returning to pre-transit values on day 2 and decreasing again on day 7 (**Figure 2A**). Plasma MDA concentrations were decreased on day 1 and 2 before returning to pre-transit values on day 7 (**Figure 2B**). Alternatively, serum NEFA concentrations increased sharply on day 1 before returning to pre-transit values by day 2 (**Figure 2C**). Serum HP concentrations were greatest on day 2, intermediate on day 1 and similar to pre-transit values by day 7 (**Figure 2D**).

Discussion

Transit involves psychological stress, food and water deprivation as well as muscle fatigue from standing for long periods of time. These physiological responses can stimulate inflammation (Marques *et al.*, 2012) and increase the production of reactive oxygen species (Piccione *et al.*, 2012) which may lead to oxidative stress if cellular antioxidants are overwhelmed. Inflammation and oxidative stress are nutritionally demanding processes due to the need for amino acids to synthesize acute phase proteins and antioxidants as well as ATP to repair or degrade oxidatively damaged molecules (Grune *et al.*, 1997; Griffith, 1999). Therefore, this study sought to investigate proactive (pre-transit) vs. reactive (post-transit) administration of

a powerful antioxidant, VC. It was hypothesized that a pre-transit VC injection would dampen transit-induced inflammation and oxidative stress and thus spare energy and protein for growth.

Although some species require exogenous supplementation of VC, cattle possess a functional form of l-gulonolactone oxidase which allows for the endogenous synthesis of VC from glucose in the liver (Bánhegyi et al, 1997). However, management practices that impose stress on cattle may increase the requirement and exceed biosynthetic capacity of VC to combat oxidative stress. Prior to transit, plasma ascorbate concentrations of steers in the current study were 18.3 ± 3.7 μM/L, which falls within the reference range (17.1 to 28.2 μM/L) for beef cattle proposed by Matsui (2012). Transit decreased plasma ascorbate concentrations by 10% in steers that did not receive a VC injection pre-transit (CON and POST; 16.3 ± 3.3 μM/L). Other production stressors have also been shown to decrease VC status in cattle. Padilla *et al.* (2006) observed a 51% decrease in plasma ascorbate concentrations when lactating Holstein cows were exposed to heat stress conditions (24ºC on the first day, 26ºC on the second day, and 28ºC for the next 12 days; 8.7 μM ascorbate/L) vs. control conditions (18ºC for 14 days; 17.7 μM ascorbate/L). Cummins and Brunner (1991) reported 17% lesser plasma ascorbate concentrations in male Holstein calves housed in metal pens versus calves housed in commercial calf hutches (24.4 vs. 29.5 μM/L); metal pens are considered to be a more stressful form of housing based on increases in plasma cortisol concentrations. Cortisol concentrations were not measured in the current study so it is unclear if there were treatment differences in this stress hormone; however, post-transit increases in cortisol concentrations of cattle have been well documented (Crookshank *et al.*, 1979; Kent and Ewbank, 1983; Marques *et al.*, 2012). Stress associated decreases in plasma VC may be a result of increased uptake by the adrenal glands to support catecholamine biosynthesis (Kipp and Rivers, 1987) or by other tissues where there is an

increased metabolic demand for VC. In contrast to CON and POST-steers, PRE-steers had 13% greater plasma ascorbate concentrations immediately post-transit relative to pre-transit concentrations. On day 2, approximately 24 h after post-transit treatment injections, POST-steers exhibited a 56% increase in plasma ascorbate concentrations relative to concentrations measured immediately after unloading. Additionally, plasma ascorbate concentrations of CON-steers increased by 21% on day 2 relative to post-transit concentrations, suggesting endogenous synthesis of VC may have been upregulated as these steers were not given exogenous VC. Liver and plasma ascorbate concentrations on day 2 followed a similar trend with the greatest concentrations observed for POST-steers and the least concentrations observed for CON-steers, with PRE-steers being intermediate.

Although plasma ascorbate concentrations returned to pre-transit values and were not different amongst treatments by day 7, effects of injectable VC on feedlot performance were observed later in the post-transit period. Steers that received VC pre-transit were 6 kg heavier than POST-steers and 7 kg heavier than CON-steers at the end of the 56-d post-transit period. Toutain *et al.* (1997) found that skeletal muscle accounted for approximately 40% of total body ascorbate in dairy calves. Vitamin C aids muscle function in several ways: acting as a cofactor for metalloenzymes involved in collagen and carnitine biosynthesis as well as providing antioxidant protection. Collagen supports muscle growth by facilitating the migration, proliferation and differentiation of myoblasts (i.e. an undifferentiated cell that can give rise to a muscle cell). Collagen undergoes rapid turnover in young animals and slows as animals age or growth rate declines (Bailey, 1985). Not surprisingly and regardless of treatment, steer ADG was greatest during the period immediately following administration of an anabolic implant (day 7 to 31; 1.99 kg/day) versus later in the trial (day 31 to 57; 1.56 kg/day). However, the ADG

advantage for steers that received a pre-transit VC injection was only observed from day 7 to 31, suggesting that supplemental VC may be necessary to support collagen turnover in rapidly growing animals. It is also interesting to note that despite steers who received VC at any timepoint having greater DMI than CON-steers, only steers that received VC pre-transit exhibited a growth response. This could indicate that PRE-steers were able to utilize dietary nutrients for growth while POST-steers may have utilized those nutrients to replenish protein and energy stores depleted during the transportation event. Another potential mechanism to explain the growth response to injectable VC is the role of VC as a cofactor in enzymes involved in carnitine biosynthesis. Carnitine is essential for transporting fatty acids into the mitochondria for catabolism and subsequent production of ATP. In the current study, post-transit serum NEFA concentrations were 210% greater than pre-transit values likely due to increased lipolysis stimulated by food deprivation (glucagon) and psychological stress (cortisol). Therefore, it is possible that there was an increased need for carnitine to utilize these released fatty acids for energy production and growth. Although carnitine metabolism was not assessed in the current study, 24 h of fasting increased activity of γ-butyrobetaine dioxygenase, a VC dependent enzyme that catalyzes the last reaction of the carnitine biosynthetic pathway, and increased concentrations of free and total carnitine in the liver and kidney of pigs (Ringseis *et al.*, 2008).

Despite treatment differences in plasma concentrations of VC, a known cellular antioxidant, there was no effect of treatment on total plasma antioxidant capacity measured by FRAP. The lack of treatment differences could be a result of VC degradation in these samples since they were not treated with a reducing agent and analysis did not occur until approximately 3 months after sample collection. Circulating antioxidant capacity has been shown to decrease in response to transit stress. Chirase *et al.* (2004) observed a 9.5% decrease in total antioxidant

capacity of crossbred beef steers (207 ± 21 kg) after a 19 h 40 min (1930 km) transit event. Similarly, antioxidant capacity in the current study was decreased 9.7% immediately post-transit relative to pre-transit values. While Chirase *et al.* (2004) reported a sustained decrease in antioxidant capacity through day 28 post-transit, antioxidant capacity returned to pre-transit values by day 2 in the current study. These conflicting results could be because the steers utilized by Chirase *et al.* (2004) were multi-sourced and assembled at an order buyer barn before transportation to the Texas A&M University beef research facility while steers utilized in the current study had been previously adapted to the diet and environmental conditions at the Iowa State University Beef Nutrition Farm before the transportation event. Chirase *et al.* (2004) also reported a 3-fold increase in serum MDA, a marker of lipid peroxidation, after transit while the current study observed lesser plasma MDA concentrations on day 1 and 2 post-transit. This discrepancy may be explained by differences in analytical methods used to determine MDA as Chirase *et al.* (2004) utilized HPLC which is more specific for MDA than the colorimetric method utilized in the current study (Del Rio *et al.*, 2005).

Transit induced an inflammatory response in the current study evidenced by increased concentrations of the acute phase protein haptoglobin. It was hypothesized that a pre-transit VC injection would mitigate the decline in antioxidant status and subsequently decrease inflammation; however, no treatment effects were observed for serum haptoglobin concentrations. Urban-Chmiel *et al.* (2011) also observed no effect of injectable VC on haptoglobin concentrations 3, 7, 14 and 21 days after Simmentaler calves (100 to 120 kg) received a subcutaneous VC injection (1.25 g/steer; 10.4 to 12.5 mg/kg BW) on the first and second day in the feedlot. Increased haptoglobin concentrations after transportation and arrival at the feedlot have been positively correlated with bovine respiratory disease (**BRD**; Godson *et al.*,

1996; Joshi *et al.*, 2018). Several studies have investigated the use of VC as an ancillary therapy at the time of BRD treatment due to the antioxidant role of VC in protecting phagocytic immune cells from the reactive oxygen species these cells produce to kill infectious pathogens. Although the effectiveness of VC as an ancillary BRD therapy has not been definitively proven, according to a survey conducted by the National Animal Health Monitoring System, approximately one of three cattle affected and treated for BRD in large feedlots (≥ 1,000 animals) are given a VC injection as part of an initial course of treatment for respiratory disease (United States Department of Agriculture, 2013). No effects of injectable VC on respiratory disease rates were observed in the current study (data not shown), likely due to most respiratory treatments occurring within 8 days of arrival (52 days prior to trial initiation and VC injection).

Vitamin C is a relatively ignored nutrient in beef production due to lack of a perceived dietary requirement. In the current study, a pre-transit VC injection mitigated the transit induced decline in plasma ascorbate concentrations and improved post-transit feedlot performance of beef steers. Due to the differential responses observed for pre- versus post-transit administration, timing of VC administration is likely an important consideration for beef producers looking to adopt this supplementation strategy. Future research should seek to determine how dose and timing of injectable VC administration affects cattle health and performance.

Implications

In the U.S. beef industry, cattle often change ownership and are transported between industry segments such as a cow-calf or stocker operation to a feedlot. Therefore, this research sought to determine if pre- versus post-transit supplementation of a powerful antioxidant (vitamin C) would differentially affect feedlot performance. Based on improved performance of steers that received a pre-transit VC injection, cow-calf or stocker operations may be able to

generate premiums and increase demand for calves given injectable VC prior to transport to a feedlot. Additionally, injectable VC offers a good return on investment for cow-calf producers looking to retain ownership.

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Table 1. Ingredient composition of common diet fed from day 1 to 57

DM, %	60
Ingredient, % DM basis	
Sweet Bran[1]	35
Corn silage	30
Dried distillers grains with solubles	18.04
Dry-rolled corn	15
Limestone	1.5
Salt	0.31
Rumensin[2]	0.015
Vitamin A and E premix[3]	0.11
Trace mineral premix[4]	0.024
Analyzed composition[5]	
CP	18.0
NDF	28.9
Ether extract	4.1
Calculated composition[6]	
Net energy for gain, Mcal/kg	1.29

[1]Branded wet corn gluten feed (Cargill Corn Milling, Blair, NE)
[2]Provided monensin (Elanco Animal Health, Greenfield, IN) at 27 g/ton
[3]Provided 2,200 IU vitamin A and 25 IU vitamin E/kg diet
[4]Provided per kilogram of diet DM: 10 mg of Cu, 30 mg of Zn, 20 mg of Mn, 0.5 mg of I, 0.1 mg of Se and 0.1 mg of Co all from inorganic sources
[5]Based on total mixed ration analysis from Dairyland, Inc., Arcadia, WI
[6]Based on National Academies of Sciences, Engineering, and Medicine (2016) reported net energy for gain values of feedstuffs

Table 2. Effect of injectable vitamin C treatment on feedlot performance by beef steers

	Treatment[1]				Treatment
	CON	PRE	POST	SEM[2]	*P*-value
Steers (*n*)	24	23	24		
day 0 BW, kg	354	357	355	3.7	0.83
day 1 BW, kg	334	335	334	3.5	0.97
Shrink[3], %	5.7	6.2	6.0	0.23	0.28
Mid BW[4], kg	390[b]	395[a]	389[b]	1.6	0.03
Final BW[4], kg	429[y]	436[x]	430[y]	2.4	0.07
Average daily gain, kg/day					
1 to 7	1.43	1.78	1.34	0.198	0.25
7 to 31	1.94[b]	2.10[a]	1.94[b]	0.054	0.05
31 to 57	1.48	1.61	1.59	0.059	0.25
1 to 57	1.67[b]	1.84[a]	1.72[b]	0.042	0.02
Dry matter intake[5], kg/day					
1 to 7	8.6	8.9	9.0	0.14	0.13
7 to 31	9.2[y]	9.6[x]	9.7[x]	0.18	0.08
31 to 57	9.3[b]	10.0[a]	9.8[a]	0.17	0.01
1 to 57	9.0[b]	9.5[a]	9.5[a]	0.14	0.02
Gain:feed, kg/kg					
1 to 7	0.164	0.198	0.150	0.022	0.31
7 to 31	0.213	0.218	0.202	0.006	0.18
31 to 57	0.161	0.160	0.164	0.006	0.89
1 to 57	0.187	0.193	0.183	0.004	0.30

d 0 BW = BW collected pre-transit; day 1 BW = BW collected post-transit; mid BW = average BW collected on day 30 and 31; final BW = average BW collected on day 56 and 57

[1]CON = 20 mL of saline administered intramuscularly (IM) immediately prior to and post-transit; PRE = 20 mL of sodium ascorbate (250 mg/mL) administered IM immediately prior to transit and 20 mL of saline immediately post-transit; POST = 20 mL of saline administered IM immediately prior to transit and 20 mL of sodium ascorbate immediately post-transit

[2]Highest SEM of any treatment reported

[3]Percent BW shrink after an 18 h (1,675 km) transit event; a 6% shrink was applied to subsequent BW to be comparable to the post-transit BW conditions

[4]Day 0 BW utilized as a covariate in analysis

[5]Average steer DMI from day -7 through -1 utilized as a covariate in analysis.

[a,b]Values with unlike superscripts differ ($P \leq 0.05$)

[x,y]Values with unlike superscripts tend to differ ($P \leq 0.10$)

Table 3. Effect of injectable vitamin C treatment on day 2 liver ascorbate concentrations and blood parameters

	Treatment[1]				Treatment
	CON	PRE	POST	SEM[2]	*P*-Value
Liver ascorbate, µg/g wet tissue	183	225	253	23.1	0.11
Blood parameters[3]					
Plasma FRAP, µM	309	313	319	7.3	0.59
Plasma MDA, µM	6.9	6.8	6.9	0.47	0.97
Serum NEFA[4,5], µM	212	215	221	12.4	0.87
Serum HP[5], µg/mL	0.94	0.80	0.53	0.225	0.21

FRAP = ferric reducing antioxidant potential; HP = haptoglobin; MDA = malondialdehyde; NEFA = non-esterified fatty acid

[1]CON = 20 mL of saline administered intramuscularly (IM) immediately prior to and post-transit; PRE = 20 mL of sodium ascorbate (250 mg/mL) administered IM immediately prior to transit and 20 mL of saline immediately post-transit; POST = 20 mL of saline administered IM immediately prior to transit and 20 mL of sodium ascorbate immediately post-transit

[2]Highest SEM of any treatment reported

[3]Based on repeated measures analysis of blood samples collected on day 0, 1, 2 and 7; day effects are shown in Figure 2

[4]Day 0 concentrations utilized as a covariate in analysis

[5]Data were natural log transformed prior to analysis

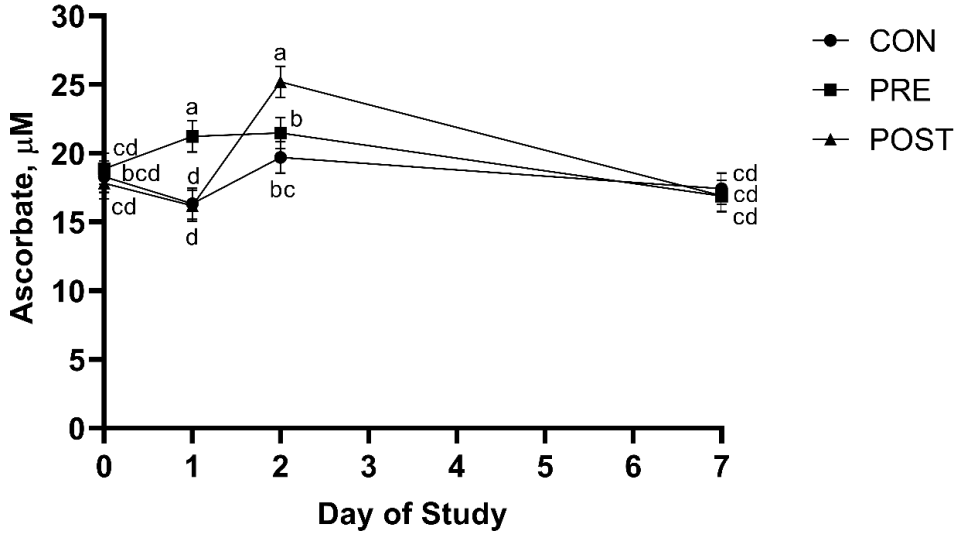

Figure 1. Effect of injectable vitamin C treatment and day of sampling relative to an 18 h (1,675 km) transit event (treatment × day; $P < 0.01$) on plasma ascorbate concentrations based on repeated measures analysis of samples collected on day 0 (pre-transit), 1 (post-transit), 2 and 7; values with unlike superscripts differ ($P \leq 0.05$) across treatments and sampling days. CON = 20 mL of saline administered intramuscularly (IM) immediately prior to and post-transit; PRE = 20 mL of sodium ascorbate (250 mg/mL) administered IM immediately prior to transit and 20 mL of saline immediately post-transit; POST = 20 mL of saline administered IM immediately prior to transit and 20 mL of sodium ascorbate immediately post-transit.

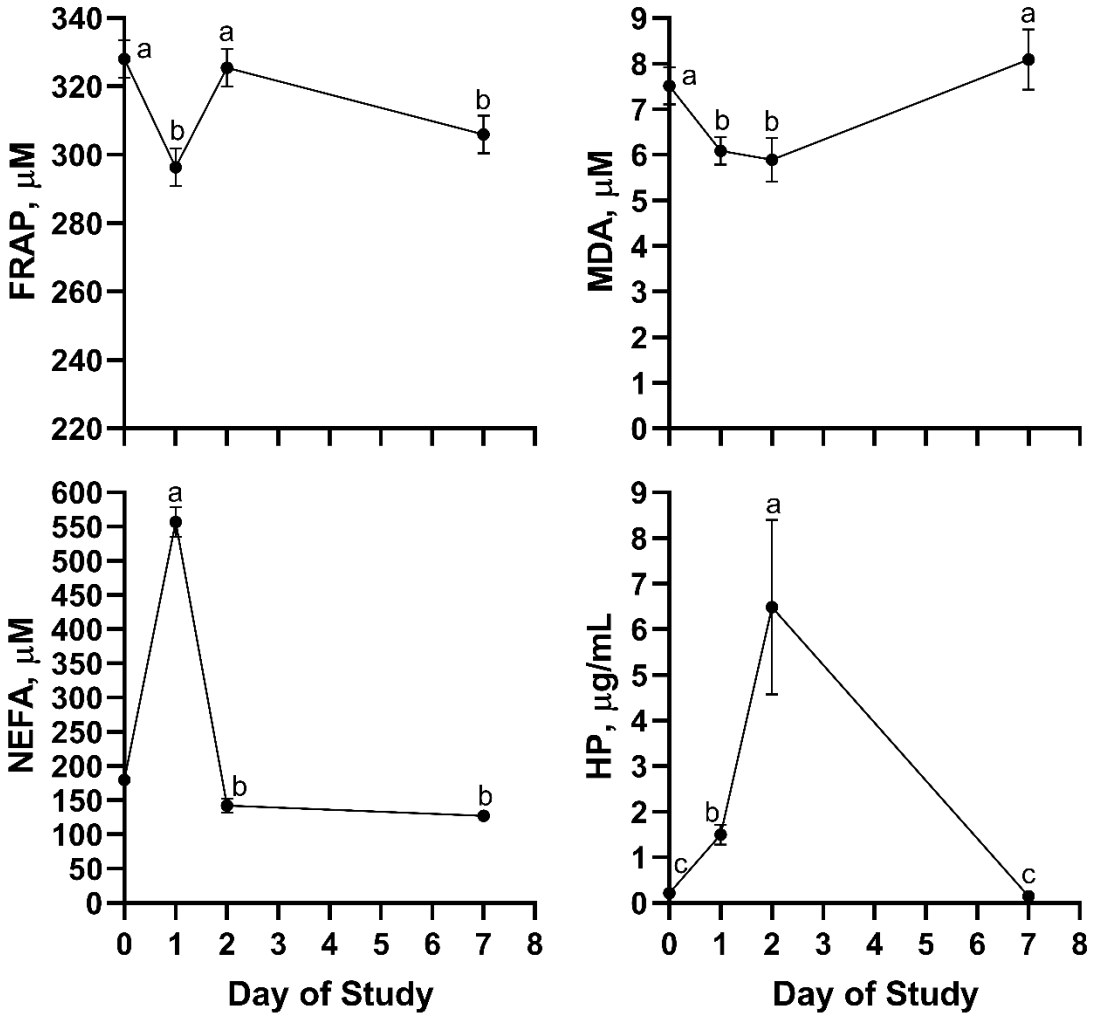

Figure 2. Effect of day ($P < 0.01$) of sampling relative to an 18 h (1,675 km) transit event on plasma ferric reducing antioxidant potential (FRAP), plasma malondialdehyde (MDA), serum non-esterified fatty acid (NEFA) and serum haptoglobin (HP) based on repeated measures analysis of samples collected on day 0 (pre-transit), 1 (post-transit), 2 and 7. Values within a panel with unlike superscripts differ ($P \leq 0.05$) across sampling days; day 0 values were used as a covariate in analysis of serum NEFA concentrations.

CHAPTER 6. TRANSIT STRESS IN BEEF CATTLE: A TALE OF TWO TISSUES

A manuscript to be submitted for publication in *The Journal of Animal Science*

E. L. Deters and S. L. Hansen

Abstract

Thirty-six Angus-cross steers (324 ± 36 kg) from a single source were used to delineate the effects of a long-distance transit event on blood and tissue metabolites, blood leukocyte profiles and tissue antioxidant enzyme activity of beef steers. On d -14, steers were stratified by BW and randomly assigned to treatments (n = 12 steers/treatment): **CON** = no transit or feed and water deprivation, **TRANS** = road transport for 18 h (1,790 km) and no access to feed or water for the duration of transit, and **DEPR** = no transit but deprived of feed and water for 18 h. From d -14 to 14 steers were housed in pens equipped with GrowSafe bunks to allow for measurement of individual DMI (n = 6 steers/pen; 2 pens/treatment). Treatments were initiated on d 0 and terminated approximately 18 h later on d 1. Blood was collected from all steers on d 0, 1, 3 and 8; steer BW was also recorded on these days and d 14. Liver and muscle (*longissimus dorsi*) biopsies were performed on all steers on d -4, 1 and 3. Data (except for tissue metabolites) were analyzed as a completely randomized design using the Mixed Procedure of SAS 9.4 (experimental unit = steer). Steer BW and DMI were analyzed as repeated measure with the fixed effects of treatment, day and treatment × day; plasma cortisol (d 1 and 3), serum haptoglobin (d 1, 3 and 8), leukocyte profiles (d 0 and 1) as well as liver and muscle superoxide dismutase (**SOD**) activity (d 1 and 3) were analyzed as single timepoints with the fixed effect of treatment. Tissue metabolite data were analyzed using the Biomarker and Pathway Analysis modules of MetaboAnalyst 4.0; significant pathways were identified based on false discovery

rate corrected *P*-values (*Q*-values). Steer BW did not differ due to treatment on d 0, 3, 8 or 14, but CON was heavier than DEPR or TRANS on d 1 (treatment × day $P < 0.01$); BW shrink from d 0 to 1 was least for CON (1.7%), intermediate for DEPR (7.9%) and greatest for TRANS (10.5%; $P < 0.01$). Steer DMI was variably affected by treatment from d 0 to 14 (treatment × day $P < 0.01$), generally driven by experimental design induced differences in DMI on d 0. Plasma cortisol concentrations did not differ due to treatment on d 1 or 3 ($P \geq 0.11$). Serum haptoglobin concentrations were greater for TRANS compared to CON or DEPR on d 1 ($P = 0.02$). From d 0 to 1, TRANS exhibited the greatest increase in neutrophil numbers ($P < 0.01$) and tended to exhibit the greatest decrease in lymphocyte numbers ($P = 0.08$) resulting in a greater increase in the neutrophil to lymphocyte ratio ($P < 0.01$). The change in monocytes and eosinophils from d 0 to 1 was greatest for DEPR and CON, respectively ($P \leq 0.01$). On d 1, total liver SOD activity tended to be greatest for both DEPR or TRANS ($P = 0.07$) but total muscle SOD activity was only greater for TRANS ($P = 0.02$), driven by a tendency for greater Mn-SOD activity ($P = 0.10$) and greater CuZn-SOD activity ($P = 0.02$). On d 3, Mn-SOD activity tended to be greatest for CON and least for TRANS ($P = 0.07$). Several metabolic pathways were identified as significant on d 1 including aminoacyl-tRNA and branched chain amino acid biosynthesis in CON and DEPR liver ($Q \leq 0.02$), unsaturated fatty acid biosynthesis in TRANS liver ($Q = 0.05$) and aminoacyl-tRNA biosynthesis in TRANS muscle ($Q = 0.04$). In conclusion, transported steers experienced a more robust inflammatory response and an increase in neutrophil numbers, both of which could contribute to greater oxidative stress and subsequent increase in antioxidant enzyme activity. The differential tissue responses for deprived versus transported steers indicate that muscle plays an important role in how cattle respond to and recover from transit stress.

Introduction

The U.S. beef industry is geographically segmented to take advantage of feed resources across the country. This means a beef animal will often be transported by road multiple times from birth to harvest. Transit is associated with numerous stressors including interaction with humans, exposure to environmental elements, feed and water deprivation as well as skeletal muscle fatigue. As a result of these psychological and physical stressors, biological pathways involved in nutrient metabolism and inflammation are activated in transported cattle (Warriss et al., 1995; Cooke et al., 2013). Although changes in circulating stress biomarkers (e.g. cortisol, acute phase proteins) have been relatively well characterized in cattle post-transit (Van Engen and Coetzee, 2018), few studies have attempted to distinguish the effects of feed and water deprivation alone or in conjunction with a long-distance transit event. Additionally, there is a paucity of research examining the physiological impacts of transit at the tissue level. Therefore, this study sought to gain insight into the effects of transit on metabolically and economically relevant tissues (liver and muscle).

The first objective of this study was to assess changes in targeted blood metabolites and leukocyte profiles of feed and water deprived steers or transported steers also deprived of feed and water. Complete metabolite profiling provides a discovery-based approach for identifying poorly understood biological pathways that may be affected by transit. The second objective of this study was to identify metabolites and metabolic pathways affected by transit in liver and muscle of beef steers. Reactive oxygen species (**ROS**) are a by-product of several biological pathways known to be activated by transit (Babior, 1973; Turrens et al., 1985) and when the production of ROS exceeds the antioxidant capacity of the cell, oxidative stress occurs (Sies, 1985). Because oxidative stress can be detrimental to immune function and cellular efficiency (Martin, 1984; Bottje et al., 2006), understanding transit-induced oxidative stress may be crucial

to understanding the negative effects of transit on cattle health and performance. Hence, the final objective of this study was to determine the effects of transit on liver and muscle antioxidant enzyme activity. It was hypothesized that transit would affect steers differently than feed and water deprivation alone.

Materials and Methods

Animals and Experimental Design

All experimental procedures were approved by the Iowa State University Animal Care and Use Committee (#19-084).

In August 2019, 40 Angus-cross steers from a single ranch were purchased from a sale barn (Dunlap, IA) and transported (~2 h; 177 km) to the Iowa State University Beef Nutrition Research Farm (Ames, IA). Steers were received into partially covered concrete pens (3.7 × 12.2 m) equipped with one GrowSafe bunk (GrowSafe Systems Ltd., Airdrie, Alberta, Canada) and one automatic waterer per pen. Upon arrival, steers were offered a corn silage and corn coproduct-based diet (**Table 1**) top-dressed with long-stem grass hay; no hay was offered after the second full day. Two full days after arrival (d -29), body weights (**BW**) were collected and steers received unique visual and electronic identification tags. On d -14 steers were weighed and the 36 steers most similar in BW (324 ± 36 kg) and health status were stratified by BW and randomly assigned to experimental treatments (n = 12 steers/treatment) and pens (described above; 6 steers/pen; 2 pens/treatment). Treatments included no transit or feed deprivation (**CON**), an 18 h road transit event during which time steers had no access to feed or water (**TRANS**) and no transit but feed and water deprivation for 18 h (**DEPR**). On d 0 (treatment initiation), CON steers were weighed (1100 h) and returned to their pens with ad libitum access to feed and water; TRANS steers were weighed (1300 h), loaded into a single compartment of a

commercial livestock trailer (Silverstar PSDCL-402, Wilson Trailer Company, Sioux City, IA; 1.30 m^2/steer) and transported for ~18 h (1,790 km); and DEPR steers were weighed (1500 h) and returned to their pens where feed had been removed and waterers drained and shut off. Treatments were stagger started by 2 h to accommodate time required for post-treatment BW, blood and tissue collection; this ensured that samples for each treatment were collected approximately 18 h after treatment initiation. On d 1 (treatment termination), CON, TRANS and DEPR steers were weighed at 0600, 0800 and 1000 h, respectively. Steers were also weighed on d 3, 8 and 14 at approximately the same time as BW collected on d 1. All BW were collected prior to feeding except for d 0 when steers had access to feed for approximately 3 h prior to treatment initiation. Individual steer intake was assessed via GrowSafe bunks from d -7 to the end of the trial (d 14) and feed efficiency (gain:feed; **G:F**) was calculated from d 1 to 14; day 14 weights were pencil shrunk based on individual steer BW shrink from d 0 to 1.

Sample Collection and Analytical Procedures

Weekly samples of the total mixed ration were collected for dry matter determination by drying in a forced air oven at 70ºC for 48 h. These dry matter values were used to calculate steer dry matter intake (**DMI**) based on individual as-fed feed disappearance recorded by the GrowSafe system. Dried total mixed ration samples were then ground in a Retsch ZM 100 grinding mill (Retsch GmbH, Haan, Germany) to pass through a 2 mm screen and subsamples were composited for analysis of nitrogen (crude protein; AOAC, 1999a; method 990.03), neutral detergent fiber (AOAC, 2005; method 2002.04) and ether extract (AOAC, 1999b; method 920.39) by a commercial laboratory (Dairyland Laboratories, Inc., Arcadia, WI). Analyzed compositions are presented in **Table 1**.

Blood and tissue collection. Blood was collected from all steers via jugular venipuncture on d -4, 0 (immediately pre-treatment), 1 (immediately post-treatment), 3 (~48 h after treatment termination) and 8 into vacuum tubes (potassium EDTA #367844, serum #366430, Becton Dickinson, Franklin Lakes, NJ) and transported to the laboratory on ice. All blood samples were collected prior to feeding except for d 0 when blood samples were collected ~3 h after feeding. Whole blood collected on d 0 and 1 was transported on ice to the Iowa State University Clinical Pathology Laboratory (Ames, IA) for determination of complete blood cell count and automated differentiation of white blood cells. Plasma from potassium EDTA tubes was removed after centrifugation (1,000 × *g* for 20 min at 4ºC), aliquoted and stored at -80ºC until further analysis. Plasma collected on d -4, 1 and 3 was analyzed for cortisol concentrations using a commercially available enzyme immunoassay kit (K003-H1/H5, Arbor Assays, Ann Arbor, MI; intra-assay CV = 3.8%, inter-assay CV = 9.7%). Serum tubes were allowed to clot at room temperature for at least 90 min prior to centrifugation (1,500 × *g* for 20 min at 4ºC), serum removal and storage at -80ºC. Serum collected on d -4, 1, 3 and 8 was analyzed for haptoglobin concentrations using a bovine specific ELISA kit (HAPT-11, Life Diagnostics, Inc., West Chester, PA; intra-assay CV = 8.8%, inter-assay CV = 7.4%).

Liver and muscle (*longissimus dorsi*) biopsies were performed on all steers on d -4, 1 and 3 using methods previously described by Engle and Spears (2000) and a modified method previously described by Pampusch et al. (2003), respectively. Liver biopsies were consistently performed between the 11th and 12th ribs on the right side of the animal while consecutive muscle biopsies were performed on alternate sides of the animal and forward (cranial) one rib space to limit tissue damage. On the day of collection, liver and muscle samples were flash frozen in liquid N, transported to the laboratory on dry ice and stored at -80ºC; tissue was then ground

with a mortar and pestle in liquid N to facilitate partitioning of the sample for various analyses. Approximately 100 mg (wet basis) of liver and muscle from d 1 and 3 was utilized for total metabolite analysis (see methods below). Liver and muscle samples (~150 mg; wet basis) from d -4, 1 and 3 were homogenized (Deters and Hansen, 2019) and analyzed for total and Mn-superoxide dismutase (**SOD**) activity using a commercially available kit (706002, Cayman Chemical, Ann Arbor, MI; liver intra-assay CV = 6.6% and inter-assay CV = 10.5%; muscle intra-assay CV = 6.7% and inter-assay CV = 11.2%). Copper/zinc-SOD activity was calculated by subtracting Mn-SOD activity from total-SOD activity. Protein concentrations of liver and muscle samples analyzed for SOD activity were determined using the Coomassie (Bradford) Protein Assay Kit (23200, Thermo Scientific, Rockford, IL; liver intra-assay CV = 3.5% and inter-assay CV = 5.2%; muscle intra-assay CV = 1.8% and inter-assay CV = 6.8%).

Metabolomics. Total metabolite analysis via gas chromatography-mass spectrometry (GC-MS) was conducted at the W. M. Keck Metabolomics Research Laboratory (Ames, IA). First, 10 µL of each internal standard (ribitol and nonadecanoic acid; 1.5 mg/mL) were added to liver and muscle samples (~100 mg; wet basis) followed by addition of 0.5 mL cold HPLC grade methanol. Samples were then sonicated, vortexed and centrifuged followed by addition of 0.4 mL chloroform. Then, 0.34 mL of HPLC grade water was added and samples were vortexed, sonicated and centrifuged. The top polar and bottom non-polar layers were transferred to separate GC vials and dried overnight in a speed vacuum. The remaining protein pellet was stored at -80ºC for future proteomics analysis; metabolite extraction was done on ice to prevent protein and metabolite degradation. Dried polar and non-polar fractions were derivatized by addition of 50 µL methoxyamine hydrochloride (20 mg/ml) in pyridine and incubation at 30ºC

for 90 min followed by addition of 70 µL of bis-trimethyl silyl trifluoroacetamide with 1% trimethylchlorosilane (BSTFA) for silylation and incubation at 60ºC for 30 min.

All samples were run on the same instrument (Agilent Technologies Model 6890 GC coupled to Model 5975 MS) and treatments were equally represented within runs (4 runs/tissue; 8 runs total) to limit instrument variability. Additionally, a hydrocarbon ladder was run along with samples in each batch of runs and was used for retention index calibration. Sample separation was obtained on an Agilent-HP5MSI column (30 m × 250 µM × 0.25 µM). The oven program was as follows: initial temperature of 70ºC for 0.5 min followed by, 10ºC/min ramp to 160ºC and 5.5ºC/min ramp to 320ºC with a final hold for 6.4 min. The inlet and the interface temperatures were maintained at 280ºC. Detection mass range was set from 40–600 m/z and the GC-MS was controlled by the Agilent ChemStation software. Metabolites were identified using total ion mass spectrum and comparison to the National Institutes of Standards and Technology mass spectral library (NIST, 2014). Metabolites were quantified by normalizing the MS peak area of each metabolite to the moles of internal standard added to each sample; ribitol and nonadecanoic acid were used to normalize polar and non-polar samples, respectively.

Statistical Analysis

Data were analyzed as a completely randomized design using the Mixed Procedure of SAS 9.4 (SAS Institute Inc., Cary, NC). Steer served as the experimental unit for all variables. Blood leukocyte profiles from d 0 and 1 as well as the change in cell number from d 0 to 1 (delta) were analyzed as single timepoints with the fixed effect of treatment. Plasma cortisol and serum haptoglobin concentrations, as well as liver and muscle SOD activity, were analyzed as single timepoints with the fixed effect of treatment; d -4 values were utilized as covariates in analysis of subsequent sampling dates. Steer BW and DMI were analyzed as repeated measures

with the fixed effects of treatment, day and treatment × day. Covariance structures were selected based on the lowest Akaike's information criterion (Littell et al., 1998). The heterogeneous autoregressive structure was utilized for analysis of BW data and the compound symmetry covariance structure was utilized for analysis of DMI data. Pre-trial (d -7 through -1) DMI served as a covariate in analysis of subsequent DMI data. To meet assumptions of normality, serum haptoglobin concentrations were log transformed prior to analysis and back transformed means and SEM are presented. Outliers were determined based on Cook's D statistic and removed if ≥ 0.50; 1 DEPR steer was removed from analysis of monocytes and muscle SOD, 3 steers (1 DEPR and 2 TRANS) were removed from analysis of eosinophils and 3 steers (1 from each treatment) were removed from analysis of serum haptoglobin and liver SOD. Significance was declared at $P \leq 0.05$ and tendencies from $0.05 < P \leq 0.10$.

Metabolomics data were analyzed using the Biomarker Analysis module of MetaboAnalyst 4.0 (Chong et al., 2018). This module allows for identification of a metabolite or set of metabolites capable of classifying conditions (in this case, experimental treatments) with high sensitivity (true positive rate) and specificity (true negative rate). Metabolites with > 80% missing values were removed from the data set and missing values were imputated using the K-Nearest Neighbor (KNN) method. A classical univariate receiver operator characteristic (ROC) curve analysis was performed comparing CON vs. TRANS and DEPR vs. TRANS. Area under the curve (AUC) can be interpreted as the probability that a classifier will rank a randomly chosen positive instance higher than a randomly chosen negative one and was used to assess the utility of individual metabolites as biomarkers where 0.9-1.0 = excellent, 0.8-0.9 = good and 0.7-0.8 = fair (Xia et al., 2013); only metabolites with AUC ≥ 0.70 are reported. The identified metabolites were then entered into the Pathway Analysis module of MetaboAnalyst 4.0. For

over-representation analysis, the Hypergeometric test was selected and for pathway topology analysis, relative betweenness centrality was selected as the node importance measure. The October 2019 Kyoto Encyclopedia of Genes and Genomics (KEGG) library for Bos taurus was selected as the pathway library. To adjust for multiple comparisons, significant pathways were identified based on false discovery rate (FDR) corrected *P*-values (i.e. *Q*-values) from the enrichment analysis.

Results

Feedlot Performance

Steer BW did not differ due to treatment on d 0, 3, 8 or 14, but, by experimental design, CON-steers were heavier than DEPR or TRANS-steers on d 1 (treatment × day $P < 0.01$; **Figure 1**). Shrink, calculated as change in BW from d 0 to 1, was greatest for TRANS (10.5%), least for CON (1.7%) and intermediate for DEPR (7.9%; SEM = 0.36; $P < 0.01$). Steer DMI was variable due to treatment throughout the trial (treatment × day $P < 0.01$; **Figure 2**). As per treatment design, CON had greater DMI on d 0 (treatment initiation) than DEPR or TRANS. After treatment termination on d 1, DMI was similar for CON and DEPR, but lesser for TRANS. In general, TRANS continued to exhibit lesser DMI than DEPR until d 8 and by the end of the trial DMI was greater for DEPR or TRANS compared to CON. Steer G:F (calculated from d 1 to 14) did not differ due to treatment (average across treatments = 0.143; SEM = 0.0185; $P = 0.96$).

Blood Metabolites and Leukocyte Profiles

The effects of treatment on blood metabolite concentrations are presented in **Table 2**. Plasma cortisol concentrations did not differ due to treatment on d 1 or 3 ($P \geq 0.11$). Serum haptoglobin concentrations were greater for TRANS compared to CON or DEPR on d 1 (*P* =

0.02) but did not differ due to treatment on d 3 or 8 ($P \geq 0.75$). The effects of treatment on blood leukocyte profiles on d 0 (immediately prior to treatment initiation) and d 1 (immediately after treatment termination) are presented in **Table 3**. Absolute leukocyte numbers did not differ due to treatment on d 0 or 1 ($P \geq 0.52$), nor was the change in leukocyte number from d 0 to 1 affected by treatment ($P = 0.20$). On d 0, the number of neutrophils, lymphocytes, monocytes and eosinophils did not differ due to treatment ($P \geq 27$). Neutrophil numbers on d 1 were greater for CON or TRANS compared to DEPR ($P < 0.01$) while the change in neutrophils from d 0 to 1 was greatest for TRANS compared to CON or DEPR ($P < 0.01$). Lymphocyte numbers on d 1 were greatest for DEPR, least for TRANS and intermediate for CON ($P = 0.05$). This resulted in a tendency for a greater change in lymphocyte number from d 0 to 1 for DEPR or CON compared to TRANS ($P = 0.08$). The neutrophil to lymphocyte ratio (**N:L**) was not different due to treatment on d 0 ($P = 0.14$). However, on d 1, the N:L ratio was greatest for TRANS, least for DEPR and intermediate for CON ($P < 0.01$), resulting in a greater change in N:L from d 0 to 1 for TRANS compared to CON or DEPR ($P < 0.01$). Monocytes were not affected by treatment on d 1 ($P = 0.31$), but the change in monocyte number from d 0 to 1 was greater for DEPR compared to CON or TRANS ($P = 0.01$). Eosinophil numbers on d 1 tended to be greatest for CON and lowest for DEPR with TRANS being intermediate ($P = 0.10$). The change in eosinophil number from d 0 to 1 was greater for CON compared to DEPR or TRANS ($P < 0.01$).

Tissue Antioxidant Enzyme Activity

The effects of treatment on liver and muscle SOD activity are presented in **Table 4** and **Table 5**, respectively. Total liver SOD activity tended to be greater for DEPR or TRANS compared to CON on d 1 ($P = 0.07$). However, there was no effect of treatment on d 1 liver Mn or CuZn-SOD activity or the ratio of Mn-SOD to total-SOD ($P \geq 0.28$). No effects of treatment

were observed on d 3 liver SOD activity (total, Mn, CuZn or ratio $P \geq 0.49$). Day 1 muscle total and CuZn-SOD activity were greatest for TRANS compared to CON or DEPR ($P = 0.02$) and Mn-SOD tended to be greatest for TRANS ($P = 0.10$). Day 3 muscle total and CuZn-SOD activity were not affected by treatment, but Mn-SOD activity tended to be greatest for CON and least for TRANS ($P = 0.07$). There was no effect of treatment on the ratio of Mn-SOD to total-SOD in muscle on d 1 or 3 ($P \geq 0.47$).

Tissue Metabolomics

Results of biomarker analysis are presented in **Table 6** (CON vs. TRANS) and **Table 7** (DEPR vs. TRANS). Significant pathways were identified based on enrichment analysis which compares the number of differentially expressed metabolites (i.e. metabolites identified as potential biomarkers) as a proportion of the total number of metabolites in a metabolic pathway. Pathway analysis identified aminoacyl-tRNA biosynthesis ($Q < 0.01$) and branched chain amino acid (valine, leucine, isoleucine) biosynthesis ($Q \leq 0.02$) as significant pathways in CON and DEPR liver on d 1. When compared to CON on d 1, biosynthesis of unsaturated fatty acids was identified as a significant pathway in TRANS liver ($Q = 0.05$) and aminoacyl-tRNA biosynthesis was identified as a significant pathway in TRANS muscle ($Q = 0.04$). No metabolic pathways were identified in liver or muscle on d 3 ($Q \geq 0.21$).

Discussion

Road transportation of cattle is virtually unavoidable due to the segmented nature of the U.S. beef industry. Transported cattle frequently display poor growth performance and increased incidence of disease upon arrival at their destination. This is likely a result of the various stressors they are exposed to during transit, including psychological stress, feed and water

deprivation and physical exertion. Because of the physical demand of standing during transport (Knowles, 1999), it was hypothesized that transported steers would experience a more robust oxidative stress response than steers simply deprived of feed and water. In support of this hypothesis, muscle SOD activity was increased in cattle immediately post-transit. Unaccustomed and/or exhaustive exercise has been shown to cause local and systemic inflammation (Fielding et al., 1993; Steensberg et al., 2000). Proinflammatory stimuli activate the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB**)** signaling pathway which has been shown to increase the transcription of Mn-SOD (Das et al., 1995) and may have contributed to the subsequent increase in Mn-SOD activity observed immediately post-transit.

Interestingly, Mn-SOD activity was lesser in muscle of transported steers on d 3 relative to control steers. Hypotheses as to why this occurred include 1) the stimuli for increased Mn-SOD transcription was removed or 2) persistent oxidative stress in transported steers resulted in nitration and/or oxidation of tyrosine residues of Mn-SOD and inhibited enzymatic activity (MacMillan-Crow et al., 1998). In support of the second hypothesis, metabolites of the pentose phosphate pathway (gluconic acid and D-ribose) were identified as potential biomarkers in TRANS muscle on d 3. The pentose phosphate pathway is involved in the production of reducing equivalents (i.e. NADPH) and so, under conditions of oxidative stress, glucose will be diverted from glycolysis to the pentose phosphate pathway (Wang et al., 2014). Although not identified as a significant metabolic pathway in the current study ($Q = 0.25$), these metabolites could indicate that transported steers were still trying to combat residual oxidative stress in the muscle 48 h after transport.

Increased oxidative stress in transported steers could be a result of increased inflammation. Arachidonic acid was identified as a potential biomarker in the liver of transported

steers immediately post-transit. This polyunsaturated fatty acid serves a substrate for the production of inflammatory mediators, known as eicosanoids, by ROS producing enzymes (lipoxygenase and cyclooxygenase; Hamberg and Samuelsson, 1967). Also, serum concentrations of the acute phase protein haptoglobin were greater in transported steers immediately post-transit and numerically greater on d 3 (~48 h after treatment termination) than their feed and water deprived counterparts. Marques et al. (2012) also observed greater plasma haptoglobin concentrations in transported cattle than feed and water restricted cattle. The acute phase response is triggered by proinflammatory cytokines (interleukin-1, interleukin-6, tumor necrosis factor-α) produced predominantly by activated macrophages (i.e. monocytes that have differentiated and migrated into tissues; Jakubzick et al., 2017). Proinflammatory cytokines also stimulate neutrophil mobilization. In the current study, neutrophil numbers were increased in transported steers from d 0 to 1. The primary function of neutrophils is to phagocytose pathogens via oxygen-dependent and oxygen independent pathways. The oxygen-dependent pathway, also known as the respiratory burst, involves activation of NADPH oxidase enzymes which produce large amounts of ROS (superoxide; Babior et al., 1973; Babior et al., 2002), further contributing to transit-induced oxidative stress.

The observed increase in neutrophil numbers, coupled with a decrease in lymphocyte numbers, resulted in a greater N:L ratio on d 1 for transported steers. It has been suggested that this ratio may be a more useful indicator of psychological stress (i.e. a neuroendocrine stress response) than circulating glucocorticoids (primarily cortisol in cattle) as concentrations of these hormones increase rapidly upon stress exposure, making it difficult to accurately quantify baseline and peak concentrations (Davis et al., 2008). Furthermore, the N:L may be particularly useful in assessing chronic rather than acute stress because immune cell population shifts occur

more slowly than changes in circulating glucocorticoids (Swan and Hickman, 2014). In the current study, plasma cortisol concentration changes were not congruent with the shift in N:L observed in transported steers. Numerically lesser cortisol concentrations immediately post-transit could be indicative of cattle becoming acclimated to transport conditions or negative feedback regulation of cortisol on the pituitary and hypothalamus. In support of this, Warriss et al. (1995) observed greater cortisol concentrations in steers transported for 5 h compared to steers transported for 10 or 15 h and suggested cortisol release is triggered by the initial psychological stress of loading and the novel environment of the truck but then progressively declines.

In accordance with the current study, neutrophilia, lymphopenia and eosinopenia have been previously observed in transported cattle (Murata et al., 1987). Similar trends in leukocyte profiles have been induced in dairy heifers through intramuscular injection of 250 IU of adrenocorticotropic hormone (Wegner and Stott, 1972), suggesting that although a cortisol response was not observed in the current study, transported steers were experiencing a neuroendocrine stress response. Stress is a major predisposing factor in the pathogenesis of bovine respiratory disease (Hodgson et al., 2005). Bovine respiratory disease represents the costliest disease of feedlot cattle in the U.S. (Griffin, 1997) and is aptly referred to as “shipping fever” because disease incidence is highest during the first 45 d after feedlot arrival (Jensen et al., 1976). Furthermore, stimulation of a neuroendocrine stress response via intravenous administration of corticotropin-releasing hormone elicited an inflammatory response in beef steers (Cooke and Bohnert, 2011). Based on N:L and haptoglobin concentrations, transported steers in the current study were experiencing a greater neuroendocrine stress response and inflammatory response compared to control or feed and water deprived steers. Transported steers

also took longer to recover feed intake during the post-treatment period. Cytokines released during an inflammatory response have been shown to act directly on the central nervous system to suppress feed intake (Plata-Salamán et al., 1988), and in combination with glucocorticoids, increase lipolysis and muscle degradation. Thus, the net effect of inflammation is inhibition of growth (Johnson, 1997).

In response to inflammation and/or a neuroendocrine stress response, aerobic metabolism of nutrients mobilized from adipose and skeletal muscle in the liver increases mitochondrial derived ROS. In the current study, steers exposed to 18 h of feed and water deprivation alone or in conjunction with transit exhibited greater liver SOD activity on d 1 compared to control steers. Feed (caloric) restriction has resulted in variable effects on oxidative stress parameters in other species. Moderate caloric restriction in rats increased liver antioxidant defense (Mn-SOD activity and glutathione concentrations) while severe caloric restriction and acute fasting increased oxidative damage and decreased SOD-activity in the liver (Stankovic et al., 2013). Based on plasma glucocorticoid concentrations and indicators of hepatocyte damage, the authors concluded moderate caloric restriction produced an adaptive stress response resulting in upregulation of antioxidant defense mechanisms, but severe caloric restriction or acute fasting resulted in liver damaged and decreased liver antioxidant capacity. Although it was expected that ruminal contents would decrease during the 18 h deprivation/transit period in the current study (Fluharty et al., 1996), nutrients were likely still being absorbed due to greater retention time of digesta in the gastrointestinal tract for ruminant versus monogastric species. Therefore, 18 h of feed and water deprivation in cattle may be classified as a moderate caloric restriction event which resulted in the greater liver antioxidant enzyme activity observed herein.

As hypothesized, steers exposed to a long-distance transit event experienced a greater oxidative stress response than steers simply deprived of feed and water for the same duration. Targeted analysis of antioxidant enzyme activity revealed greater SOD activity in the muscle of transported steers on d 1. Additionally, discovery-based metabolomics identified metabolites involved in inflammation (arachidonic acid) and oxidative stress protection (gluconic acid and D-ribose) as potential biomarkers in the liver and muscle of transported steers. Based on haptoglobin concentrations and the N:L, differences in oxidative stress responses for deprived and transported steers may have been driven by a greater inflammatory and neuroendocrine stress response in transported steers. Inflammation and neuroendocrine stress are known to hinder growth and immune function. However, less is known about how transit-induced oxidative stress affects feedlot health and performance, despite evidence that ROS can impair immune cell and respiratory function (Martin, 1984; Wright et al., 1994) and cellular efficiency (Bottje et al., 2006). Therefore, further characterization of transit-induced oxidative stress in beef cattle is warranted.

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Table 1. Composition of common diet fed to all steers

Dry matter (DM), % as fed basis	55
Ingredient, % DM basis	
Corn silage	40
Sweet Bran[1]	40
Dried distillers grains with solubles	18.05
Limestone	1.5
Salt	0.31
Rumensin[2]	0.0135
Vitamin A and E premix[3]	0.1
Trace mineral premix[4]	0.024
Analyzed composition[5], %	
Crude protein	18.6
Neutral detergent fiber	31.7
Ether extract	4.7
Calculated composition	
Net energy for gain[6], Mcal/kg	1.34

[1]Branded wet corn gluten feed (Cargill Corn Milling, Blair, NE)
[2]Provided monensin (Elanco Animal Health, Greenfield, IN) at 27 g/ton
[3]Provided 2,200 IU vitamin A and 25 IU vitamin E/kg diet
[4]Provided per kg of diet DM: 10 mg of Cu, 30 mg of Zn, 20 mg of Mn, 0.5 mg of I, 0.1 mg of Se, and 0.1 mg of Co, all from inorganic sources
[5]Based on total mixed ration analysis from Dairyland, Inc., Arcadia, WI
[6]Net energy for gain based on NASEM (2016) reported NEg values of feedstuffs

Table 2. Effect of treatment on blood metabolite concentrations of beef steers

	Treatment[1]				*P*-value
	CON	DEPR	TRANS	SEM[2]	Treatment
Plasma cortisol, ng/mL					
d -4[3]	41.0	53.9	51.5	-	-
d 1	47.8	49.8	31.8	6.50	0.11
d 3	37.2	41.6	36.4	5.04	0.73
Serum haptoglobin[4], µg/mL					
d -4[3]	0.21	0.20	0.25	-	-
d 1	0.44[b]	0.66[b]	1.65[a]	0.514	0.02
d 3	3.27	3.67	4.62	2.599	0.91
d 8	0.20	0.26	0.24	0.064	0.75

[1]CON = steers remained in home pens with ad libitum access to feed and water; DEPR = steers remained in home pens but were deprived of feed and water for approximately 18 h; TRANS = steers were transported on a single commercial livestock trailer for approximately 18 h (1,790 km) with no access to feed or water for the duration of transit
[2]Highest SEM of any treatment reported
[3]Day -4 values utilized as covariates in analysis of subsequent sampling days
[4]Data were log transformed prior to analysis; back transformed means and SEM are presented
[a,b]Within a row, means with unlike superscripts differ ($P \leq 0.05$)

Table 3. Effect of treatment on blood[1] leukocyte profiles of beef steers

	Treatment[2]				*P*-value
Cell number ×10^3/μL	CON	DEPR	TRANS	SEM[3]	Treatment
Leukocytes					
d 0	11.19	11.28	11.48	0.624	0.94
d 1	10.02	9.66	10.56	0.556	0.52
Delta[4]	-1.16	-1.63	0.92	0.272	0.20
Neutrophils					
d 0	4.34	3.69	4.23	0.378	0.44
d 1	4.11^a	2.92^b	5.01^a	0.409	<0.01
Delta[4]	-0.23^b	-0.77^b	0.78^a	0.245	<0.01
Lymphocytes					
d 0	5.56	6.20	5.70	0.330	0.37
d 1	4.64^b	5.38^a	4.25^c	0.312	0.05
Delta[4]	-0.91^x	-0.82^x	-1.45^y	0.208	0.08
Neutrophil:Lymphocyte					
d 0	0.79	0.60	0.76	0.069	0.14
d 1	0.92^b	0.55^c	1.27^a	0.119	<0.01
Delta[4]	0.13^b	0.05^b	0.51^a	0.093	<0.01
Monocytes					
d 0	0.79	0.72	0.86	0.059	0.27
d 1	0.74	0.91	0.75	0.091	0.31
Delta[4]	-0.06^b	0.19^a	-0.11^b	0.073	0.01
Eosinophils					
d 0	0.25	0.22	0.29	0.039	0.37
d 1	0.26^x	0.14^y	0.21^{xy}	0.042	0.10
Delta[4]	0.02^a	-0.08^b	-0.09^b	0.026	<0.01

[1]Blood was collected immediately prior to treatment initiation on d 0 and immediately after treatment termination on d 1

[2]CON = steers remained in home pens with ad libitum access to feed and water; DEPR = steers remained in home pens but were deprived of feed and water for approximately 18 h; TRANS = steers were transported on a single commercial livestock trailer for approximately 18 h (1,790 km) with no access to feed or water for the duration of transit

[3]Highest SEM of any treatment reported

[4]Delta = change in cell number from d 0 to 1

[a,b]Within a row, means with unlike superscripts differ ($P \leq 0.05$)

[x,y]Within a row, means with unlike superscripts tend to differ ($P \leq 0.10$)

Table 4. Effect of treatment on liver superoxide dismutase activity of beef steers

	Treatment[1]				*P*-value
U/mg protein[2]	CON	DEPR	TRANS	SEM[3]	Treatment
Day -4[4]					
Total	143.4	140.1	140.2	-	-
Mn	72.6	68.6	71.9	-	-
CuZn	70.8	71.5	68.3	-	-
Ratio[5]	0.50	0.50	0.52	-	-
Day 1					
Total	125.6[y]	145.1[x]	140.7[x]	5.81	0.07
Mn	63.4	71.6	61.0	5.57	0.38
CuZn	62.7	73.1	79.6	7.38	0.28
Ratio[5]	0.50	0.50	0.45	0.040	0.56
Day 3					
Total	133.6	139.3	140.3	8.77	0.85
Mn	65.7	67.6	62.3	5.27	0.77
CuZn	67.2	71.8	78.5	8.12	0.62
Ratio[5]	0.50	0.49	0.45	0.034	0.49

[1]CON = steers remained in home pens with ad libitum access to feed and water; DEPR = steers remained in home pens but were deprived of feed and water for approximately 18 h; TRANS = steers were transported on a single commercial livestock trailer for approximately 18 h (1,790 km) with no access to feed or water for the duration of transit

[2]One unit (U) = the amount of enzyme required to dismutate 50% of the superoxide radical

[3]Highest SEM of any treatment reported

[4]Day -4 values utilized as covariates in analysis of subsequent sampling days

[5]Ratio = Mn-SOD/Total-SOD

[x,y]Within a row, means with unlike superscripts tend to differ ($P \leq 0.10$)

Table 5. Effect of treatment on muscle superoxide dismutase activity of beef steers

	Treatment[1]				*P*-value
U/mg protein[2]	CON	DEPR	TRANS	SEM[3]	Treatment
Day -4[4]					
Total	17.7	17.1	18.5	-	-
Mn	9.1	9.6	10.1	-	-
CuZn	8.6	7.5	8.4	-	-
Ratio[5]	0.53	0.56	0.55	-	-
Day 1					
Total	14.6^{b}	13.5^{b}	18.7^{a}	1.24	0.02
Mn	9.5^{y}	9.0^{y}	11.7^{x}	0.93	0.10
CuZn	5.3^{b}	4.4^{b}	6.9^{a}	0.64	0.02
Ratio[5]	0.63	0.68	0.63	0.033	0.50
Day 3					
Total	17.6	15.3	15.6	1.01	0.20
Mn	11.9^{x}	10.4^{xy}	9.2^{y}	0.80	0.07
CuZn	5.9	4.7	6.3	1.05	0.54
Ratio[5]	0.67	0.69	0.61	0.052	0.47

[1]CON = steers remained in home pens with ad libitum access to feed and water; DEPR = steers remained in home pens but were deprived of feed and water for approximately 18 h; TRANS = steers were transported on a single commercial livestock trailer for approximately 18 h (1,790 km) with no access to feed or water for the duration of transit

[2]One unit (U) = the amount of enzyme required to dismutate 50% of the superoxide radical

[3]Highest SEM of any treatment reported

[4]Day -4 values utilized as covariates in analysis of subsequent sampling days

[5]Ratio = Mn-SOD/Total-SOD

[a,b]Within a row, means with unlike superscripts differ ($P \leq 0.05$)

[x,y]Within a row, means with unlike superscripts tend to differ ($P \leq 0.10$)

Table 6. Biomarker analysis for liver and muscle comparing CON and TRANS-steers[1]; area under the curve[2] values for each metabolite shown in parentheses

Liver		Muscle	
CON	TRANS	CON	TRANS
Day 1[3]			
L-Proline (0.81)[a] L-Alanine (0.75)[a] L-Leucine (0.75)[ab] L-Lactic acid (0.75) 4-Hydroxyproline (0.74) Allose (0.71) L-Valine (0.71)[ab]	L-Glutamic acid (0.87) Palmitic acid (0.87)[c] Phosphoric acid (0.79) Alpha-tocopherol (0.76) Oleic acid (0.76)[c] L-Cystine (0.75) Myo-Inositol (0.74) Arachidonic acid (0.70)[c]	L-Proline (0.74) 2-Alpha-aminobutyric acid (0.71)	Gluconic acid (0.93) Acrylic acid (0.85) D-Fructose (0.81) Ornithine (0.78) L-Threonine (0.77)[a] Niacinamide (0.76) Glycine (0.76)[a] β-Alanine (0.75) O-Phosphoethanolamine (0.74) Glycolic acid (0.73) L-Aspartic acid (0.72)[a] L-Tryptophan (0.72)[a] Glycine (0.72)
Day 3			
Niacinamide (0.76) Myristic acid (0.74) Citric acid (0.73) Hydroxylamine (0.73)	D-Fructose (0.75) 3-Hydroxybutyric acid (0.73) D-Mannose (0.73) Maleic acid (0.71) Cholesterol (0.70)	Heptadecanoic acid (0.72) Succinic acid (0.71)	Gluconic acid (0.88) D-Ribose (0.85) D-Maltose (0.79) Urea (0.78) L-Lysine (0.74) Niacinamide (0.72)

[1]CON = steers remained in home pens with ad libitum access to feed and water; TRANS = steers were transported on a single commercial livestock trailer for approximately 18 h (1,790 km) with no access to feed or water for the duration of transit

[2]Area under the curve values indicate utility of individual metabolites as biomarkers where 0.9-1.0 = excellent, 0.8-0.9 = good and 0.7-0.8 = fair (Xia et al., 2013)

[3]Day 1 samples collected immediately after treatment termination

[a]Metabolite is a pathway compound in aminoacyl-tRNA biosynthesis

[b]Metabolite is a pathway compound in valine, leucine and isoleucine biosynthesis

[c]Metabolite is a pathway compound in biosynthesis of unsaturated fatty acids

Table 7. Biomarker analysis for liver and muscle comparing DEPR and TRANS-steers[1]; area under the curve[2] values for each metabolite shown in parentheses

Liver		Muscle	
DEPR	TRANS	DEPR	TRANS
Day 1			
D-Mannose (0.94)	Ribose (0.76)		Glycine (0.75)
4-Hydroxyproline (0.92)			Phosphoric acid (0.71)
L-Leucine (0.92)[ab]			
L-Proline (0.88)[a]			
L-Alanine (0.86)[a]			
L-Valine (0.86)[ab]			
L-Alpha-aminobutyric acid (0.78)			
L-Serine (0.78)[a]			
Inosine (0.77)			
L-Lactic acid (0.76)			
Phosphoric acid (0.76)			
L-Threonine (0.75)[ab]			
L-Tyrosine (0.75)[a]			
Urea (0.75)			
Myo-inositol (0.73)			
Allose (0.72)			
L-Isoleucine (0.72)[ab]			
Arabinofuranose (0.71)			
Day 3			
Carbamic acid (0.79)	L-Cystine (0.77)	Cyanuric acid (0.70)	Gluconic acid (0.78)
Niacinamide (0.72)	L-Threonine (0.77)		Serine (0.78)
L-Alanine (0.72)	Myo-inositol (0.73)		3-Hydroxybutyric acid (0.75)
Uridine (0.71)	Acrylic acid (0.72)		D-Ribose (0.75)
	Arachidonic acid (0.71)		4-Hydroxyproline (0.73)
	Ornithine (0.70)		Urea (0.72)

[1]DEPR = steers remained in home pens but were deprived of feed and water for approximately 18 h; TRANS = steers were transported on a single commercial livestock trailer for approximately 18 h (1,790 km) with no access to feed or water for the duration of transit

[2]Area under the curve values indicate utility of individual metabolites as biomarkers where 0.9-1.0 = excellent, 0.8-0.9 = good and 0.7-0.8 = fair (Xia et al., 2013)

[3]Day 1 samples collected immediately after treatment termination

[a]Metabolite is a pathway compound in aminoacyl-tRNA biosynthesis

[b]Metabolite is a pathway compound in valine, leucine and isoleucine biosynthesis

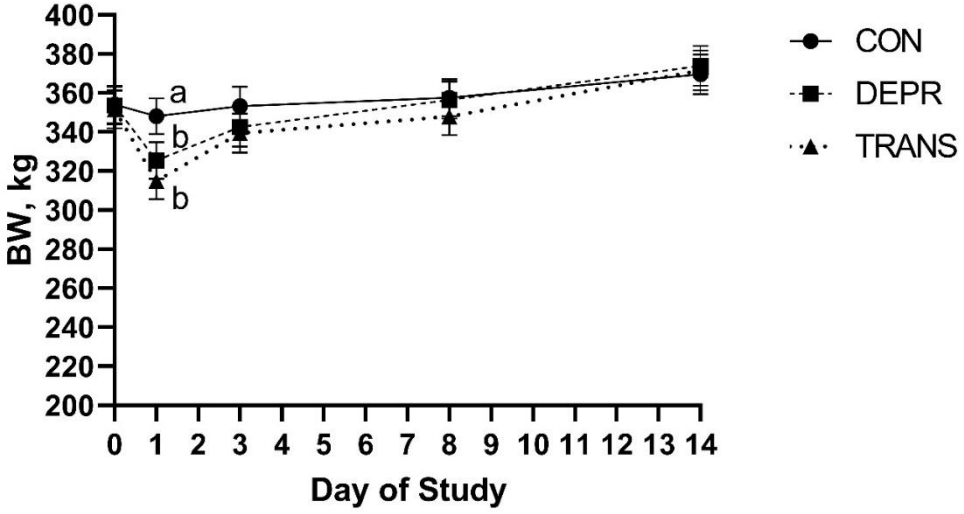

Figure 1. Effect of treatment and day on steer body weight (BW; treatment × day $P < 0.01$). Control (CON) steers remained in home pens with ad libitum access to feed and water, deprived (DEPR) steers remained in home pens but were deprived of feed and water for approximately 18 h and TRANS steers were transported on a single commercial livestock trailer for approximately 18 h (1,790 km) with no access to feed or water for the duration of transit. Treatments were initiated on d 0 and terminated on d 1. Unlike superscripts indicate a difference ($P \leq 0.05$) between treatments within day of study.

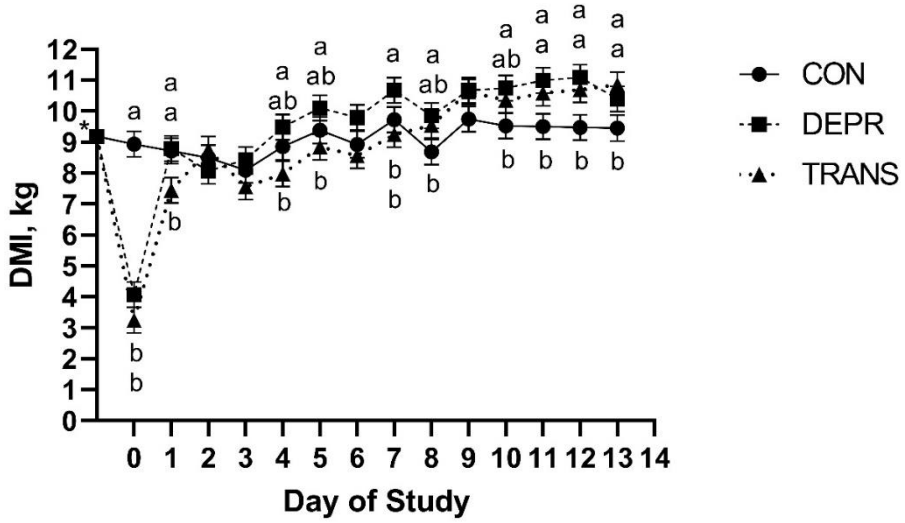

Figure 2. Effect of treatment and day on steer dry matter intake (DMI; treatment × day $P < 0.01$). Control (CON) steers remained in home pens with ad libitum access to feed and water, deprived (DEPR) steers remained in home pens but were deprived of feed and water for approximately 18 h and TRANS steers were transported on a single commercial livestock trailer for approximately 18 h (1,790 km) with no access to feed or water for the duration of transit. Treatments were initiated on d 0 and terminated on d 1. Unlike superscripts indicate a difference ($P \leq 0.05$) between treatments within day of study. *Pre-trial DMI (d -7 through -1) was used as a covariate in analysis.

CHAPTER 7. GENERAL CONCLUSIONS

The first objective of this research was to gain a better understanding of how stress, associated with feedlot receiving and/or transit, influence ROS producing pathways and cellular antioxidants in beef steers. Newly received cattle frequently experience periods of feed and water deprivation during marketing and transportation to a feedlot. Cattle may also self-restrict feed intake upon arrival if they have not been adapted to a feed bunk or to the feedstuffs that are offered. Feed deprivation leads to a decline in blood glucose concentrations and depletion of liver glycogen stores, which increases blood glucagon and decreases blood insulin. This hormonal profile stimulates lipolysis and increases circulating concentrations of non-esterified fatty acids (Perea et al., 1995). In chapter 5, serum non-esterified fatty acid concentrations of beef steers were increased over 200% immediately after an 18 h transit event, providing evidence that long-distance transit stimulates mobilization of lipid reserves. Feedlot receiving and transit also involve the psychological stress associated with handling and exposure to novel environmental stimuli. Psychological stress activates two hormonal pathways: 1) the sympathetic-adrenal-medullary pathway which stimulates an acute increase in heart rate, blood pressure and hepatic glycogenolysis and 2) the hypothalamic-pituitary-adrenal pathway which exerts several effects on peripheral tissues including regulation of glucose metabolism, inhibition of protein synthesis and stimulation of proteolysis (Baxter and Forsham, 1972). Aerobic metabolism of mobilized nutrients in response to these hormonal stimuli contribute to mitochondrial ROS production due to increased electron flow through the electron transport chain and subsequent leakage onto oxygen to form superoxide radicals.

Glucocorticoid hormones (i.e. cortisol) are the main effector molecules of the neuroendocrine stress response and thus, are commonly measured indicators of stress in cattle.

However, cortisol concentrations did not increase in steers subjected to feed restriction (chapter 3 and 6) or long-distance transit (chapter 6). Alternatively, the neutrophil to lymphocyte ratio, also an indicator of the neuroendocrine stress response, was increased in transported steers compared to ad libitum-fed control steers or steers deprived of feed and water (chapter 6). Neutrophils contribute to ROS production due to their phagocytic mechanism. Stressful production scenarios frequently experienced by newly received cattle such as vaccination, fasting and transport have also been shown to stimulate inflammation in cattle (Arthington et al., 2008; Cappellozza et al., 2011; Arthington et al., 2013). In chapters 5 and 6, an 18 h transit event elicited an inflammatory response, evidenced by increased concentrations of the acute phase protein haptoglobin. In both studies, haptoglobin concentrations were elevated immediately post-transit and peak concentrations were observed 24 h (chapter 5) or 48 h (chapter 6) post-transit before returning to pre-transit values by d 7 or 8. Transported steers also exhibited a more robust inflammatory response than steers simply deprived of feed and water (chapter 6). Inflammation increases ROS production by activating neutrophils and stimulating eicosanoid biosynthesis. In chapter 6, arachidonic acid, which serves as a precursor for eicosanoid biosynthesis, was identified as a potential biomarker in the liver of transported steers.

Several studies presented herein provide evidence that stress associated with feedlot receiving, feed and water deprivation or long-distance transit affects cellular antioxidants. In chapter 3, steers that entered the feedlot with adequate serum VE status (4.0 mg/L) declined to marginal status (2.7 mg/L) within 26 d when they did not receive supplemental VE. Others have also observed decreases in VE status in response to receiving period stress (Carter et al., 2005) and transit stress (Chirase et al., 2001). In chapter 5, a 10% decrease in plasma ascorbate concentrations and ferric reducing antioxidant potential, an indicator of total antioxidant

capacity, was observed in beef steers immediately after a long-distance transit event. While concentrations of nonenzymatic antioxidants have been shown to decrease in response to stress, superoxide dismutase activity has generally increased. Specifically, red blood cell lysate Mn-SOD activity was increased 24 h post-transit (chapter 4), liver total-SOD activity was increased immediately after an 18 h feed and water deprivation or transit event (chapter 6) and muscle total, Mn and CuZn-SOD activity was increased immediately post-transit (chapter 6). Gene transcription of antioxidant enzymes is under redox regulation so, when ROS are elevated, transcription of antioxidant enzymes increases and could contribute to the increase in antioxidant enzyme activity noted in these studies. It is also worth noting that neuroendocrine stress (adrenocorticotropic hormone; Chinn et al., 2002) and inflammation (lipopolysaccharide, interleukin-1, tumor necrosis factor; Visner et al., 1990) have been shown to upregulate transcription of Mn-SOD. In an attempt to maintain homeostasis, both neuroendocrine and inflammatory pathways are activated in response to a long-distance transit event, suggesting antioxidant enzymes play an important role in how cattle respond to and recover from transit. As a result of receiving period and/or transit stress, the activation of ROS producing pathways coupled with a depletion of cellular antioxidants could culminate in oxidative stress.

Although several studies have indicated negative effects of oxidative stress on efficiency at the cellular level, the impact of oxidative stress on growth performance and production efficiency at the whole animal level is not well understood. Observations from this research indicate oxidative stress, and ultimately oxidative stress modulation, can influence producer profitability and sustainability. For instance, steers with greater liver glutathione concentrations prior to a long-distance transit event exhibited greater ADG and G:F for the first 30 d post-transit (chapter 4). Additionally, steers administered an injection of a water-soluble antioxidant (VC)

prior to a long-distance transit event exhibited greater ADG for the 56-d post-transit period (chapter 5). Thus, improving antioxidant status prior to a stressful event, such as transit, could be beneficial in improving growth rates and decreasing days on feed. Most of the markers of neuroendocrine stress, inflammation and oxidative stress presented herein returned to baseline by d 7 or 8, yet longer term growth responses have been observed. Although the effects of transit on muscle physiology of beef cattle has not been well defined, oxidative stress in the muscle may be influencing cattle growth. In chapter 6, lesser Mn-SOD activity was observed in the muscle of steers 48 h after a long-distance transit event while metabolites of the pentose phosphate pathway were increased, which could indicate residual oxidative stress. Because cattle may receive a growth stimulating anabolic implant within 48 h after feedlot arrival, it is worth investigating how oxidative stress, particularly in the muscle, may impact the response to growth promoting technologies.

The second objective of this research was to determine how various nutritional supplements would influence antioxidant defense as well as feedlot health and performance. When dietary VE, a lipid soluble antioxidant, was supplemented to newly received beef steers for 28 d at increasing doses (0 IU/kg DM, 25 IU/kg DM, 500 IU/steer daily or 1,000 IU/steer daily), VE status was linearly improved (chapter 3); however, liver concentrations of the endogenous antioxidant glutathione were linearly decreased. Moreover, there was a tendency for a quadratic effect of VE on liver Mn-SOD activity at the end of the trial, driven by greatest activity for steers supplemented 500 IU VE/d and lesser activity for steers supplemented 1,000 IU VE/d. Other cellular antioxidants, such as glutathione and VC, are required to recycle VE to its reduced state (Rietjens et al., 2002). Thus, lesser glutathione concentrations for steers supplemented VE at 1,000 IU/d may have resulted in greater buildup of the oxidized VE radical,

leading to a prooxidant cellular environment and subsequent inactivation of Mn-SOD. Dose was also an influential factor when steers were supplemented a SCFP at 0, 12 or 18 g·steer^{-1}·day^{-1} (chapter 4). Sixteen days after dietary supplementation began, a quadratic effect of SCFP on liver glutathione concentrations was observed, driven by greater concentrations for steers supplemented SCFP at 12 g·steer^{-1}·day^{-1} (the manufacturer's current recommended dose for receiving cattle). A similar quadratic response of SCFP on red blood cell lysate glutathione concentration in beef steers was observed by Deters et al. (2018). Although there is evidence that SCFP have anti-inflammatory and antioxidant properties, higher doses of SCFP may be proinflammatory (Jensen et al., 2008), resulting in increased ROS production and depletion of endogenous antioxidants. These data suggest over supplementation of exogenous antioxidant nutrients could be detrimental to endogenous antioxidant defense mechanisms.

Timing of nutritional supplementation also influences the response to supplementation. In chapter 3, greater ADG and G:F for steers supplemented VE at 500 IU/d was not observed until the second half of the trial (d 14 to 27). Additionally, positive effects of VE on BVDV type 1 antibody titers were observed on d 26 but not 14, suggesting a lag time to improve status is needed. Alternatively, no effects of timing were observed when steers were supplemented SCFP at 18 g·steer^{-1}·day^{-1} for 19 d prior to a long-distance transit event and for 58 d post-transit or only supplemented SCFP at 18 g·steer^{-1}·day^{-1} during the post-transit period (chapter 4). It is possible timing would have affected the response to SCFP supplementation if the lesser dose (12 g·steer^{-1}·day^{-1}) had been used to test this hypothesis, as the lesser dose positively influenced steer antioxidant defense and receiving period performance. In chapter 5, beef steers were administered an intramuscular injection of VC, before or after a long-distance transit event. This supplementation strategy was chosen as a quick and effective method to increase VC status.

Steers that received VC pre-transit did not show a decline in plasma ascorbate concentrations post-transit and exhibited greater ADG over the 56 d post-transit period than steers that received no VC or steers that received VC immediately post-transit. Although a post-transit VC injection increased plasma ascorbate concentrations and post-transit DMI, growth rate and final BW for these steers were similar to control steers. Based on these data, timing considerations for different oxidative stress modulation strategies should include length of time required to influence antioxidant status and proactive (pre-stress) versus reactive (post-stress) supplementation. The second consideration is particularly important as calves often change ownership and are exposed to numerous stressors between the cow-calf and feedlot sectors.

Collectively, the data in this dissertation support the overall hypothesis that cattle exposed to stress, whether that be feedlot receiving or specifically transit, are at greater risk for developing oxidative stress. Despite some clues that oxidative stress can be detrimental to feedlot performance and improving antioxidant defense can help overcome this, further research is needed to better understand the long-term implications of oxidative stress on cattle health and performance. In order to do so, efforts should be made to identify appropriate oxidative stress biomarkers and establish a reference panel for cattle. Further refinement of the nutritional strategies utilized herein is also needed to optimize dose and timing of supplementation for oxidative stress modulation. Classifying the type of cattle (age, nutritional background, health status, stress exposure) that would benefit most from oxidative stress modulation could help beef producers make more strategic decisions about implementing these nutritional strategies. With regards to transit stress, transit duration will likely influence the oxidative stress response and warrants further investigation.

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