THE EFFECTS OF ANTI-PHOSPHOLIPASE A2 ANTIBODY SUPPLEMENTATION ON FEED EFFICIENCY, ANIMAL PERFORMANCE, AND THE ACUTE PHASE RESPONSE OF BACKGROUND AND FINISHING BEEF CATTLE DIETS

By

VITOR RODRIGUES GOMES MERCADANTE

A THESIS PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE

UNIVERSITY OF FLORIDA

2012

To my grandfather, Oripes

ACKNOWLEDGMENTS

I am deeply thankful to Dr. G. Cliff Lamb for allowing me the opportunity to come to UF, and for believing in my potential when he invited me to work in his group during my Masters program. Throughout the years he has been much more than just an advisor, he is a great mentor, a role model and a good friend. I would also like to thank Dr. Nicolas DiLorenzo, he is an outstanding researcher who has been extremely helpful during my research trials and became a dear friend. In addition, thank you to Dr. John D. Arthington for all support, friendship and many good laughs! I consider myself extremely lucky and blessed for having committee members that were deeply involved in my formation allowing me to learn so much and grow as a professional and as a person. For that I will always be grateful.

My desire to further my studies came during my training in vet school, under the influence of Dr. Vasconcelos, “Zequinha”. He was my first advisor, and introduced me to research and extension. It was through him that I met Dr. Lamb and came to the U.S.A on an internship program. Zequinha is a great man with strong values, and I’m very proud to say that I was one of his students and that he is a personal friend.

During the past two years I have made many good friends. My incredible lab mates Dr. Guilherme Marquezini, Tera Black, Kalyn Waters, Darren Henry, and Francine Messias. We worked as true team, and together we have made collecting samples, working cattle and even milking beef cows a lot more fun! Also, thank you to Philipe Moriel for his friendship and for helping me with lab work. To all my friends in the Animal Sciences Department, the amazing NFREC crew in Marianna, and all others

who are just too many to list here. I am eternally thankful for the support and good moments we shared. You all have made grad school a lot easier for me!

I cannot thank my family enough for the unconditional support throughout the years. My father Guilherme, my mother Ângela, and my brothers Rafa and João are always present and very close to me even when thousands of miles away. My beloved grandparents Oripes and Gija, who are the reason I am passionate about agriculture. Thanks to my in-laws Hans and Waleria, for the friendship and kindness. And lastly, my wife Paula, who is my best friend and the love of my life! She is the fuel that keeps me going and my drive to succeed in life.

TABLE OF CONTENTS

page

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AA	Arachidonic acid
ACTH	Adrenocorticotropic hormone
ADF	Acid detergent fiber
ADG	Average daily gain
aPLA2	Anti-phospholipase A2
aPLA2 0.2%	aPLA2 supplementation at 0.2% of diet DM treatment
aPLA2 0.4%	aPLA2 supplementation at 0.4% of diet DM treatment
APP	Acute-phase proteins
APR	Acute-phase response
CON	Control treatment
CP	Crude protein
cPLA2	Cytosolic phospholipase A2
CV	Coefficient of variation
DM	Dry matter
DMI	Dry matter intake
FCR	Feed conversion ration
FE	Feed efficiency
GH	Growth hormone
IGF-I	Insulin-like growth factor-I
IL-1	Interleukin 1
IL-6	Interleukin 6
LED	Light-emitting diode
LPS	Lipopolysaccharides

MT	Monensin and tylosin supplementation treatment
NDF	Neutral detergent fiber
PLA2	Phospholipase A2
PUFA	Poly unsaturated fatty acids
RFI	Residual feed intake
sPLA2	Secretory phospholipase A2
TDN	Total digestible nutrient
TLR	Toll like receptors
TMR	Total mixed ration
TNF-α	Tumor necrosis factor-alpha
WBC	White blood cells count

Abstract of Thesis Presented to the Graduate School
of the University of Florida in Partial Fulfillment of the
Requirements for the Degree of Master of Science

THE EFFECTS OF ANTI-PHOSPHOLIPASE A2 ANTIBODY SUPPLEMENTATION ON FEED EFFICIENCY, ANIMAL PERFORMANCE, AND THE ACUTE PHASE RESPONSE OF BACKGROUND AND FINISHING BEEF CATTLE DIETS

By

Vitor Rodrigues Gomes Mercadante

December 2012

Chair: Graham Cliff Lamb
Co-chair: Nicolas DiLorenzo
Major: Animal Sciences

To determine whether supplementation of anti-phospholipase A2 antibody (aPLA2) animal performance, and the acute phase response of transportation, and background and finishing beef cattle diets, two experiments were conducted. Experiment I: Individual performance and daily dry matter intake (DMI) was measured on 70 cross-bred weaned calves during a 70-d period using a GrowSafe system (GrowSafe Systems Ltd., Alberta, Canada) at the University of Florida NFREC Feed Efficiency Facility (FEF). Calves were fed a growing diet, and were blocked by weight and sex, and then randomly assigned to pens to receive either no additional supplement (CON, n = 35) or receive a supplement at an inclusion rate of 0.6% of estimated daily DMI of aPLA2 antibody (aPLA2; n = 35). After the 70-d feed efficiency (FE) trial calves were loaded into a commercial livestock trailer and were driven for ≈ 1.600 km during 24 hr. Upon return to the FEF, calves were relocated to the same pens and groups, and received the same diets and treatments. Blood samples from each calf were collected on days 0, 1, 3, 5, 7, 14, 21 and 28 relative to transportation and were analyzed for determination of concentrations of plasma ceruloplasmin and haptoglobin. Initial BW

(242.0 ± 3.7 kg; *P* = 0.92), BW d 70 (313.0 ± 4.1 kg; *P* = 0.79), and ADG (1.01 ± 0.02 kg; *P* = 0.95) were similar between treatments. However, daily DMI was greater (*P* = 0.01) for CON (8.53 ± 0.15 kg) than aPLA2 (9.18 ± 0.15 kg). In addition, RFI was greater (*P* = 0.002) for CON (0.389 ± 0.110 kg/d) than aPLA2 calves (-0.272 ± 0.110 kg/d). After transportation there were no differences between treatments on BW loss (26.0 ± 0.6 kg; *P* = 0.86), BW d 28 (339.0 ± 4.1 kg; *P* = 0.72), ADG (1.28 ± 0.03 kg/d; *P* = 0.72), G:F (0.164 ± 0.004; *P* = 0.83), and concentrations of plasma haptoglobin (0.08 ± 0.02; *P* = 0.41). However, concentration of plasma ceruloplasmin was greater (*P* < 0.001) for CON calves (14.3 ± 0.3) compared to aPLA2 calves (13.0 ± 0.3). Experiment II: Individual daily DMI was measured on 80 cross-bred steers during a 141-d period at the FEF. On d 0, steers were blocked by BW and randomly assigned to receive a growing forage diet containing the following treatments: 1) no additive (CON; n = 20); 2) 30 mg of monensin and 8.8 mg of tylosin per kg of diet DM (MT; n = 20); 3) same as CON, but including aPLA2 at 0.4% of the diet DM (BB0.4%; n = 20); 4) same as CON, but including aPLA2 at 0.2% of the diet DM (BB0.2%; n = 20). On d 60 steers were transitioned into grain-based diet (90% concentrate) over a 21 d ′step-up′ period while continuing to receive their supplement treatments, and were maintained on the high-grain diet until the end of the trial on d 141. On d 0, d 60, d 81, and d 141 individual shrunk BW was recorded. Blood samples were collected on d 60, 63, 65, 67, 70, 72, 74, 77, 79, 81, and 84, and for determination of concentration of plasma ceruloplasmin, haptoglobin, and blood differentials. No treatment differences were detected on overall performance, BW on d 141 (388.0 ± 5.1 kg, *P* = 0.79), ADG (1.25 ± 0.02 kg/d, *P* = 0.33), average daily DMI (7.1 ± 0.1 kg, *P* = 0.43), and RFI (-1.25 ± 0.06 kg/d, *P* = 0.61).

However, during the growing forage diet period daily DMI tended (P = 0.07) to be lower for aPLA2 0.2% and 0.4% treatments (6.51 ± 0.25 kg and 6.70 ± 0.25 kg, respectively) compared to CON (7.38 ± 0.25 kg) treatment, with MT (7.09 ± 0.25 kg) treatment being intermediate. Steers from aPLA2 0.2% and aPLA2 0.4% treatments (-0.12 ± 0.13 kg/d and -0.22 ± 0.13 kg, respectively) had lower (P < 0.05) RFI than CON (0.31 ± 0.13 kg/d) steers, with MT (0.05 ± 0.13 kg/d) steers being intermediate. During the grain-based diet period, the aPLA2 0.2% (-0.12 ± 0.10 kg/d), aPLA2 0.4% (0.36 ± 0.10 kg/d), and MT (0.10 ± 0.10) steers had greater (P = 0.04) RFI than CON (-0.37 ± 0.10 kg/d) steers. During the transition to grain-based diet phase WBC were greater (P = 0.04) for aPLA2 0.2% (13.61 ± 0.42 k/µL) than aPLA2 0.4% and MT treatments (12.16 ± 0.42 and 12.37 ± 0.42 k/µL, respectively), with CON being intermediate (12.87 ± 0.42 k/µL), and concentrations of lymphocytes also were greater (P = 0.01) for aPLA2 0.2% (7.66 ± 0.28 k/µL) than aPLA2 0.4% and MT treatments (6.71 ± 0.28 and 6.70 ± 0.28 k/µL, respectively), with CON being intermediate (7.11 ± 0.28 k/µL). Concentrations of plasma ceruloplasmin was reduced (P < 0.0001) for CON (22.2 ± 0.8 mg/dL) steers compared to aPLA2 (24.4 ± 0.8 mg/dL) treatments, and concentrations of plasma haptoglobin was reduced (P < 0.05) for CON (0.18 ± 0.05 mg/mL) steers compared to aPLA2 (0.26 ± 0.05 mg/mL) treatments. Mean daily DMI was negatively correlated with concentrations of plasma ceruloplasmin and haptoglobin. In conclusion, beef cattle supplemented with aPLA2 had improved FE when fed growing forage diets, but not grain-based diets, and had reduced concentrations of plasma ceruloplasmin after 24 hr transportation. However, CON steers had reduced concentrations of plasma ceruloplasmin and

haptoglobin during the transition to grain-based diet compared to steers on aPLA2 and MT treatments.

CHAPTER 1
INTRODUCTION

In a world where the population continues to grow, global agriculture will need to increase its production by 70% in order to feed the projected 9.1 billion people by 2050. Of the production increase, 80% will need to come from increases in yields, and only 20% from expansion of land (FAO, 2009). To fulfill the demand for food, more specifically beef, efficiency needs to be a primary focus of the beef production chain. Efficiency can be defined as the ability to accomplish a task with a minimum expenditure of time and energy, and it is commonly presented as a ratio of outputs to inputs. Many different measures of efficiency can be applied to beef production systems and feed efficiency (FE) is one of those.

Rising feed costs, global competition, and societal concerns about energy policy and the environment have created new economic challenges for the beef industry, since nearly two-thirds of the costs of producing beef is directly tied to the cost of feed inputs (Arthur and Herd, 2005). In order to achieve FE and more efficient body weight gain, feedlots in the United States have developed unique feeding and management strategies. The combination of extensive grain processing with greater consistency in quality of grain feeds compared to roughages, and the necessity to achieve greater productivity for increased demand in beef, lead feedlots to utilize diets with decreased concentrations of roughage (Galyean et al., 2011). Greater demands for grain in ethanol production and export markets associated with recent droughts have caused decreased grain production, resulting in increased global grain prices. Thus, feedlots and stocker cattle operations need to focus on optimizing grain utilization through processing, finding alternative energy sources, selection of more feed efficiency animals and

development of strategies to improve FE while maintaining high performance (Galyean et al., 2011).

In addition to the economic benefits of improving overall FE of beef production, there is an important environmental benefit. Manure is an inevitable byproduct of livestock production and feed intake and manure output have a strong positive relationship. Reduction in greenhouse gas emissions from enteric fermentation in cattle is a high priority, plus enteric methane formation represents an energy loss to the animal; thus, improving FE is a novel way of reducing feed costs, methane production, and nitrogen excretion without compromising growth rates and the economic viability of beef systems (Hegarty et al., 2007).

The use of antibiotics has greatly impacted animal agriculture by improving FE and productivity of animal protein production systems. However, antimicrobial resistance is a growing public issue and the use of antibiotics as growth promotants seems to be subjected to future limitations and more restricted regulations (Galyean et al., 2011). The addition of antibiotics to cattle diets modifies the rumen micro-flora, improving rumen fermentation (Richardson et al., 1976). However, there is also a positive effect on animal health, with decreased acidosis and rumenitis (Nagaraja and Titgemeyer, 2007). The activation of the immune system is costly in terms of animal growth and FE, with redirection of nutrients and consumption of body reserves of energy and protein (Johnson, 1997). Novel strategies using avian derived antibodies to modulate and reduce the activation of the immune system have been successfully used to improve performance and FE of livestock (Cook, 2011).

Therefore, the development of alternative strategies and novel technologies to improve FE and sustain animal performance, ensuring the economic viability of beef production enterprises and the supply of the global growing demand for beef, are of vital importance and need further investigation.

CHAPTER 2
LITERATURE REVIEW

Quantification of Feed Efficiency

The relationship between feed intake and its utilization by the animal relies on the complexity of biological processes and interactions with the environment. Thus, selection of cattle based solely on feed intake is rarely used (Arthur and Herd, 2005). The complexity to determine the energetic efficiency of cattle has resulted in multiple measurements of FE to be developed, such as feed conversion ratio (FCR; Brody, 1945), residual feed intake (RFI; Koch et al., 1963), partial efficiency of growth (Kellner, 1909), relative growth rate (Fitzhugh, Jr. and Taylor, 1971) and the Kleiber ratio (Kleiber, 1947) (Nkrumah et al., 2004).

Feed Conversion Ratio

Feed conversion ratio is the most common measurement of FE, due to its ease of calculation, and it is also referred to as feed to gain. It is the ratio between the feed consumed to the amount of body weight gain over a specific period of time (Brody, 1945). In beef cattle, FCR has been reported to be highly correlated with growth (Archer et al., 1999), and as a selection tool FCR has the potential to increase growth rate in growing animals. However, selection for FCR could also result in a larger mature size of the herd, with consequent increased feed intake (Nkrumah et al., 2007). Therefore, selection for FCR may result in unfavorable effects on overall production system efficiency (Archer et al., 1999; Nkrumah et al., 2004).

Residual Feed Intake

The concept of RFI was first used to adjust feed intake for body weight and weight gain, dividing feed intake in two portions, the expected feed intake for a given level of

performance, and the residual portion. The residual portion may be used to identify animals that deviate from their expected feed intake, with animals having negative RFI values being more efficient (Archer et al., 1999). However, differences in RFI have been associated with differences in carcass fatness, carcass leanness, and meat quality, and although the results in the literature are inconsistent, a reduction in carcass merit due to selection for lower RFI animals may not be desirable to the beef cattle industry (Herd et al., 2003; Nkrumah et al., 2004, 2007).

Immune System

The immune system has evolved to protect the organism from pathogens and generates an variety of cells and molecules capable of specifically recognizing and eliminating foreign invaders and cancerous cells, all of which act together in a dynamic network (Kindt, 2007). There are two types of immunity that collaborate to protect the organism, innate immunity and adaptive immunity.

The innate immunity is less specific and provides the first line of defense against invading pathogens (Lippolis, 2008). The innate immune system includes physical, chemical, and cellular barriers. The key cells that play a role in the innate immune response are neutrophils, macrophages, monocytes, natural killer cells, dendritic cells, and cells that release inflammatory mediators, such as basophils, mast cells and eosinophils (Kindt, 2007; Carroll and Forsberg, 2007).

Adaptive immunity is highly specific and capable of recognizing and eliminating specific antigens (Kindt, 2007). The adaptive immunity is further subdivided into humoral and cell-mediated. Humoral immunity is mediated by B-lymphocytes, which produce antibodies specific to antigens and become memory cells. In cell-mediated

immunity, the T-lymphocytes provide protection against intracellular pathogens and tumor cells (Galyean et al., 1999).

Although the innate and adaptive immune systems are classified as distinct, they do not operate independently, but rather in conjunction with one another and with help of molecules and nonimmune cells in a complex network at the initiation of the inflammatory response (Lippolis, 2008). The toll-like receptors (TLR) are a family of cell surface receptors that bind to various molecules specific to pathogens, and act as some of the earliest surveillance mechanisms against infections. The TLR interact with the pathogen associated molecular patterns, which are different components of bacterial cell walls, such as lipopeptides and lipopolysaccharides (Parker et al., 2007; Lippolis, 2008). Upon stimulation the TLR acts to stimulate the cell to respond to infection and facilitates cellular responses via signaling pathways. The complex interactions among these cells are mediated by regulatory proteins called cytokines. Cytokines regulate and modulate a variety of cell functions and physiological processes, including the inflammatory response (Kindt, 2007; Parker et al., 2007; Carroll and Forsberg, 2007; Lippolis, 2008).

Acute-Phase Response

The acute-phase response (APR) is an important component of the innate immune system, which is characterized by various reactions and physiological changes of the body in response to disturbances of its homeostasis, such as infections, disease, stress, and trauma (Carroll and Forsberg, 2007; Cooke and Bohnert, 2011). The body's characteristic reactions during the APR includes fever, shift in liver metabolism and gene regulation, plasma mineral alterations, and changes in behavior, such as lethargy, anorexia, hyperalgesia (Johnson, 1997), and decreased social and sexual behavior

(Cooke et al., 2009a). The APR is stimulated by the release of proinflammatory cytokines from macrophages and monocytes at the site of inflammation or infection. The initial release of proinflammatory cytokines is augmented by their paracrine actions, which cause further release of these cytokines and eventually results in a systemic release of cytokines (Carroll and Forsberg, 2007; Carroll et al., 2009b).

Cytokines

The term cytokine was originally used to distinguish a group of immunoregulatory proteins from other cellular growth factors. Unlike hormones, cytokines are not produced by specialized cells, but rather by a number of diverse cells types, such as white blood cells, T-helper cells, dendritic cells, monocytes and macrophages, but can also be secreted by other nonimmune cells in response to stimuli, such as injury, trauma, infection and stress. Likewise, there is not one specific cell type that is the sole target of most cytokines (Johnson, 1997; Kindt, 2007; Parker et al., 2007; Lippolis, 2008).

The APR is stimulated by the release of the pro-inflammatory cytokines tumor necrosis factor-α (TNF-α), interleukin 1 (IL-1), and interleukin-6 (IL-6) from macrophages and monocytes at the site of inflammation or infection (Johnson, 1997). These proinflammatory cytokines function as chemo-attractants inducing expression of adhesion molecules, which cause responding immune cells to localize to the site of infection, acting locally to amplify the cellular immune response, but they can also act systemically changing behavior, metabolism, and neuroendocrine secretions (Johnson, 1997; Carroll and Forsberg, 2007; Lippolis, 2008).

Acute-Phase Proteins

The initial release of proinflammatory cytokines is augmented by their paracrine actions resulting in their systemic release and stimulating production and secretion of the acute-phase proteins (APP) from the liver. Under normal circumstances, the APP have various biological functions, such as proteinase inhibitors, enzymatic functions, coagulation proteins, metal binding proteins, and transport proteins (Petersen et al., 2004; Carroll and Forsberg, 2007; Carroll, 2008). However, during an inflammatory response, the proinflammatory cytokines mediate the hepatocyte production of the APP leading to a dramatic change in APP concentration. The APP can be classified according to the magnitude of their increase (positive APP) or decrease (negative APP) in serum concentrations during the APR. Positive APP such as haptoglobin and ceruloplasmin are induced primarily by IL-6 and are characterized by a later increase in serum concentrations reaching its peak 24 to 48 hr for haptoglobin, and 72 to 168 hr for ceruloplasmin, after the initiation of the APR and may remain elevated as long as two weeks (Arthington et al., 2003; Eckersall and Bell, 2010). Other positive APP such as serum amyloid A, fibrinogen, and C-reactive protein are primarily induced by IL-1 and are characterized by an early increase in serum concentrations, occurring within four hours of the initiation of the APR and also have a rapid normalization of their serum levels, and negative APP such as albumin and transferrin, may have a 10 to 30% decrease in serum concentrations within 24 hr of the APR initiation (Petersen et al., 2004).

Haptoglobin has numerous biological functions, but its primarily function is to bind free hemoglobin released from damaged erythrocytes, forming stable complexes in the blood and restricting the availability of free iron to invading bacteria, reducing their

growth capacity, thus having a bacteriostatic effect (Petersen et al., 2004; Eckersall and Bell, 2010; Ceciliani et al., 2012). Ceruloplasmin is the major copper-carrying protein in the blood and also participates in iron homeostasis. Copper deficiency has been correlated with immune suppression in cattle, with decreased concentrations of plasma ceruloplasmin (Arthington et al., 1996).

In cattle, increased serum concentrations of haptoglobin and ceruloplasmin were found after experimentally induced inflammation (Carroll et al., 2009b, 2011; Cooke and Bohnert, 2011; Cooke et al., 2012b), trauma and castration (Petersen et al., 2004; Warnock et al., 2012; Ceciliani et al., 2012), inflammatory diseases and vaccination (Petersen et al., 2004; Ganheim et al., 2007; Eckersall and Bell, 2010), road transportation, weaning and commingling (Arthington et al., 2003, 2008; Fike and Spire, 2006; Qiu et al., 2007; Araujo et al., 2010), and when feeding backgrounding and finishing diets (Berry et al., 2004a; Ametaj et al., 2009; Zebeli et al., 2010).

Phospholipase A2

Phospholipase A2 (PLA2) are a complex family of potent inflammatory enzymes that are upregulated upon extracellular stimuli. The PLA2 enzymes catalyze the hydrolysis of the sn-2 position of membrane glycerophospholipids, yielding free fatty acids and lysophospholipids (Kudo and Murakami, 2002). In mammals, secretory PLA2 type IIA (sPLA2) is a potent inflammatory marker that is upregulated by endotoxins and proinflammatory cytokines, and it is recognized as one of the first line of defenses against microbial infection, playing a central role as one of the first steps that triggers the inflammatory cascade. Inflammatory effector cells such as neutrophils and macrophages store sPLA2 in secretory granules and release it upon cellular activation. The sPLA2 also has an antimicrobial capacity by causing the disruption of bacteria,

through hydrolysis of the bacterial membrane phospholipids. The sPLA2 cleaves the fatty acid located in the sn-2 position on the outer leaflet of the cell membranes, releasing free fatty acids, such as arachidonic acid (AA). The release of sPLA2 upregulates cytosolic PLA2 (cPLA2), and cPLA2 cleaves AA from the sn-2 position on the inner leaflet of the cell membrane, releasing free AA in the cytosol. Arachidonic acid acts, via cyclo-oxygenase and lipoxygenase pathways, to serve as substrate to production of eicosanoids, such as prostaglandins, leukotrienes, and thromboxanes that are potent proinflammatory mediators (Kudo and Murakami, 2002; Cook, 2004; Nevalainen et al., 2008).

In mice, sPLA2 secretion in the lumen of the gastrointestinal tract (GIT) has been reported to be stimulated by endotoxins, and its activity to have a great impact on the content and properties of the GIT phospholipid barrier, resulting in an increase in its permeability to bacterial membrane lipopolysaccharides (LPS; Rozenfeld et al., 2001; Zayat et al., 2008).

Stress and Immunity

The concept of stress was introduced in the 1930's into the medical community by Hans Selye, who proposed that regardless of the stimuli, the body's response would always be in the same physiologic manner in an effort to maintain homeostasis. The sum of all physical, emotional, or mental stimuli that disturb an individual's homeostasis are named stressors and elicit coordinated physiologic responses in order to maintain homeostasis, primarily by activation of the hypothalamic-pituitary-adrenal axis and the sympathetic nervous system (Pacák and Palkovits, 2001).

Upon exposure to stressors, corticotropin-releasing hormone and vasopressin are secreted in the hypothalamus, stimulating adrenocorticotropic hormone (ACTH)

secretion from the anterior pituitary gland, ACTH then stimulates production of steroids from the adrenal gland, cholesterol uptake and the release of glucocorticoids, such as cortisol, from the adrenal cortex (Friend, 1991). Glucocorticoids are essential for the maintenance and restoration of homeostasis by inducing metabolism of carbohydrates and proteins, regulating the growth and reproductive axes, stress responses, and the immune functions (Cooke and Bohnert, 2011). However, excessive concentrations of glucocorticoids in cattle have been linked to reduced rates of reproduction, suboptimal growth and performance, reduced milk production and suppression of immune function by reducing the production and release of various cytokines, including the pro-inflammatory cytokines IL-1, IL-6, and TNF-α (Friend, 1991; Johnson, 1997; Cooke and Bohnert, 2011).

Glucocorticoids also enhance synthesis and secretion of catecholamines, which control several physiological processes. The most important catecholamines secreted by the adrenal medulla are adrenaline, noradrenaline, and dopamine. The main effects of catecholamines consist of increased heart rate, pupil and bronchiole dilatation, vasoconstriction in the skin and gut, vasodilatation in muscles, and increases glucose production by the liver via gluconeogenesis, all of which compose the flight or fight response, also catecholamines control the stress response via regulation of ACTH release from the pituitary gland and stimulation of cortisol secretion from the adrenal cortex (Carroll and Forsberg, 2007).

Throughout the beef production cycle, cattle experience several environmental, managerial, and nutritional stressors that can negatively impact productivity. In livestock, stressors may be assigned to three categories: psychological, physical, and

physiological. Psychological stress usually is associated with fear when experiencing commingling, restraining, and exposure to new environments. Thermal stress, hunger and thirst, pain, and disease represent physical stress, whereas physiological stress results from deviations in homeostasis, such as nutrient deficiencies and endocrine disorders (Friend, 1991; Grandin, 1997). The immune system response to stress is dependent on the type of stress encountered. Acute stress usually is immunoenhancing, in a manner to prepare the body to potential infection and ultimately activation of the APR. However, chronic stress is immunosuppressive and long term exposure to glucocorticoids shifts the immune system from a preparatory into a suppressive mode (Carroll and Forsberg, 2007).

Transportation Stress

Transportation is one of the most common and intense acute physical stressors that cattle encounter throughout the beef production cycle. The event of transportation leads to physiological, nutritional, and immunological changes that affect subsequent health and performance of cattle, with decreased feed intake, weight loss, increased heart and respiration rate, increased blood concentrations of cortisol, increased concentrations of catecholamines, and activation of the APR with production and secretion of proinflammatory cytokines and APP (Loerch and Fluharty, 1999; Arthington et al., 2003; Fike and Spire, 2006; Duff and Galyean, 2007; Araujo et al., 2010). Following transportation, total white blood cell (WBC) counts and differentials are altered, with an increase in neutrophils and monocytes and decrease in lymphocytes, with a consequent increase in neutrophil to lymphocyte ratio (Ishizaki and Kariya, 2010; Hulbert et al., 2011).

Development of immunosuppression, due to chronic stress related to transportation combined with commingling and weaning stress, also may lead to increased morbidity and mortality of bovine respiratory disease in newly received feedlot steers. Negatively affecting feedlot performance and carcass merit, resulting in substantial economic losses (Galyean et al., 1999; Duff and Galyean, 2007).

Concentrate Diet Levels and Stress

Digestion of feedstuffs in the retilculorumen occurs in an anaerobic ecosystem, where microbes convert fermentable substrates into organic acids, which are then absorbed through the rumen wall. In beef cattle fed high concentrate diets, rumen pH usually ranges from 5.6 to 6.5, and fluctuates depending on intake of fermentable carbohydrates, an animals capacity to provide buffer through saliva production, and the rates of absorption and utilization of the organic acids. Rumen pH is critical for the normal function of the rumen because of its profound effects on rumen microbial populations, fermentation products and physiological functions, such as motility and absorptive functions (Nagaraja et al., 1985). When feeding high-grain diets, ruminal pH can fall below 5.6, having significant impact on ruminal function and animal health, leading to the development of acidosis and rumenitis (Nagaraja and Titgemeyer, 2007).

Endotoxin or LPS is a cell wall component of gram-negative bacteria. Death and disintegration of bacteria in the rumen are normal events and endotoxin is commonly present in rumen fluid. The rumen fluid concentration of endotoxin is greater in grain-fed compared to forage-fed cattle, due to greater quantities of gram-negative bacteria and greater rates of bacterial death caused by lower rumen pH values (Nagaraja et al., 1978a; b; Andersen et al., 1994). The development of acidosis with accumulation of endotoxins in rumen fluid may lead to inflammation and degenerative processes of the

rumen and intestinal mucosa, resulting in translocation of endotoxins into the bloodstream with activation of the APR and release of inflammatory mediators, such as AA metabolites and proinflammatory cytokines (Andersen et al., 1994; Gozho et al., 2007; Emmanuel et al., 2007, 2008; Khafipour et al., 2009; Ametaj et al., 2009; Dong et al., 2011).

Beef cattle in feed yards undergo acidotic challenges when they are transitioned from forage-based into grain-based diets. The effects of the change in diet on feed intake, rumen pH and physiological responses, such as the activation of the APR, are dependent on the percentage increase in concentrate feeds and the time allowed between diet changes for the rumen to adapt (Berry et al., 2004a; Nagaraja and Titgemeyer, 2007; Ametaj et al., 2009).

Immune Regulation of Growth

The immune system has a priority in energy and nutrient utilization relative to animal growth. Upon activation of the immune system, the organism diverts nutrients away from growth and development, redirecting it into defense processes. The redirection of nutrients is followed by an immediate decrease in feed intake, wasting of skeletal muscle, increase lipolysis, and production of APP by the liver (Johnson, 1997; Cook, 2011).

A mechanism to explain the anorectic and metabolic effects of immunological challenged livestock animals, with reduced performance and feed efficiency, was proposed and stated that pathogens and LPS stimulate leukocyte production of proinflammatory cytokines, leading to anorexia and fever by acting directly on the central nervous system, and mediating systemic activation of cytokine receptors on nonimmune tissues such as, muscle and adipose, with increased lipolysis, muscle

catabolism, hepatic production of APP, increased glucocorticoids, increased corticosteroids, reduced growth hormone (GH) and insulin-like growth factor-I (IGF-I) secretion (Johnson, 1997; Spurlock, 1997; Gifford et al., 2012).

Muscle protein degradation is mediated by IL-1, IL-6, and TNF-α in order to boost liver synthesis of APP. At least 60% of the amino acids used for hepatic synthesis of APP are derived from body protein reserves (Johnson, 1997). Although IL-1 and TNF-α may increase uptake of amino acids in vivo, only IL-6 has been shown to stimulate uptake of amino acids in vitro and is considered the primary mediator of this metabolic response to inflammation (Johnson, 1997; Spurlock, 1997). It appears that IL-1 and TNF-α stimulate hepatic production of APP indirectly, by increasing production and secretion of IL-6 (Klasing et al., 1987; Johnson, 1997; Spurlock, 1997; Gifford et al., 2012).

Nutrient intake is decreased during immune challenges, leading to a shift from accretion to mobilization of adipose tissue, with an increase in plasma triglycerides in order to sustain the energy demand of the organism. Hepatic fatty acid synthesis is increased by TNF-α, and TNF-α decreases the activity of the lipoprotein lipase enzyme on adipose tissues acting in synergism with IL-1, stimulating lipolysis and resulting in hypertriglyceridemia and increased very-low-density lipoproteins, that transport fatty acids to peripheral tissues for utilization (Johnson, 1997; Elsasser et al., 2008; Gifford et al., 2012).

In cattle, decreased concentrations of GH and IGF-1 upon immunological challenge with injection of LPS, are correlated with greater plasma concentrations of IL-1 and TNF-α (Spurlock, 1997; Carroll, 2008; Elsasser et al., 2008). Peripheral

inflammatory responses may stimulate production of proinflammatory cytokines in the brain via stimulation of vagal afferent nerves. The proinflammatory cytokines act in conjunction with each other to depress feed intake, alter social and sexual behavior and inducing fever (Johnson, 1997; Spurlock, 1997). However, IL-1 has been shown to be more potent in suppressing nutrient intake, altering glucose metabolism, and inducing behavior associated with illness, such as lethargy and depression, and TNF-α is more potent at inducing lipolysis and muscle degradation (Johnson, 1997). In addition, IL-6 is known as the primary cytokine responsible for the shift in hepatic protein synthesis towards the production of APP (Johnson, 1997; Carroll, 2008).

Strategies to Improve Performance and Feed Efficiency

Feed is a major cost to beef production systems, and in order to achieve FE and maintain adequate body weight gain and performance, beef production systems have developed unique feeding and management strategies to manipulate and improve the efficiency of rumen fermentation and overall animal health. These strategies aim to minimize the activation of the immune system and to maximize performance and feed efficiency (Loerch and Fluharty, 1999; Duff and Galyean, 2007; Cook, 2011).

Management Strategies

Upon arrive to a feedlot, beef calves have been exposed to multiple stressors that lead to physiological, metabolic and behavior responses consequently resulting in reduced nutrient intake, decreased performance and increased morbidity and mortality of diseases, such as bovine respiratory disease (Loerch and Fluharty, 1999; Duff and Galyean, 2007). Management practices of beef calves prior to entry to the feedlot involve a complete preconditioning program to ensure that animals have been weaned greater than 30 days prior to shipping. The preconditioning program usually involves

vaccination program, castration of bull calves, and acclimation to feed and water bunks, thereby reducing the stress response associated with these events once arriving at the feedlot (Duff and Galyean, 2007). In addition, strategies to improve health and performance after feedlot arrival are extremely important to ensure proper nutrient intake and immunological support, allowing achievement of high performance rates after arrival (Peterson et al., 1989; Loerch and Fluharty, 1999; Duff and Galyean, 2007).

Early weaning, at 70 days of age, followed by concentrate supplementation has been shown to reduce the APR associated with transportation and entry to the feedlot, compared to calves weaned directly before transport and feedlot entry. The early weaned steers also had improved feedlot performance, with greater average daily gain (ADG) and greater FCR than control steers (Arthington et al., 2008). Similarly in an alternative study, early weaned beef calves had decreased concentrations of pro-inflammatory cytokines followed by LPS challenge compared to normal weaned calves, and early weaned calves had higher concentrations of interferon-γ, indicating that the innate immune system of early weaned calves may be more competent at recognizing and eliminating the endotoxin (Carroll et al., 2009a).

Temperament has been shown to also impact animal performance. Animals with greater temperament scores, more aggressive or excitable, had decreased ADG and dry matter intake (DMI; Grandin, 1997). Strategies to acclimate animals to handling facilities and personnel, as well as selection for calm temperament, have been successfully used to reduce temperament scores, decrease the APR associated with handling, and consequently increase animal performance (Voisinet et al., 1997; Cooke et al., 2009a,b; 2012a).

Dietary Strategies

Diets with higher nutrient density for newly received feedlot calves have been used in order to compensate for the lower feed intake of those animals. Diets must be formulated to supply sufficient nutrients so that the animal does not use its own body reserves (Berry et al., 2004b). A series of studies using diets with high crude protein and energy contents for newly arrived feedlot cattle were reviewed, and indicated that calves receiving diets with greater concentrate and protein density, during the first weeks after feedlot entry, had increased overall performance and improved health (Loerch and Fluharty, 1999; Berry et al., 2004a; b; Duff and Galyean, 2007).

Supplementation of rumen-protected polyunsaturated fatty acids (PUFA) has been shown to modulate the immune response in cattle, with reduced concentration of APP after transportation and feedlot entry. However, PUFA supplementation had a negative impact on ADG and DMI (Araujo et al., 2010).

Concentrations of most minerals needs to be increased in receiving diets to compensate for the lower DMI of newly received calves. A number of minerals, specifically selenium, copper, zinc, and chromium, have been shown to be important for the support and maintenance of the immune system in cattle (Duff and Galyean, 2007). Mineral deficiencies can affect immunity by reducing antibody production and responses, cell mediated immunity, and NK cell activity (Carroll and Forsberg, 2007). However, it remains unclear whether mineral supplementation beyond physiological requirements enhances immunity of livestock, and the results appears to be dependent on source, organic or inorganic, and on interactions with vitamins (Galyean et al., 1999; Carroll and Forsberg, 2007; Duff and Galyean, 2007).

Antibiotics as Growth Promotants

The use of antibiotics in animal feeds as antimicrobial therapy for disease and as growth promotants has increased since the early 1950's. Several experiments in poultry revealed that antibiotic supplementation had no effect on animal performance in germ-free animals (Cook, 2004, 2011). Monensin, a carboxylic polyether ionophore antibiotic, naturally produced by strains of *Streptomyces cinnamonensis* (Haney, M E and Hoehn, 1967), selectively inhibits gram-positive bacteria, modifying the rumen flora and metabolism, improving digestive efficiency of cattle. Feeding monensin increases the rumen concentration of propionic acid and decreases concentrations of acetate and butyrate acids (Richardson et al., 1976), it also reduces the production of methane, thus enhancing animal performance from the improved retention of carbon and energy in rumen fermentation, increased digestibility of dry matter, starches and amino acids (Bergen and Bates, 1984; Beckett et al., 1998). Monensin treatment has also been successfully used to decrease the risk of acidosis and sub-acute acidosis in cattle consuming high concentrate diets, due to reduction in lactic acid concentration in the rumen (Nagaraja et al., 1985; Burrin and Britton, 1986; Nagaraja and Titgemeyer, 2007).

Recently in a meta-analysis on the effects of feeding monensin for growing and finishing beef cattle, authors concluded that monensin improved FE, reduced DMI, improved ADG, and a linear effect of monensin dose was found for FE (higher dose improving FE), DMI (higher dose reducing DMI), and for ADG (higher dose reducing ADG; Duffield et al., 2012).

Feeding high grain diets may result in the development of liver abscesses in cattle. Generally, the incidence of liver abscesses averages from 12 to 32% in most feedlots

(Nagaraja and Chengappa, 1998). Liver abscesses are a major economic liability to the beef industry, as the number one cause for liver condemnation in the United States, and even more important by its great impacts on animal performance with reduced DMI, weight gain, decreased FE, and decreased carcass dressing percentage (Meyer et al., 2009). Tylosin is an antibiotic effective primarily against Gram-positive bacteria, and its inclusion on high grain diets for finishing cattle has been shown to reduce the incidence of liver abscesses, as well as improve ADG, FE and carcass dressing percentage (Galyean et al., 1992; Stock et al., 1995; Nagaraja and Chengappa, 1998; Meyer et al., 2009).

Antibodies as Feed Additives

Public health concerns that the use of antibiotics in animal agriculture may generate microbial resistance resulted in the development of alternative strategies to manipulate the gastrointestinal micro-flora and enhance animal performance (Salyers, 2002; Cook, 2004). The use of avian antibodies to modify ruminal bacteria populations and manipulate rumen fermentation to improve animal performance and FE, is a novel technology and an alternative to the use of antibiotics for livestock diets (Cook, 2004, 2011; Cook and Trott, 2010). Feeding avian egg antibodies is a possible alternative because immunoglobulin Y (IgY), the main source of avian immunoglobulin, is resistant to heat, acid digestion, and proteolysis (Shimizu et al., 1988). Binding activity of purified IgY was reduced only when heated at 70°C for 15 minutes. Egg antibodies retained over 85% of their antirotavirus neutralizing activity at a pH of 2 for one hour, even though the antibody binding capacity was reduced by 50% (Cook and Trott, 2010). In addition, the hen offers many advantages for the production of polyclonal antibodies,

with low cost mass production and a capacity to develop effective antibodies for specific targets (Cook, 2011).

Avian-derived polyclonal antibodies targeting specific rumen bacteria populations have been used to prevent deleterious effects associated with these bacteria, improving animal performance. Polyclonal antibody preparations against *Streptococcus bovis* and *Fusobacterium necrophorum* were successfully used as feed additives to beef steers receiving high grain finishing diets, to reduce rumen acidosis and liver abscess that are related to higher counts of those bacteria (Nagaraja and Titgemeyer, 2007). Steers receiving the polyclonal antibody preparation against *Streptococcus bovis* also had increased FCR, but had decreased dressing percentage (DiLorenzo et al., 2006, 2008). An alternative study compared a polyclonal antibody preparation against numerous rumen microorganisms with supplementation of monensin and found a tendency in the reduction of liver abscess for the group receiving the antibody, and similar feedlot performance between monensin and antibody supplementation (Pacheco et al., 2012).

Antibody Against sPLA2

The sPLA2 plays an important role in endotoxin-induced prostaglandin synthesis in intestinal epithelial cells, mediating the disruption of the intestinal barrier, a potent proinflammatory mediator leading to the activation of the immune system with production of proinflammatory cytokines and the APP (Cook, 2004, 2011). An avian antibody specific against intestinal sPLA2, anti-phospholipase A2 (aPLA2) was developed to regulate the intestinal inflammatory response and the negative effects of the activation of the APR on animal growth and FE (Cook, 2011). In a series of studies with poultry, swine, and fish the inclusion of aPLA2 improved FE and growth, without increased incidence of infectious diseases (Yang et al., 2003; Cook, 2004, 2011;

Schwartz et al., 2006; Barry and Yang, 2008). However, to our knowledge no studies on the use of aPLA2 as a feed additive for cattle diets have been reported.

Rationale

The activation of the immune system with production and secretion of proinflammatory cytokines and acute phase proteins is costly in terms of energy and animal performance, with decreased ADG, DMI, and FE, establishment of fever and immediate redirection of nutrients, muscle waste, and lipolysis. Feed is a major input in beef production systems, and with feedstuff prices increasing FE is a primary concern to maintain sustainability of beef production in an economic manner. Alternative strategies to the use of antibiotics, due to increased public concern and environmental agencies with development of bacterial resistance, need to be addressed. The use of specific egg antibodies targeting proinflammatory enzymes, such as the aPLA2, blocking the APR at the intestinal level are a novel approach to improve animal performance and FE. Thus, the objective of this research was to elucidate the effects of aPLA2 supplementation on performance and feed efficiency of beef cattle receiving background and finishing diets, and the effects of aPLA2 supplementation on the acute phase response due to transportation and feeding high concentrate diets.

CHAPTER 3
EFFECTS OF INCLUSION OF ANTI-PHOSPHOLIPASE A2 ANTIBODY TO BACKGROUNDING DIETS ON PERFORMANCE, FEED EFFICIENCY AND THE ACUTE PHASE RESPONSE OF GROWING BEEF CALVES

Rising feed costs, global competition, and societal concerns about energy policy and the environment have created new economic challenges for the beef industry, since nearly two-thirds of the costs of producing beef is directly tied to the cost of feed inputs (Arthur and Herd, 2005). Thus, beef cattle operations need to focus on optimizing feed utilization through finding alternative energy sources, selection for feed efficient animals and development of strategies to improve feed efficiency (FE) while maintaining high performance rates (Galyean et al., 2011).

The relationship between feed intake and its utilization by the animal relies on the complexity of biological processes and interactions with the environment. The immune system has a priority in energy and nutrients utilization relative to animal growth. Upon activation of the immune system and the acute phase response (APR), the organism diverts nutrients away from growth and development, redirecting it into defense processes followed by immediate decrease in feed intake, wasting of skeletal muscle, increase lipolysis and production of acute phase proteins (APP) by the liver, plasma mineral alterations, fever and changes in behavior (Johnson, 1997; Cook, 2011; Cooke and Bohnert, 2011). The APR is stimulated by the release from macrophages and monocytes of the pro-inflammatory cytokines tumor necrosis factor-α (TNF-α), interleukin 1 (IL-1), and interleukin-6 (IL-6) at the site of inflammation or infection (Johnson, 1997).

Transportation is one of the most common and intense acute physical stressors that cattle encounter throughout the production cycle and leads to physiological,

nutritional and immunological changes that can affect subsequent health and performance (Loerch and Fluharty, 1999; Arthington et al., 2003; Fike and Spire, 2006; Duff and Galyean, 2007; Araujo et al., 2010). A theory explaining the mechanism by which immunological challenged livestock have poorer performance and FE, hypothesizes that pathogens and lipopolysaccharides (LPS) translocate into the bloodstream through activation of secretory phospholipase A2 (sPLA2) in the intestinal lumen, and once in the bloodstream LPS stimulates leukocyte production of proinflammatory cytokines with further activation of the APR, leading to anorectic and physiological responses that have direct effects on animal performance (Cook, 2011). The sPLA2 is a potent inflammatory marker that is upregulated by LPS and proinflammatory cytokines. Upon activation, sPLA2 up-regulates cytosolic phospholipase A2 (cPLA2) that will cleave arachidonic acid (AA) from the sn-2 position of the cell membrane. Free AA on the cytosol will then serve as substrate for production of eicosanoids such as prostaglandins and leukotrienes, that are potent inflammatory mediators (Kudo and Murakami, 2002; Cook, 2011). Feed efficiency and growth was improved in a series of studies with poultry, swine and fish using an egg-derived antibody against sPLA2 (aPLA2) as a feed additive (Yang et al., 2003; Cook, 2004, 2011; Schwartz et al., 2006; Barry and Yang, 2008). However, the use of aPLA2 as a feed additive for cattle diets has not been reported.

Therefore, the potential exists that cattle that consume aPLA2 may have decreased inflammatory response, and consequently enhanced performance and improved FE. The objective of this study was to determine whether supplementation of

aPLA2 would alter the overall performance of growing beef calves receiving a background diet, and the APR associated with transportation.

Materials and Methods

Animals and Treatments

Phase I. Seventy cross-bred weaned beef calves (steers, n = 53; and heifers, n = 17) were allocated in group pens (108 m2/pen; 2 pens with 11 animals each, and 4 pens of 12 animals each) at the University of Florida North Florida Research and Extension Center, Feed Efficiency Facility (FEF), in Marianna, FL. Calves were submitted to a 70-d feed efficiency trial and individual daily DMI was measured using the GrowSafe system (GrowSafe Systems Ltd., Alberta, Canada). Each pen in the FEF was equipped with two GrowSafe feed bunks. Calves were blocked by weight and sex and then randomly assigned to pens to receive treatments. A 21-d period of adaptation to facilities and diets preceded the 70-d FE trial. Body weight (BW) was recorded at 14-d intervals over the 70-d period.

Calves were assigned to receive a growing TMR diet (0.6 Mcal NEg/kg DM, 13% CP) containing 31% corn gluten feed pellets, 30.5% soy hulls, 3.0% molasses, 34% bahiagrass hay, 0.5% mineral supplement, 0.5% salt and 0.5% limestone (Table 3-1). Calves received either an additional supplement of estimated daily DMI containing an anti-phospholipase A2 antibody (BIG BEEF, Aova Technologies, Madison, WI; aPLA2, n = 35) at an inclusion rate of 0.6% or no additional supplement (CON, n = 35). The antibody supplement was formulated using a premix with soy hulls containing the aPLA2 at a concentration of 30%, and the antibody premix was included in the TMR diet at a 2% level.

Phase II. Immediately after completion of the 70-d feed efficiency trial, on d 0 calves were loaded into a commercial livestock trailer and driven within the state of FL for ≈ 1,600 km during a 24-hr period, before returning and being unloaded at the FEF, on d 1. Due to a limit in the maximum weight of the livestock trailer, four calves (two of each treatment, the heaviest and lightest calves) were not loaded into the trailer and were subsequently excluded from the statistical analysis for Phase II. Upon return to the FEF, calves were relocated to the same pens and groups, and received the same diets and treatments of Phase I. Body weight was determined on d 0 before shipping and upon arrival on d 1, and a shrunk BW (16 hr following removal from feed) at the final of Phase II, on d 28.

Ultrasonic Carcass Traits

Phase II. On d 28 of Phase II, ultrasound was used to determine fat thickness (BF) using an Aloka real-time ultrasound scanner (3.5-MHz linear array transducer, Aloka 500V, Corimetrics Medical Systems, Inc., Wallingford, CT) and image capturing software. Scanning was performed on the right side of each animal, between the 12th and 13th ribs. Two measurements were taken per animal at each scanning session. Final BF values were calculated as the average between the two measurements recorded.

Temperament Traits

Phase I. Temperament traits evaluated were chute score (CS) and exit velocity (EV). The subjective measurement of the behavioral response to restraint within the squeeze chute was assigned on a 1 to 5 scale (1 = calm, docile and quiet; 2 = restless; 3 = nervous; 4 = excited and flighty; 5 = aggressive) by the same trained evaluator at all measurement sessions. Exit velocity was the speed (m/s) at which each animal exited

the squeeze chute and passed by light-emitting diode (LED) optical sensors placed at a distance of 1.83m. Both traits were measured on 14 d intervals during the 70-d feed efficiency trial.

Blood Collection and Analyses

Phase II. Blood samples from each calf were collected from the jugular vein into 10 mL evacuated glass vials containing 143 IU of Na heparin (Vacutainer, Becton Dickinson Inc., Franklin Lakes, NJ) on days 0, 1, 3, 5, 7, 14, 21 and 28 relative to transportation. Blood samples were immediately placed on ice, and centrifuged at 1,500 x *g*, at 4°C for 15 min. The plasma was transferred by pipette into polypropylene vials (12mm x 75mm; Fisherbrand, Thermo Fisher Scientific Inc., Waltham, MA) and stored at -20°C until further analysis.

A spectrophotometer (ThermoSpectronic Genesys 20; Thermo Fisher Scientific Inc., Waltham, MA) was used to determine concentrations of plasma ceruloplasmin. The plasma ceruloplasmin oxidase activity was measured in duplicate samples using colorimetric procedures as described in the literature (Demetriou et al., 1974). Concentrations of ceruloplasmin were expressed as mg/dL (King, 1965). The intra and interassay coefficient of variation (CV) for ceruloplasmin were 2.1 and 4.8%, respectively. A microplate spectrophotometer (Power Wave 340; BioTek Instruments, Inc., Winooski, VT) was used to determine concentrations of plasma haptoglobin in duplicate samples by measuring haptoglobin/hemoglobin complexing by the estimation of differences in peroxidase activity as described previously (Makimura and Suzuki, 1982) and results are expressed as arbitrary units from the absorption reading at 450 nm of wavelength x 100. The intra and interassay CV for haptoglobin were 5.9 and 4.9%, respectively.

Feed Sample Collection and Analyses

Representative samples of each treatment TMR diet were taken before feeding and at 28-d intervals throughout Phases I and II. All samples were bagged and frozen immediately after collection until drying. All feed samples analyzed for nutritive values were dried at 55°C for 48 h in a forced air oven. At conclusion of the drying period all samples were ground in a Wiley mill (Arthur H. Thomas Company, Philadelphia, PA, USA) using a 1.0 mm screen. After grinding, samples were composited for analysis on an equal weight basis. The composited samples were analyzed for DM, CP, TDN, ADF, NDF, Ca and P in duplicate by a commercial laboratory using NIR procedures (Dairy One Forage Laboratory, Ithaca, NY).

Statistical Analyses

Phase I. Linear regression of BW against day on trial was used to establish ADG, using the SLOPE function of EXCEL (Microsoft, Redmond, WA). Gain to feed ratio was computed as the ratio of ADG to daily DMI. Residual feed intake was calculated as the actual DMI minus expected DMI, with expected DMI derived from the regression of actual DMI on ADG and mid-test metabolic body weight using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC).

The repeated measures statement of the MIXED procedure of SAS was used to analyze differences in CS and EV. The statistical model included treatment, day, treatment by day interaction and sex, with day as the repeated variable (d 0, 14, 28, 42. 56 and 70), animal as subject and block as random effect.

The MIXED procedure of SAS was used to identify differences in initial BW, final BW, ADG, daily DMI, G:F and RFI. The statistical model included treatment, pen within

treatment and sex. Statistical differences were reported at $P < 0.05$ and tendencies were identified when $P < 0.10$, with means being reported as LS means ± SEM.

Phase II. Individual calf ADG was determined by the difference between the final shrunk BW and the initial shrunk BW upon arrival on d 1, divided by the number of days on trial. Body weight loss (BWL) after transportation was calculated as the difference between BW on d 0 and BW on d 1, the percentage of BWL (%BWL) was calculated as the ratio between BW d 0 and BWL multiplied by 100, and G:F was calculated as the ratio of ADG to daily DMI. The MIXED procedure of SAS was used to identify differences in BW d0, BW d 1, BWL, %BWL, daily DMI, G:F and BF. The statistical model included treatment, pen within treatment and sex.

The repeated measures statement of the MIXED procedure of SAS was used to analyze differences in concentrations of haptoglobin and ceruloplasmin. The statistical model included treatment, day, sex and treatment by day interaction, with day as the repeated (d 0, 1, 3, 5, 7, 14, 21 and 28), animal as the subject and block as random effect. For differences in daily DMI after transportation, the repeated measures statement of the MIXED procedure of SAS was used. The statistical model included treatment, day, sex and treatment by day interaction, with day as repeated (d 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 and 14), animal as the subject and block as random effect. The GLM procedure of SAS was used to assess the correlation between concentrations of ceruloplasmin and haptoglobin with DMI on d 0, 1, 3, 5, 7 and 14 following transportation. Statistical differences were reported at $P < 0.05$ and tendencies were identified when $P < 0.10$, with means being reported as LS means ± SEM.

Results and Discussion

Phase I. Animal performance and FE data for Phase I are summarized in Table 3-2. There were no differences between treatments in initial BW, final BW, and ADG during Phase I. Mean daily DMI during the 70-d FE trial was significantly greater ($P < 0.01$) for CON (9.18 ± 0.15 kg) compared to aPLA2 (8.53 ± 0.15 kg). Daily DMI was greater ($P < 0.01$) for CON calves during wk 1, 2, 7, and 8, and tended ($P < 0.10$) to be greater during wk 9 and 10 of the 70 d trial, compared to calves in the aPLA2 treatment (Figure 3-1). Supplementation of aPLA2 reduced daily DMI, while maintaining similar ADG and final BW to CON calves, improving FE. Residual feed intake was significantly lower ($P < 0.01$) for aPLA2 calves (-0.272 ± 0.110 kg/d) than for CON calves (0.389 ± 0.110). There was also a tendency ($P = 0.09$) for the aPLA2 treatment to have greater G:F than the CON treatment (0.117 ± 0.003 and 0.110 ± 0.003, for aPLA2 and CON, respectively). Calves receiving aPLA2 supplementation had a 7% reduction in average daily DMI compared to CON calves (Table 3-2). Similarly, supplementation of aPLA2 improved FE and growth of broilers by 3.8 and 5.3%, respectively (Cook, 2004, 2011). Several other reports of improvements on FE and growth of swine and fish supplemented with aPLA2 have been reported, without increasing morbidity and mortality due to infectious diseases (Yang et al., 2003; Schwartz et al., 2006; Barry and Yang, 2008).

Germ-free and antibiotic fed chickens have decreased thickness and weight of the intestine that is largely attributed to accumulation of immune cells and enzymes in order to build a defense barrier against microorganisms on the intestinal lumen (Lev and Forbes, 1959; Drew et al., 2003; Cook, 2004). Lipopolysaccharides are commonly present in rumen fluid, and translocation of LPS into the bloodstream can occur,

especially under immunological stress and ruminally acidotic conditions (Andersen et al., 1994; Gozho et al., 2007; Emmanuel et al., 2007, 2008; Khafipour et al., 2009; Ametaj et al., 2009; Dong et al., 2011). The translocation of LPS is mediated by sPLA2, with disruption of the intestinal phospholipid barrier, release of free AA in the cytosol and increased production of prostaglandins, with further activation of the APR and production of proinflammatory cytokines (Johnson, 1997; Rozenfeld et al., 2001; Zayat et al., 2008; Cook, 2011). Regulation of the intestinal immune stimulation could reduce intestinal thickness improving nutrient absorption, reduce the energy costs associated with the maintenance of the intestinal immune barrier, and reduce the detrimental effects of the activation of APR with production of proinflammatory cytokines over animal performance, explaining the decreased DMI and FE improvement observed on calves treated with aPLA2.

It has been shown that cattle with more excitable temperament (greater CS and EV) have increased secretion and circulating concentrations of adrenocorticotropic hormone (ACTH) and cortisol, via activation of the hypothalamic-pituitary-adrenal axis (Curley et al., 2008). Glucocorticoids, such as cortisol, are essential to the maintenance of homeostasis and act on the regulation of carbohydrate and protein metabolisms, and on the regulation of growth and reproductive axes. However, excessive levels of cortisol have been linked with suboptimal growth and performance of cattle (Friend, 1991; Johnson, 1997), and cattle with calmer temperament during handling have increased ADG compared with cattle that are agitated during routine handling (Voisinet et al., 1997). A day effect ($P < 0.01$) was detected for CS and EV, with an increase in EV (Figure 3-3), and decreased CS (Figure 3-2) during the 70 d FE trial for both treatments.

There was no treatment effect on EV, however calves receiving aPLA2 had reduced ($P < 0.05$) CS (Table 3-2), indicating an improvement in temperament and perhaps alleviating the negatives effects on animal performance associated with the activation of the hypothalamic-pituitary-adrenal axis and greater concentrations of cortisol; however concentrations of cortisol were not measured in this experiment.

Phase II. Animal performance and FE data for Phase II are summarized in Table 3-3. There were no differences in BW before (d 0) and after (d 1) 24 hr transportation, and no differences in final BW at d 28. No difference in mean BWL after 24 hr transportation (26.2 ± 0.6 kg and 25.9 ± 0.6 kg for CON and aPLA2 calves, respectively) was detected. Weight loss after transportation of beef calves range from 6 to 10%, and the amount of weight loss was directly dependent on body condition prior to transportation, pre-transit diets and duration of transport (Fike and Spire, 2006). The percentage of BW loss after 24 hr transportation did not differ ($P = 0.73$) between treatments, calves from both treatments had a 8.1 ± 0.1% reduction in BW during transportation.

There were no differences in ADG, average DMI, and G:F between treatments during the 28-d following 24 hr transportation (Table 3-3). Beef calves have decreased DMI after transportation, especially within the first two weeks following transport, and the decrease in DMI was even more dramatically when there was a combination of stressors such as transport, commingling, and introduction to new diets that have greater contents of concentrate (Loerch and Fluharty, 1999; Arthington et al., 2003; Duff and Galyean, 2007). There was no difference ($P = 0.26$) in the decrease in DMI as a result of transportation from d 0 to d 1 (2.43 ± 0.15 kg) (Table 3-3). However, the

decrease in average daily DMI over the two weeks following transportation was not different. Calves in the aPLA2 and CON treatments had similar decrease in average daily DMI (10 and 13% decrease, respectively) within the first two weeks following transportation compared with average daily DMI on the two weeks that preceded transport. In this study calves were maintained on the same diets and group pens during pre- and post-transport periods, eliminating the stress associated with commingling and dietary changes, which may have attributed similar decreases in DMI following transportation.

There were a treatment ($P = 0.02$) and a treatment × day ($P < 0.0001$) effect on daily DMI during the first 15 d following transportation (Figure 3-4). Calves consuming the aPLA2 supplement had reduced average DMI compared to CON calves (8.45 ± 0.21 kg and 8.82 ± 0.20 kg, for aPLA2 and CON, respectively) during the first 15 d following transportation. Calves in the CON group had greater daily DMI than aPLA2 calves on d 1, 6 and 8 and lower daily DMI on d 7. The reduced DMI during the first 15 d following transportation of aPLA2 calves is likely a result of the lower DMI during Phase I, and reinforce the hypothesis that calves receiving the aPLA2 have reduced energy requirements for maintenance of the intestinal local immune response allowing more available energy and nutrients for the animal to cope with stress and physiological processes, resulting in reduced DMI and increased FE.

The APR is stimulated by the release from macrophages and monocytes of the proinflammatory cytokines TNF-α, IL-1, and IL-6 at the site of inflammation, but they may also act systemically changing behavior, metabolism, neuroendocrine secretions, inducing skeletal muscle waste and lipolysis (Johnson, 1997; Carroll and Forsberg,

2007; Lippolis, 2008). An ultrasonography measurement of BF at d 28 was performed to determine fat thickness between the 12th and 13th ribs; however, no difference (P = 0.26) in BF was detected between treatments (Table 3-3).

Concentrations of haptoglobin and ceruloplasmin peaked on d 3 (day effect, P < 0.0001) after transportation indicating that calves experienced an APR (Arthington et al., 2008). Activation of the APR is a normal immunological reaction of the organism to stress, and is characterized by increased concentrations of proinflammatory cytokines and APP (Johnson, 1997). Although CON calves had numerically greater concentrations of haptoglobin at the peak on d 3, supplementation of aPLA2 did not affect (P = 0.41) concentrations of plasma haptoglobin after 24 hr transportation and no treatment × day interaction existed (P = 0.21; Figure 3-5). In addition, no treatment × day interaction was detected (P = 0.98) for concentrations of plasma ceruloplasmin. However, a treatment effect (P < 0.001) existed for concentrations of plasma ceruloplasmin after transportation. Calves receiving aPLA2 supplementation had reduced concentrations of plasma ceruloplasmin throughout the 28 d that followed transportation compared to CON calves (Figure 3-6), indicating that aPLA2 supplementation successfully reduced the APR to 24 hr transportation.

Independent of treatments, mean concentrations of plasma ceruloplasmin was negatively correlated (P < 0.001) with average daily DMI during the first two weeks after transportation (Figure 3-7), but no correlation (P = 0.77) between average daily DMI and concentrations of plasma haptoglobin was detected. Greater concentrations of ceruloplasmin after transportation have been negatively correlated with ADG, and

positively correlated with concentrations of cortisol (Arthington et al., 2003; Cooke et al., 2009a; Araujo et al., 2010).

Conclusion

Beef calves receiving a growing diet supplemented with aPLA2 had reduced average daily DMI, while maintaining similar ADG than CON calves, resulting in lower RFI and improved FE during the 70-d trial. Supplementation of aPLA2 improved temperament by reducing CS of calves, and had reduced concentrations of plasma ceruloplasmin after 24 hr transportation, indicating a reduction in the magnitude of the APR and its negative effects on animal performance.

Table 3-1. Nutrient composition of background diets fed to beef calves during a 70-d feed efficiency trial.

	Treatments[1]	
Composition	aPLA2	CON
DM, %	92.0	92.8
CP, % DM	12.0	13.1
NE_g[2], Mcal/kg DM	0.6	0.6
NDF, % DM	63.5	60.1
ADF, % DM	36.1	35.4
TDN, % DM	56.0	57.0
Calcium, % DM	0.6	0.6
Phosphorus, % DM	0.4	0.4

[1] aPLA2 = inclusion of aPLA2 supplement at 0.6% of the diet DM; CON = no additive.
[2] Net energy for gain.

Table 3-2. The effects of aPLA2 supplementation on animal performance and feed efficiency of growing beef cattle receiving backgrounding diets.

	Treatments[1]			
Item	aPLA2	CON	SEM	*P*-value
Initial BW[2], kg	242.0	241.6	3.7	0.92
Final BW[2], kg	312.6	314.0	4.1	0.79
ADG[3], kg	0.99	1.00	0.02	0.95
Daily DMI[4], kg	8.53	9.18	0.15	0.01
G:F	0.117	0.110	0.003	0.09
RFI[5], kg/d	-0.272	0.389	0.110	0.002
CS[6]	2.21	2.32	0.05	0.03
EV[7], m/s	0.76	0.71	0.05	0.25

[1] aPLA2 = inclusion of aPLA2 supplement at 0.6% of the diet DM; CON = no additive.
[2] Live BW.
[3] Calculated using linear regression of BW against day on trial.
[4] Average daily DMI during the 70-d feed efficiency trial.
[5] Residual feed intake.
[6] Based on a 1 to 5 scale, with 1 being docile and 5 being aggressive.
[7] Measure of seconds taken for animal to travel 1.83 m from squeeze chute.

Table 3-3. The effects of aPLA2 supplementation on animal performance, feed efficiency and concentrations of plasma acute-phase proteins after 24 hr transportation.

	Treatments[1]			
Item	aPLA2	CON	SEM	*P*-value
BW d 0[2], kg	317.2	315.6	3.9	0.73
BW d 1[3], kg	291.3	289.4	3.7	0.65
BW loss[4], kg	25.9	26.2	0.6	0.86
% BW loss[5]	8.2	8.3	0.2	0.73
BW d 28[6], kg	339.9	337.9	4.1	0.72
ADG, kg	1.27	1.29	0.03	0.72
Daily DMI[7], kg	8.73	8.95	0.15	0.45
DECDMI[8], kg	2.60	2.25	0.15	0.26
G:F	0.165	0.163	0.004	0.83
BF[9], mm	1.91	2.08	0.01	0.26
Haptoglobin[10], mg/mL	0.08	0.09	0.02	0.41
Ceruloplasmin[11], mg/dL	13.0	14.3	0.3	<0.001

[1] aPLA2 = inclusion of aPLA2 supplement at 0.6% of the diet DM; CON = no additive.
[2] Shrunk BW (16 hrs separation from feed) immediately before 24 hr transportation.
[3] BW immediately after 24 hr transportation.
[4] Difference in BW between d 0 and 1.
[5] Percentage BW loss after 24 hr transportation relative to BW on d 0.
[6] Shrunk BW (16 hr separation from feed).
[7] Average daily DMI during the 28 d following transportation.
[8] Decrease in DMI on d 1 of Phase II relative to d 70 of Phase I.
[9] Fat thickness at d 28 (measured between the 12th and 13th ribs by ultrasonography).
[10] Least square mean of concentration of plasma haptoglobin during Phase II.
[11] Least square mean of concentration of plasma ceruloplasmin during Phase II.

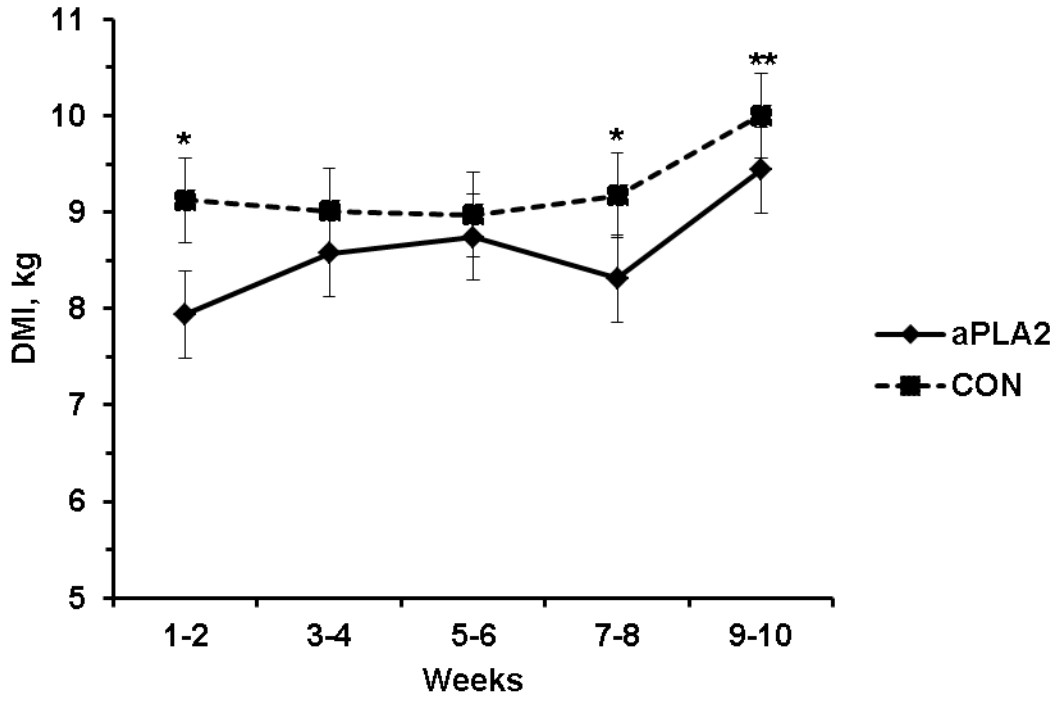

Figure 3-1. Average daily DMI calculated on a biweekly basis during the 70-d feed efficiency trial. * Means differ (P < 0.01). ** Means tend to differ (P = 0.06).

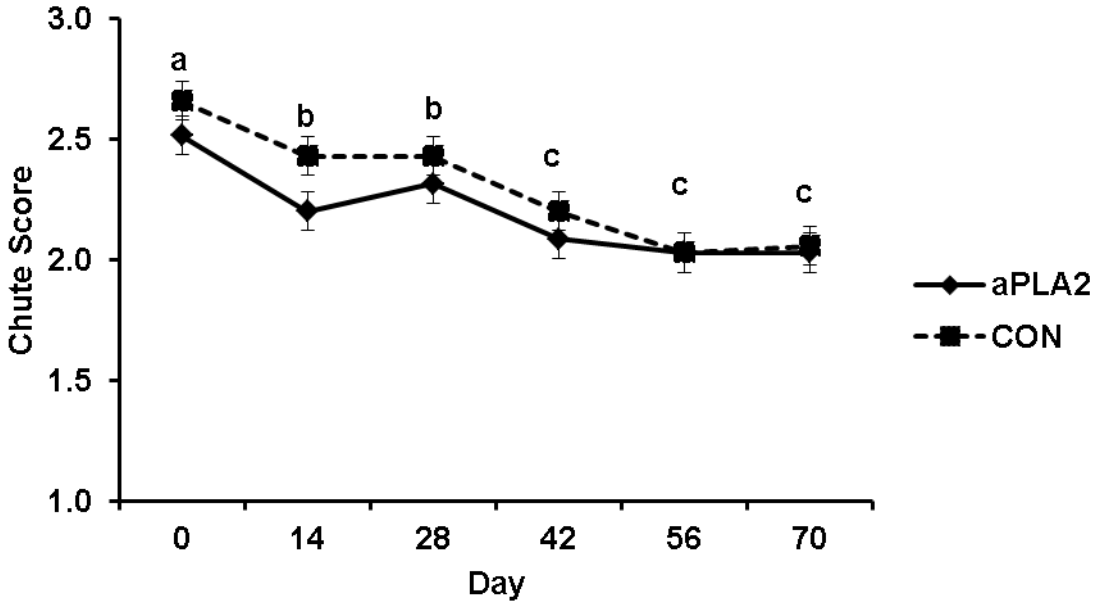

Figure 3-2. Mean chute score (on 5 point scale, with 1 being calm and 5 being aggressive) by day of beef calves during a 70-d feed efficiency trial. a,b,c Overall day means differ ($P < 0.01$).

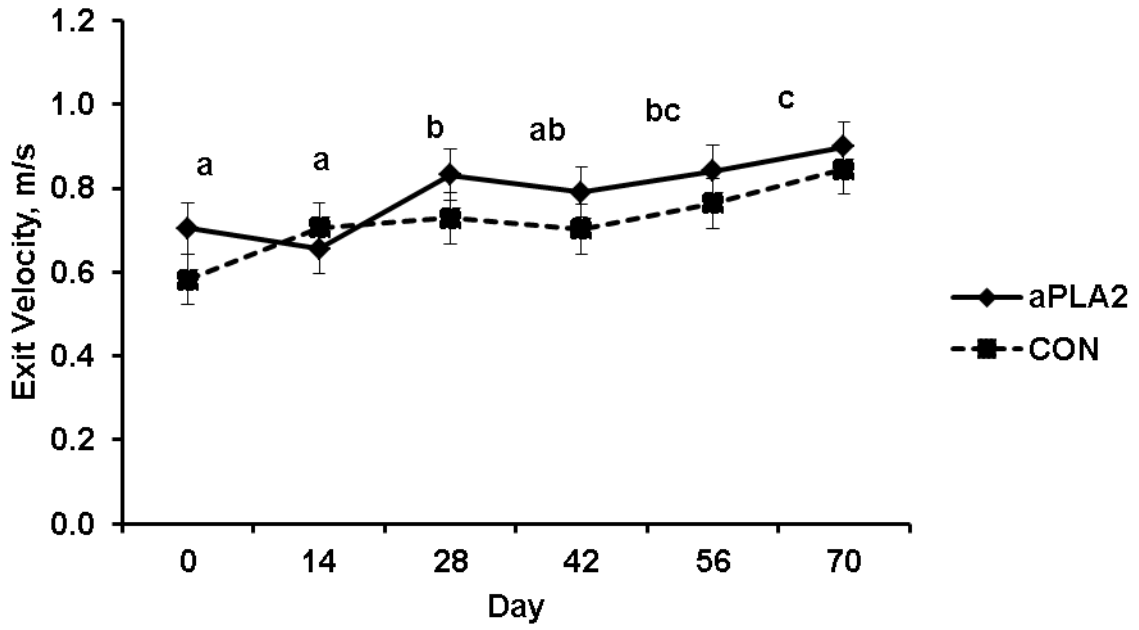

Figure 3-3. Mean exit velocity (seconds for a calf to travel 1.83 m from squeeze chute) by day of beef calves during a 70-d feed efficiency trial. a,b,c Overall day means differ ($P < 0.01$).

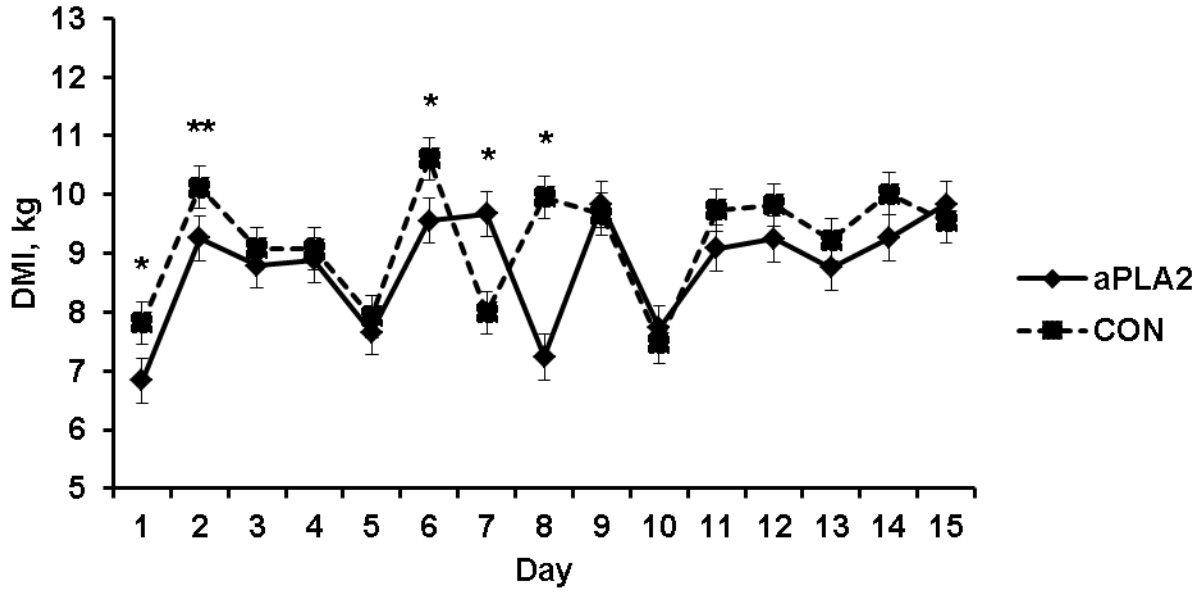

Figure 3-4. Average daily DMI of beef calves during 15 d following 24 hr transportation. * Means differ within day ($P < 0.001$). ** Means tend to differ within day ($P = 0.06$).

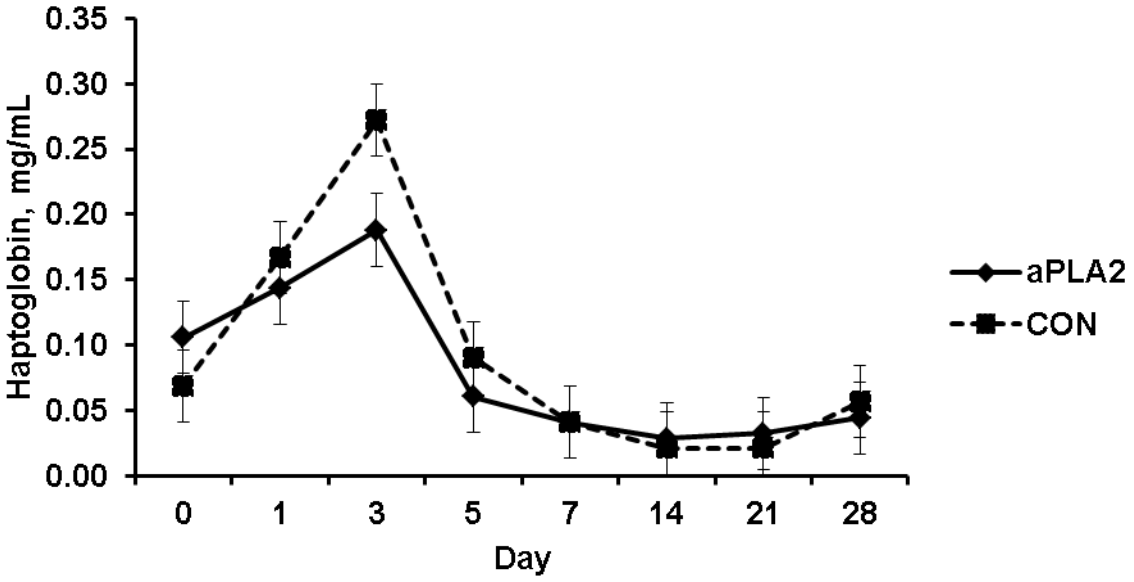

Figure 3-5. Concentration of plasma haptoglobin by day of beef calves following 24 hr transportation (treatment effect, P = 0.41; treatment by day effect, P = 0.21).

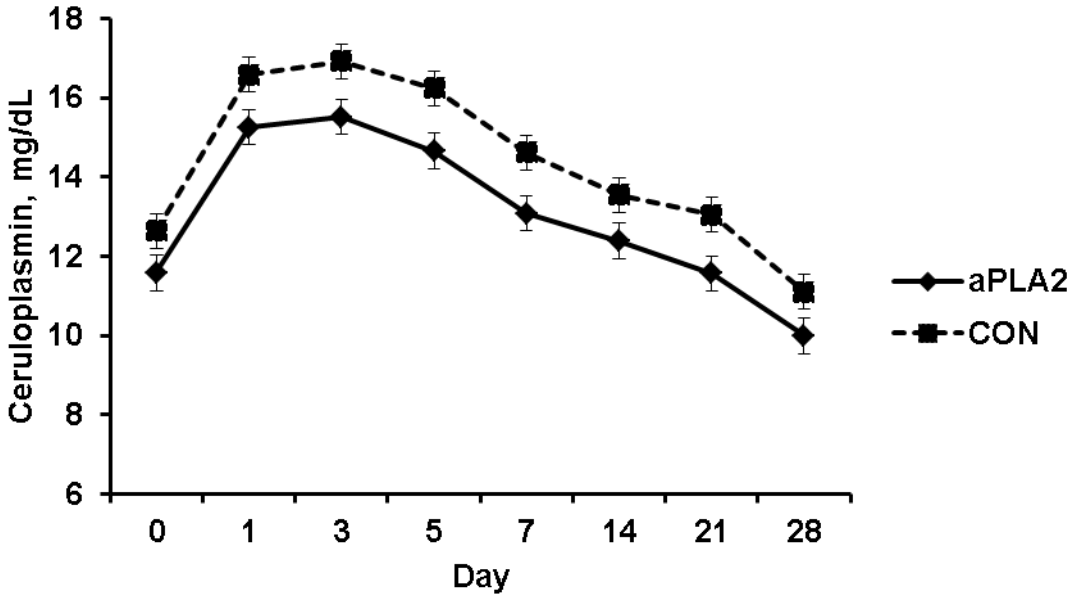

Figure 3-6. Concentration of plasma ceruloplasmin by day of beef calves following 24 hr transportation (treatment effect, $P < 0.001$).

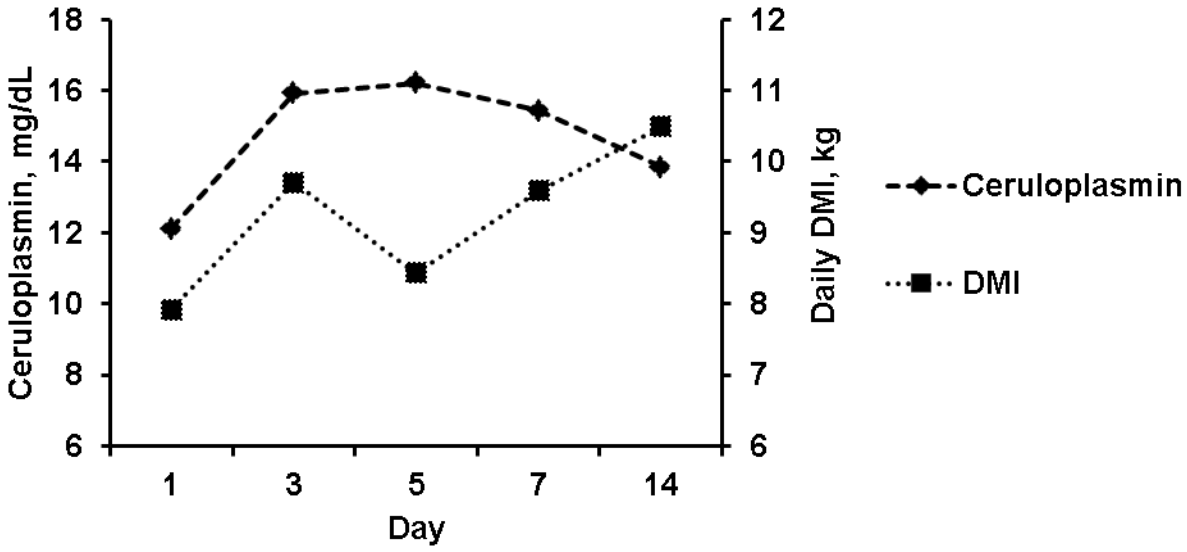

Figure 3-7. Correlation between mean concentration of plasma ceruloplasmin and average daily DMI of beef calves after 24 hr transportation, when combining treatments ($P < 0.001$, $R2 = 0.035$).

CHAPTER 4
EFFECTS OF ANTI-PHOSPHOLIPASE A2 ANTIBODY SUPPLEMENTATION ON DRY MATTER INTAKE, FEED EFFICIENCY, ACUTE PHASE RESPONSE AND BLOOD DIFFERENTIALS OF STEERS FED FORAGE AND GRAIN-BASED DIETS

In order to achieve feed efficiency (FE) and more efficient body weight gain, feedlots in the United States have developed unique feeding and management strategies. The extensive grain processing and higher consistency in quality of grains feeds in comparison to roughages, aimed at achieving higher productivity, resulted in feedlots to using diets consisting of greater concentrates. However, greater demands for grain in ethanol production and export markets have a large impact on future global grain markets, resulting in increased grain prices. Thus, feedlots and stocker cattle operations will need to focus on optimizing grain utilization, finding alternative energy sources, selecting animals with greater FE and development of strategies to improve FE while maintaining high performance rates (Galyean et al., 2011).

Beef cattle in feed yards undergo acidotic challenges when they are transitioned from forage-based into grain-based diets, resulting in decreased ruminal pH and physiological responses that are directly impacted by the percentage increase in concentrate and the time allowed for ruminal adaptation to occur between diet changes (Berry et al., 2004a; Nagaraja and Titgemeyer, 2007; Ametaj et al., 2009). Lipopolysaccharide (LPS) is a cell wall component of gram-negative bacteria that is commonly present in ruminal fluid. Grain-fed cattle have greater concentrations of LPS in rumen fluid compared to forage-fed cattle, due to greater quantities of gram-negative bacteria and greater rates of bacterial death caused by lower ruminal pH values (Nagaraja et al., 1978a; b; Andersen et al., 1994).

The combination between the development of ruminal and intestinal mucosa inflammation with increased concentrations of LPS in the rumen fluid, results in translocation of LPS into the bloodstream with activation of the immune system through the acute phase response (APR) and release of inflammatory mediators (Andersen et al., 1994; Gozho et al., 2007; Emmanuel et al., 2007, 2008; Khafipour et al., 2009; Ametaj et al., 2009; Dong et al., 2011). The secretory phospholipase A2 (sPLA2) enzyme is a potent proinflammatory mediator and plays an important role in LPS-induced prostaglandin synthesis in intestinal epithelial cells, mediating the disruption of the intestinal barrier (Cook, 2011). The APR is stimulated by the release from macrophages and monocytes of the pro-inflammatory cytokines TNF-α, IL-1 and IL-6 at the site of inflammation, resulting in redirection of nutrients into defense processes, development of fever, alterations in behavior, decreased feed intake, wasting of skeletal muscle, increased lipolysis and hepatic production of acute phase proteins (APP) (Johnson, 1997; Cook, 2011; Cooke and Bohnert, 2011).

Feeding monensin and tylosin selectively inhibits ruminal gram-positive bacteria, with consequent improved digestive efficiency of cattle consuming high-grain diets, due to increased ruminal concentration of propionic acid, improved FE, ADG, reduced DMI and lower incidence of acidosis and liver abscesses in cattle (Richardson et al., 1976; Nagaraja et al., 1985; Burrin and Britton, 1986; Galyean et al., 1992; Nagaraja and Titgemeyer, 2007; Meyer et al., 2009). However, antimicrobial resistance is a growing public issue and the use of antibiotics as growth promotants for beef cattle seems to be subjected to future limitations and more restricted regulations (Galyean et al., 2011).

Recently, an egg derived antibody against intestinal sPLA2, anti-phospholipase A2 (aPLA2), was developed to regulate the intestinal inflammatory response and the negative effects of the activation of the APR on animal growth and FE, and it has been successfully used as a feed additive for poultry, swine, and fish, with improved FE and growth (Yang et al., 2003; Cook, 2004, 2011; Schwartz et al., 2006; Barry and Yang, 2008).

Therefore, the potential exist that steers consuming aPLA2 may have decreased inflammatory response due to a transition into high-grain diets, and consequently enhanced performance and improved FE. The objectives of this study was to determine whether supplementation of aPLA2 would alter animal performance, voluntary DMI, FE, plasma concentration of APP and blood differentials (BD) of steers transitioned from a high-forage into a high-grain diet.

Materials and Methods

Animals and Treatments

Eighty cross-bred steers were allocated in group pens (108 m2/pen; 8 pens with 10 animals each) at the University of Florida North Florida Research and Extension Center, Feed Efficiency Facility (FEF), in Marianna, FL. Individual daily DMI was measured using the GrowSafe system (GrowSafe Systems Ltd., Alberta, Canada). Each pen in the FEF was equipped with two GrowSafe feed bunks. Steers were blocked by weight and then randomly assigned to pens to receive treatments. A 14-d period of adaptation to facilities and diets preceded the initiation of the 141 d trial. Shrunk BW (16 hr following removal from feed) was measured on d 0, prior to initiation of diet change on d 60, immediately following diet change on d 81 and at conclusion of the study on d 141.

Following the adaptation period, the experiment was divided in three phases (Figure 4-1). In Phase I steers were fed a growing diet (0.80 Mcal NEG/kg of DM and 13% CP) comprised of 69% concentrate, 31% bermudagrass hay and a vitamins and minerals supplement containing the following treatments: 1) no additive (CON; n = 20); 2) inclusion of 30 mg of monensin and 8.8 mg of tylosin per kg of diet DM (MT; n = 20); 3) inclusion of a aPLA2 supplement at 0.2% of the diet DM (aPLA2 0.2%; n = 20); 4) inclusion of aPLA2 supplement at 0.4% of the diet DM (aPLA2 0.4%; n = 20). Phase II, on d 60 all steers were transitioned into a grain-based diet using a 21 d “step-up” period with three steps from 69% concentrate to 75% (step 1), 85% (step 2) and 90% concentrate (step 3). Phase III, on d 81 all steers received a 90% concentrate diet (74% cracked corn; 1.32 Mcal NEg/kg of DM, 11.4% CP) for 60 d until the conclusion of the experiment on d 141. A soybean meal-based premix containing 0.2, 0.4% of aPLA2, or no antibody, was mixed to the treatment diets at 1% of the diet DM. In addition, two pelleted supplements were included at a 5% of the diet DM to deliver either no antibiotics (CTL and all aPLA2 containing diets), or 30 mg of monensin plus 8.8 mg of tylosin per kg of DM (MT diet). Steers were maintained in the same group pens throughout the 141 d trial. The nutrient content of all diets are presented on Table 4-1.

Blood Collection and Analyses

Phase II. Blood samples from each steer were collected from the jugular vein into 10 mL evacuated glass vials containing 143 IU of Na heparin (Vacutainer, Becton Dickinson Inc., Franklin Lakes, NJ) on days 0, 3, 5, 7, 10, 12, 14, 17, 19 and 21 relative to the initiation of the Phase II “step-up” period. Blood samples were immediately placed on ice, and centrifuged at 1,500 × g, at 4°C for 15 min. The plasma was transferred by

pipette into polypropylene vials (12mm x 75mm; Fisherbrand, Thermo Fisher Scientific Inc., Waltham, MA) and stored at -20°C until further analysis.

A spectrophotometer (ThermoSpectronic Genesys 20; Thermo Fisher Scientific Inc., Waltham, MA) was used to determine plasma ceruloplasmin concentration. The plasma ceruloplasmin oxidase activity was measured in duplicate samples using colorimetric procedures as described in the literature (Demetriou et al., 1974). Concentrations of ceruloplasmin were expressed as mg/dL (King, 1965). The intra and interassay coefficient of variation (CV) for ceruloplasmin were 6.0 and 10.4%, respectively. A microplate spectrophotometer (PowerWave 340; BioTek Instruments, Inc., Winooski, VT) was used to determine concentrations of plasma haptoglobin in duplicate samples by measuring haptoglobin/hemoglobin complexing by the estimation of differences in peroxidase activity as described previously (Makimura and Suzuki, 1982) and results are expressed as arbitrary units from the absorption reading at 450 nm × 100. The intra and interassay CV for haptoglobin were 7.0 and 11.3%, respectively.

Blood differentials, total red blood cell count (RBC), hematocrit (HCT), hemoglobin (HGB), total white blood cell count (WBC), neutrophils, lymphocytes and neutrophil:lymphocyte ratio (N:L), monocytes, eosinophils and total platelets count were assessed using a hematology cell counter (IDEXX ProCyte DX Hematology Analyzer, Westbrook, ME), and individual whole blood samples. Blood samples were collected from the jugular vein into 2.0 mL polypropylene evacuated tubes containing 3.6 mg of K2 EDTA (BD Hemogard, Becton Dickinson Inc., Franklin Lakes, NJ), and immediately stored on ice until analysis, all samples were analyzed within 8 hr of collection.

Feed Sample Collection and Analyses

Representative samples of each treatment TMR diet were taken before feeding, at 28-d intervals throughout the 141 d trial. All samples were bagged and frozen immediately after collection until drying. All feed samples analyzed for nutritive values were dried at 55°C for 48 hr in a forced air oven. At conclusion of the drying period all samples were ground in a Wiley mill (Arthur H. Thomas Company, Philadelphia, PA, USA) using a 1.0 mm screen. After grinding, samples were composited for analysis on an equal weight basis. The composited samples were analyzed for DM, CP, TDN, ADF, NDF, Ca, and P in duplicate by a commercial laboratory using NIR procedures (Dairy One Forage Laboratory, Ithaca, NY).

Ultrasonic Carcass Traits

On d 0 and d 141, ultrasound was used to determine fat thickness (BF) and *Longissimus dorsi* muscle area (REA) using an Aloka real-time ultrasound scanner (3.5-MHz linear array transducer, Aloka 500V, Corimetrics Medical Systems, Inc., Wallingford, CT) and image capturing software. Scanning was performed on the right side of each animal, between the 12^{th} and 13^{th} ribs. There were two measurements taken per animal at each scanning session. Final BF and REA values were calculated as the average between the two measurements recorded.

Statistical Analyses

Individual steer ADG was determined by the difference between the shrunk BW at the end and the beginning of each phase, divided by the number of days on trial. Gain to feed ratio (G:F) was computed as the ratio of ADG to daily DMI. Residual feed intake (RFI) was calculated as the actual DMI minus expected DMI, with expected DMI derived

from the regression of actual DMI on ADG and mid-test metabolic body weight using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC, 2011).

The MIXED procedure of SAS was used to identify differences in BW, ADG, average daily DMI, G:F, RFI, BF and REA. The statistical model included treatment, pen within treatment. The repeated measures statement of the MIXED procedure of SAS was used to analyze differences in concentrations of haptoglobin and ceruloplasmin, BD, and daily DMI during the phase II “step-up” period. The statistical model included treatment, day, and treatment × day interaction, with day as the repeated (d 0, 3, 5, 7, 10, 12, 14, 17, 19 and 21), animal as the subject and block as random effect. The decrease in DMI (DECDMI) immediately after each change in diet during phase II was analyzed using the repeated measures statement of the MIXED procedure of SAS. The statistical model included treatment, day and treatment by day interaction, with day as the repeated variable (d 1, 8 and 15), animal as the subject and block as random.

The CONTRAST function of the GLM procedure of SAS was used to conduct orthogonal contrasts between the aPLA2 0.2% and aPLA2 0.4% (0.2/0.4), both aPLA2 treatment combined against MT (aPLA2/MT), and both aPLA2 plus MT treatments against CON treatment (TRT/CON) on daily DMI, and concentrations of ceruloplasmin and haptoglobin, and BD during Phase II. The statistical model included treatment, day and the treatment × day interaction. The GLM procedure of SAS was used to assess the correlation between concentrations of haptoglobin and ceruloplasmin with DMI during phase II on d 0, 3, 5, 7, 10, 12, 14, 17, 19 and 21. Statistical differences were reported at $P < 0.05$ and tendencies were identified when $P < 0.10$, with means being reported as LS means ± SEM.

Results and Discussion

Animal Performance

High concentrate diets have been linked to the activation of the APR in cattle (Emmanuel et al., 2008; Ametaj et al., 2009), which may negatively affect animal growth and FE as a result of decreased DMI, wasting of skeletal muscle and lipolysis (Johnson, 1997; Cook, 2011). However, in the current experiment there were no statistical differences among treatments in measurements of performance and FE (Table 4-2). No treatment differences were detected in BW on d 0 (212.0 ± 4.0 kg, P = 0.94), BW on d 141 (388.0 ± 5.1 kg, P = 0.79), ADG (1.25 ± 0.02 kg/d, P = 0.33), BF on d 0 (0.96 ± 0.04 cm, P = 0.89) and on d 141 (1.43 ± 0.11 cm, P = 0.73), REA on d 0 (25.3 ± 3.4 cm2, P = 0.49) and on d 141 (46.4 ± 2.6 cm2, P = 0.51), G:F (0.18± 0.01, P = 0.98), average daily DMI (7.1 ± 0.1 kg, P = 0.43), and RFI (-1.25 ± 0.06 kg/d, P = 0.61).

Phase I. Animal performance and FE data during Phase I are summarized in Table 4-3. There were no differences on BW at d 60 (259.0 ± 4.4 kg; P = 0.84) and G:F (0.11 ± 0.01; P = 0.16) among treatments. However, there was a tendency (P = 0.08) for the MT treatment to have greater ADG compared to the aPLA2 0.2%, whereas CON and aPLA2 0.4% treatments were intermediate. Feeding monensin and tylosin improves DM digestive efficiency of cattle, increasing ruminal concentrations of propionic acid and decreasing concentrations of acetate and butyrate (Richardson et al., 1976; Beckett et al., 1998), increasing glucose supply to the animal, thus improving FE, reducing DMI, and improving ADG of growing and finishing cattle (Duffield et al., 2012).

Similar to the results from Phase I of Chapter 3 (Table 3-2), aPLA2 supplementation tended to reduce daily DMI, and significantly reduced RFI compared to

CON steers, improving FE of steers fed forage-based diets. Daily DMI tended (P = 0.07) to be lower for both aPLA2 0.2% and 0.4% treatments compared to CON treatment, with MT treatment being intermediate. Steers from both aPLA2 treatments had lower RFI than CON steers (P < 0.05). Steers in the aPLA2 0.4% treatment had the lowest RFI, and MT steers had intermediate RFI values compared to other treatments. Regulation of the intestinal inflammatory response by blocking intestinal sPLA2, reduces the nutrient and energy costs of the immune intestinal barrier, therefore reducing the maintenance requirements and DMI of the animal, with an overall improvement in FE (Cook, 2011).

Phase II. Performance and FE data during Phase II are summarized in Table 4-4. There were no differences among treatments on BW on d 81 (297.0 ± 5.0 kg, P = 0.46), ADG (1.82 ± 0.16 kg/d, P = 0.17) and G:F (0.26 ± 0.02, P = 0.47) during Phase II. However, similar to Phase I average daily DMI was significant lower (P = 0.02) for aPLA2 0.4% steers compared to MT and CON steers, daily DMI for steers on aPLA2 0.2% was intermediate.

Daily DMI was impacted by diet changes during Phase II. Supplementation of aPLA2 and monensin and tylosin reduced DMI of steers during the transition to grain-based diets. Using contrast analysis to assess differences in daily DMI during Phase II, there were no differences (P = 0.18) in daily DMI between the aPLA2 treatments (Figure 4-3). However, when comparing both aPLA2 treatments combined against CON treatment (Figure 4-4), and both aPLA2 plus the MT treatments combined against CON (Figure 4-5), CON steers had significantly greater (P < 0.0001) daily DMI during Phase II.

Dry matter intake was decreased (day effect, $P < 0.0001$) on the day after diet change (d 1, 8 and 15) across all treatments. The mean DECDMI on the day after change in diets were significantly lesser ($P = 0.03$) for the MT compared with CON steers, and both aPLA2 treatments were intermediate (Table 4-4).

A treatment × day effect ($P < 0.001$) was also detected for DECDMI (Figure 4-2). On d 1, CON steers had the greatest DECDMI compared to aPLA2 0.2% and MT steers. There was a tendency ($P = 0.06$) for aPLA2 treatments to have lesser DECDMI then CON, with MT steers having intermediate DECDMI on d 8. However, on d 15 DECDMI was significantly greater for both aPLA2 treatments than MT, and CON steers had reduced DECDMI compared to aPLA2 0.2% steers. The lower DMI of MT steers is likely a reflection of the inhibition of gram-positive bacteria in the rumen (Richardson et al., 1976) during the transition to grain-based diets, increasing ruminal propionate concentration, and improving ruminal function, decreasing the negative impacts of the ruminal adaptation to higher concentrate contents of the diets on DMI and animal performance (Nagaraja et al., 1985).

Phase III. Animal performance data during Phase III are summarized in Table 4-5. Supplementation of monensin and tylosin have been successfully used to decrease the risk of acidosis and sub-acute acidosis in cattle consuming high-grain diets, due to reduction in lactic acid concentration in the rumen, reducing the negative impact of acidosis on ruminal function (Nagaraja et al., 1985; Burrin and Britton, 1986; Nagaraja and Titgemeyer, 2007). Monensin also increases ruminal concentration of propionic acid, increasing hepatic production of glucose (Richardson et al., 1976; Bergen and Bates, 1984), thus improving animal performance and FE of cattle (Duffield et al.,

2012). However, in the current experiment there were no differences in BW at d 141, ADG, average daily DMI and G:F among treatments.

Residual feed intake was significantly greater (P = 0.04) for aPLA2 0.4% treatment compared to CON. Steers in the aPLA2 0.4% treatment had the greatest RFI (0.36 ± 0.10 kg/d) and CON steers had the lowest RFI (-0.37 ± 0.10 kg/d), with aPLA2 0.2% and MT steers remaining intermediate (-0.12 ± 0.10 and 0.10 ± 0.10 kg/d, respectively). Feeding high-grain diets reduces ruminal pH, and facilitated the development of acidosis and rumenitis (Nagaraja and Titgemeyer, 2007). Egg antibodies were shown to retain over 85% of their anti-rotavirus neutralizing activity at a pH 2 for one hour, even though the antibody binding capacity was reduced by 50% (Cook and Trott, 2010). However, the effects of long exposures to lower pH, such as the rumen when feeding high-grain diets, on egg antibody function have not been reported.

Perhaps the failure of aPLA2 supplementation to improve FE during Phase III is a result of the denaturation of the antibody due to exposure of lower pH in the rumen, directly affecting the quantity of active antibody passing through the rumen into the intestine and its capacity to block the intestinal inflammatory response, thereby alleviating the negatives effects of the activation of the immune system over animal performance.

Acute-Phase Proteins and Blood Differentials

Blood differentials and APP data are summarized in Table 4-6. There were no significant differences among treatments on mean RBC (8.96 ± 0.30 M/µL, P = 0.23), HCT (34.58 ± 0.82 %, P = 0.27), HGB (11.58 ± 0.46 g/dL, P = 0.46), neutrophils (3.56 ± 0.23 k/µL, P = 0.21), N:L (0.53 ± 0.03, P = 0.84), monocytes (2.42 ± 0.71 k/µL, P = 0.82) eosinophils (0.22 ± 0.04 k/µL, P = 0.64) and platelets (406.9 ± 34.2, P = 0.31).

Transition from forage to grain-based diets has been shown to activate the APR of cattle (Berry et al., 2004a; Ametaj et al., 2009), through the development of acidosis and rumenitis with increased concentrations of LPS in the rumen fluid and translocation of LPS to the bloodstream (Nagaraja and Titgemeyer, 2007; Emmanuel et al., 2007, 2008). The APR activates leukocytes such as macrophages, increasing the production of pro-inflammatory cytokines and resulting in a systemic inflammatory response, with development of leukocytosis, which is marked by an increase in lymphocytes (Johnson, 1997). Mean WBC and lymphocytes among treatments were within the normal interval for cattle (Merck Veterinary Manual, 2010). However, WBC were significantly greater ($P = 0.04$) for aPLA2 0.2% (13.61 ± 0.42 k/μL) than aPLA2 0.4% and MT treatments (12.16 ± 0.42 and 12.37 ± 0.42 k/μL, respectively), with CON being intermediate (12.87 ± 0.42 k/μL). Concentrations of lymphocytes also were significantly greater ($P = 0.01$) for aPLA2 0.2% (7.66 ± 0.28 k/μL) than aPLA2 0.4% and MT treatments (6.71 ± 0.28 and 6.70 ± 0.28 k/μL, respectively), with CON being intermediate (7.11 ± 0.28 k/μL).

No differences in mean concentrations of plasma ceruloplasmin and haptoglobin between treatments during Phase II were detected (Table 4-6). There was a day effect ($P < 0.01$) for concentrations of both haptoglobin and ceruloplasmin in Phase II indicating that steers experienced an APR during the transition to grain-based diets (Arthington et al., 2003; Emmanuel et al., 2007; Ametaj et al., 2009). Concentrations of plasma ceruloplasmin and haptoglobin peaked after each diet change (Figure 4-6 and Figure 4-7, respectively), and there was a treatment × day effect ($P = 0.01$) on concentrations of plasma ceruloplasmin during Phase II (Figure 4-6). On d 10, steers in the aPLA2 0.4% treatment had greater ($P < 0.05$) concentrations of ceruloplasmin than

steers in MT and CON treatments. On d 17, steers in the aPLA2 0.2% treatment had greater ($P < 0.05$) concentrations of ceruloplasmin than CON and MT treatments. On d 21, aPLA2 0.2% steers had greater ($P = 0.02$) concentrations of ceruloplasmin than CON steers, with MT and aPLA2 0.4% steers being intermediate. No treatment × day effect was detected for concentrations of plasma haptoglobin (Figure 4-7).

Concentrations of plasma ceruloplasmin was reduced ($P < 0.0001$) for CON steers compared to aPLA2 treatments (Figure 4-8), and compared to aPLA2 plus the MT treatments (Figure 4-9). Concentrations of plasma haptoglobin was reduced ($P < 0.05$) for CON steers compared to aPLA2 treatments (Figure 4-10). When combining all treatments, mean concentrations of plasma ceruloplasmin (Figure 4-11) and haptoglobin (Figure 4-12) were negatively correlated ($P < 0.001$) with mean daily DMI during Phase II, in agreement with previously reports (Arthington et al., 2003; Cooke et al., 2009a; Araujo et al., 2010), and our findings from Phase II on Chapter 3.

Conclusion

Supplementation of aPLA2 improved FE of steers fed a growing diet containing 31% forage (DM basis), but not when feeding grain-based diets. Steers from both aPLA2 treatments and MT steers had reduced DMI during the 21 d transition period from forage-based to grain-based diets. Steers on aPLA2 0.4% and MT treatments had decreased WBC and concentration of lymphocytes during the transition period compared to aPLA2 0.2% steers, with CON steers being intermediate. However, CON steers had reduced concentrations of plasma ceruloplasmin and haptoglobin during the transition to grain-based diet compared to steers on aPLA2 and MT treatments. Mean daily DMI was negatively correlated with concentrations of plasma ceruloplasmin and haptoglobin.

Table 4-1. Nutrient composition of diets fed to steers during a transition from a forage-based to grain-based diet using a three steps adaptation period over 21 d.

	Treatments[1]															
	aPLA2 0.2%				aPLA2 0.4%				MT				CON			
Composition, DM	HF[2]	Stp1[3]	Stp2[4]	HG[5]	HF	Stp1	Stp2	HG	HF	Stp1	Stp2	HG	HF	Stp1	Stp2	HG
DM, %	92.4	92.9	93.9	93.7	92.6	93.2	93.3	94.0	93.3	93.4	93.5	93.7	92.2	93.1	93.4	93.3
CP, %	13.9	10.4	13.2	13.5	13.1	11.5	13.2	13.5	13.9	10.6	13.2	15.0	15.9	11.4	13.5	13.8
NE_g[6], Mcal/kg	0.8	0.9	1.1	1.2	0.8	1.0	1.1	1.2	0.8	1.0	1.1	1.1	0.8	1.0	1.1	1.2
ADF, %	32.0	22.7	13.6	9.2	32.4	22.3	18.5	9.7	35.3	20.9	13.7	17.6	29.3	20.3	17.5	11.3
NDF, %	58.1	43.9	28.1	21.6	59.2	10.3	31.5	16.9	57.8	39.2	29.7	31.5	54.3	40.7	33.6	22.2
TDN, %	63.0	68.0	73.0	75.0	63.0	69.0	71.0	77.0	63.0	69.0	72.0	71.0	65.0	69.0	71.0	75.0
Ca, %	0.9	0.7	0.8	0.6	0.8	0.8	1.0	0.9	0.9	0.7	1.0	1.0	1.0	0.8	0.9	0.8
P, %	0.5	0.4	0.4	0.4	0.5	0.4	0.4	0.4	0.5	0.4	0.4	0.5	0.6	0.4	0.4	0.4

[1] aPLA2 0.2% = inclusion of a aPLA2 supplement at 0.2% of the diet DM; aPLA2 0.4% = inclusion of a aPLA2 supplement at 0.4% of the diet DM; MT = inclusion of 30 mg of monensin and 8.8 mg of tylosin per kg of diet DM; CON = no additive.
[2] Growing diet (31% bahiagrass hay and 69% concentrate) fed during Phase I.
[3] Step-1 diet (75% concentrate) fed during Phase II.
[4] Step-2 diet (85% concentrate) fed during Phase II.
[5] High-grain diet (90% concentrate) fed during step-3 on Phase II and Phase III.
[6] Net energy for gain.

Table 4-2. Overall animal performance, feed efficiency and ultrasound carcass traits of steers transitioned from a forage-based to grain-based diets using a 21 d three steps "step-up" adaptation period during a 141 d trial.

	Treatments[1]					
Item	aPLA2 0.2%	aPLA2 0.4%	MT	CON	SEM	*P*-value
BW[2] d 0, kg	211.0	210.0	216.0	210.0	4.0	0.94
BW[2] d 141, kg	380.0	384.0	392.0	393.0	5.1	0.79
ADG[3], kg	1.20	1.23	1.25	1.30	0.02	0.33
G:F	0.18	0.17	0.17	0.18	0.01	0.98
Daily DMI[4], kg	6.8	7.1	7.2	7.2	0.1	0.43
RFI[5], kg/d	-0.14	0.01	0.04	0.09	0.06	0.61
BF[6] d 0, cm	0.95	0.91	1.01	0.97	0.04	0.89
BF[6] d 141, cm	1.47	1.38	1.42	1.44	0.11	0.73
REA[7] d 0, cm^2	25.3	23.7	26.4	25.6	3.4	0.49
REA[7] d 141, cm^2	45.4	45.9	46.7	47.5	2.6	0.51

[1] aPLA2 0.2% = inclusion of a aPLA2 supplement at 0.2% of the diet DM; aPLA2 0.4% = inclusion of a aPLA2 supplement at 0.4% of the diet DM; MT = inclusion of 30 mg of monensin and 8.8 mg of tylosin per kg of diet DM; CON = no additive.
[2] Shrunk BW (16 hr separation from feed).
[3] ADG calculated as the difference between BW on d 0 and d 141, divided by number of days on trial.
[4] Average daily DMI during the 141 d trial.
[5] Residual feed intake.
[6] Fat thickness measured between the 12th and 13th ribs by ultrasonography.
[6] *Longissimus dorsi* area measured between the 12th and 13th ribs by ultrasonography.

Table 4-3. Animal performance and feed efficiency of steers fed a forage-based diet during Phase I.

	Treatments[1]					
Item	aPLA2 0.2%	aPLA2 0.4%	MT	CON	SEM	*P*-value
BW[2] d 0, kg	211.0	210.0	216.0	210.0	4.0	0.94
BW[2] d 60, kg	253.0	258.0	260.0	264.0	4.4	0.84
ADG[3], kg	0.69	0.79	0.83	0.81	0.04	0.08
Daily DMI[4], kg	6.51	6.70	7.09	7.38	0.25	0.07
G:F	0.11	0.12	0.12	0.11	0.01	0.15
RFI[5], kg/d	-0.12[a]	-0.22[a]	0.05[ab]	0.31[b]	0.13	0.03

[a,b] Significant differences of least squared means within a row ($P < 0.05$).
[1] aPLA2 0.2% = inclusion of a aPLA2 supplement at 0.2% of the diet DM; aPLA2 0.4% = inclusion of a aPLA2 supplement at 0.4% of the diet DM; MT = inclusion of 30 mg of monensin and 8.8 mg of tylosin per kg of diet DM; CON = no additive.
[2] Shrunk BW (16 hrs separation from feed).
[3] ADG calculated as the difference between BW on d 0 and on d 60, divided by number of days on trial.
[4] Average daily DMI during the 60 d of Phase I.
[5] Residual feed intake.

Table 4-4. Animal performance and feed efficiency of steers transitioned from a forage-based to a grain-based diet during the 21 d "step-up" adaptation period of Phase II.

	Treatments[1]					
Item	aPLA2 0.2%	aPLA2 0.4%	MT	CON	SEM	*P*-value
BW[2] d 60, kg	253.0	258.0	260.0	264.0	4.4	0.84
BW[2] d 81, kg	298.0	285.0	297.0	308.0	5.0	0.46
ADG[3], kg	2.14	1.29	1.76	2.10	0.16	0.17
Daily DMI[4], kg	7.15[ab]	6.52[a]	7.21[b]	7.64[b]	0.13	0.02
G:F	0.30	0.20	0.25	0.28	0.02	0.47
DECDMI[5], kg	-2.37[ab]	-2.61[ab]	-1.59[a]	-3.18[b]	0.40	0.03

[a,b,c] Significant differences of least squared means within a row ($P < 0.05$).
[1] aPLA2 0.2% = inclusion of a aPLA2 supplement at 0.2% of the diet DM; aPLA2 0.4% = inclusion of a aPLA2 supplement at 0.4% of the diet DM; MT = inclusion of 30 mg of monensin and 8.8 mg of tylosin per kg of diet DM; CON = no additive.
[2] Shrunk BW (16 hrs separation from feed).
[3] ADG calculated as the difference between BW on d 60 and on d 81, divided by number of days on trial.
[4] Average daily DMI during the 21 d of Phase II.
[5] Average decrease in DMI immediately after change in diet.

Table 4-5. Animal performance and feed efficiency of steers fed a grain-based diet during Phase III.

	Treatments[1]					
Item	aPLA2 0.2%	aPLA2 0.4%	MT	CON	SEM	*P*-value
BW[2] d 81, kg	298.0	285.0	297.0	308.0	5.0	0.46
BW[2] d 141, kg	380.0	384.0	392.0	393.0	5.1	0.79
ADG[3], kg	1.37	1.65	1.58	1.42	0.06	0.23
Daily DMI[4], kg	7.14	7.61	7.41	6.98	0.11	0.13
G:F	0.19	0.22	0.21	0.20	0.01	0.78
RFI[5], kg/d	-0.12[ab]	0.36[a]	0.10[ab]	-0.37[b]	0.10	0.04

[a,b] Significant differences of least squared means within a row ($P < 0.05$).
[1] aPLA2 0.2% = inclusion of a aPLA2 supplement at 0.2% of the diet DM; aPLA2 0.4% = inclusion of a aPLA2 supplement at 0.4% of the diet DM; MT = inclusion of 30 mg of monensin and 8.8 mg of tylosin per kg of diet DM; CON = no additive.
[2] Shrunk BW (16 hrs separation from feed).
[3] ADG calculated as the difference between BW on d 81 and on d 141, divided by number of days on trial.
[4] Average daily DMI during the 60 d of Phase III.
[5] Residual feed intake.

Table 4-6. Blood differentials and concentrations of plasma acute phase proteins of steers transitioned from a forage-based to grain-based diet over a 21 d "step-up" adaptation period during Phase II.

Item[2]	Treatments[1]				SEM	*P*-value
	aPLA2 0.2%	aPLA2 0.4%	MT	CON		
RBC, M/µL	9.01	8.79	9.25	8.78	0.30	0.23
HCT, %	34.34	34.00	35.76	34.22	0.82	0.27
HGB, g/dL	11.45	11.54	11.89	11.42	0.43	0.46
WBC, k/µL	13.61[a]	12.16[b]	12.37[b]	12.87[ab]	0.42	0.04
Neutrophils, k/µL	3.79	3.27	3.53	3.63	0.23	0.21
Lymphocytes, K/µL	7.66[a]	6.71[b]	6.70[b]	7.11[ab]	0.28	0.01
N:L	0.52	0.51	0.53	0.54	0.03	0.84
Monocytes, k/µL	1.99	1.94	2.81	2.94	0.71	0.82
Eosinophils, k/µL	0.25	0.18	0.20	0.25	0.04	0.64
Platelets, k/µL	407.19	387.36	448.41	384.64	34.20	0.31
Ceruloplasmin, mg/dL	24.43	24.28	23.39	22.20	0.83	0.21
Haptoglobin, mg/mL	0.28	0.23	0.19	0.18	0.05	0.42

[a,b] Significant differences of least squared means within a row ($P < 0.05$).

[1] aPLA2 0.2% = inclusion of a aPLA2 supplement at 0.2% of the diet DM; aPLA2 0.4% = inclusion of a aPLA2 supplement at 0.4% of the diet DM; MT = inclusion of 30 mg of monensin and 8.8 mg of tylosin per kg of diet DM; CON = no additive.

[2] RBC = red blood cell count; HCT = hematocrit; HGB = hemoglobin; WBC = white blood cell count; N:L = neutrophil to lymphocyte ratio.

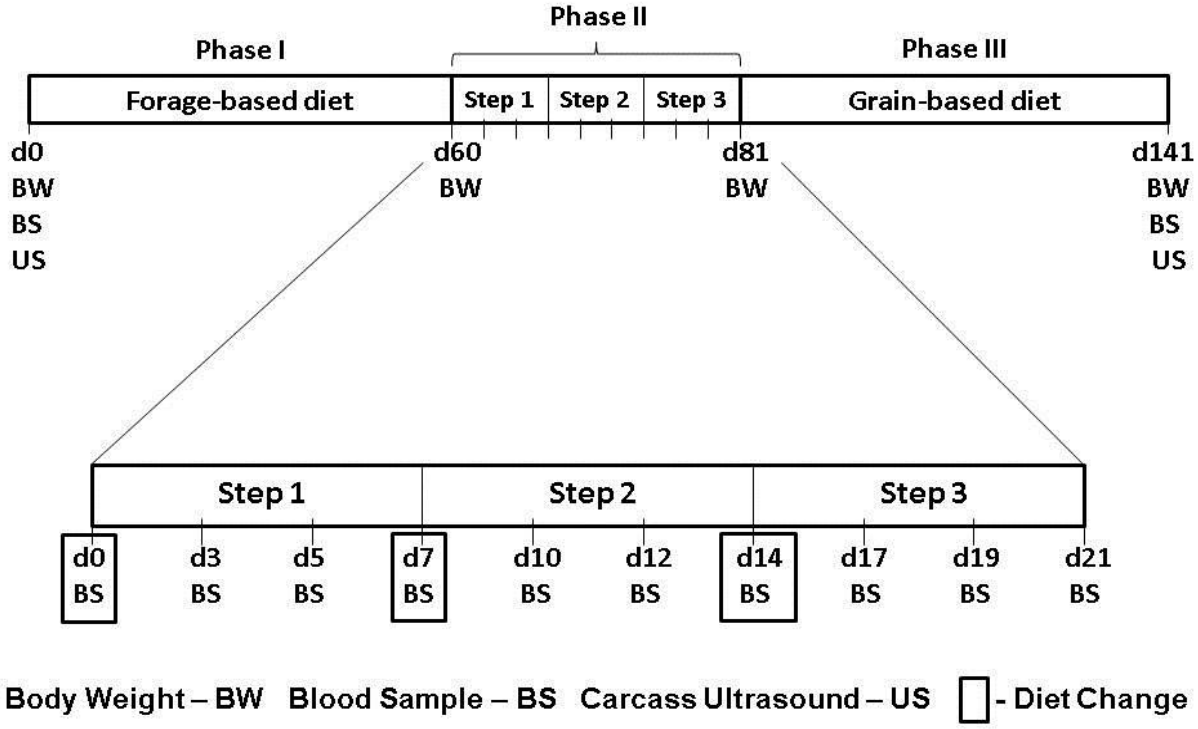

Figure 4-1. Experiment outline of steers transitioned from a forage-based to a grain-based diet using a 21 d “step-up” adaption period.

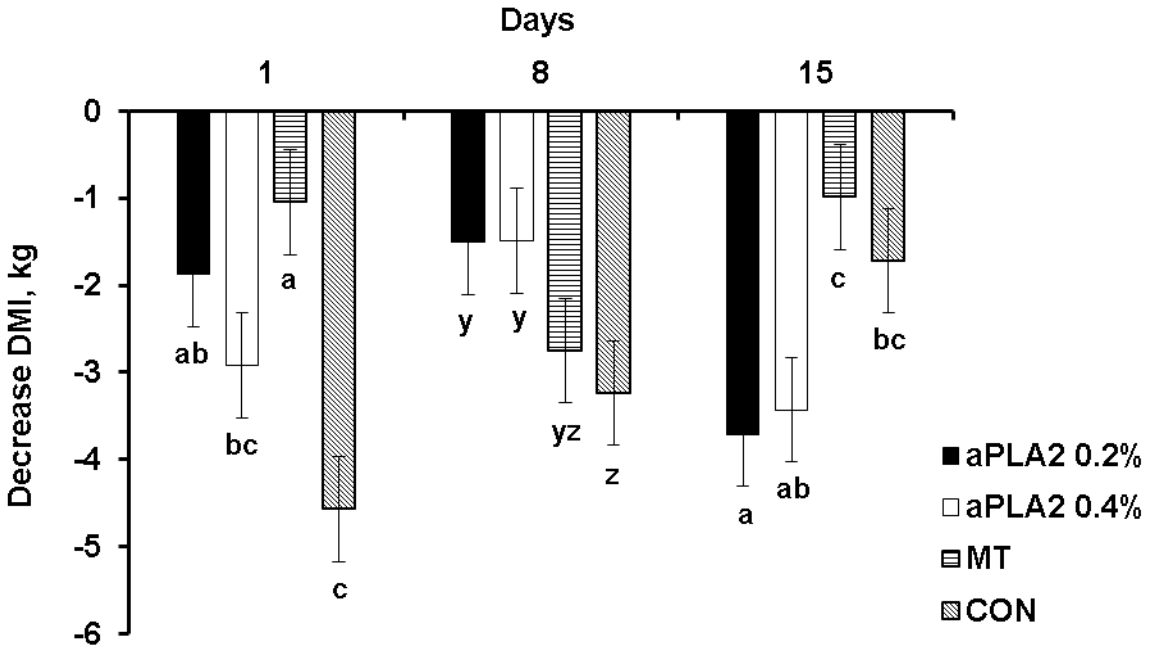

Figure 4-2. Decrease in daily DMI after diet change on d 1 (step 1), 8 (step 2), and 15 (step 3) of steers transitioned from a forage-based to grain-based diet over a 21 d “step-up” adaption period during Phase II. (abc least square means differ, $P < 0.001$; yz least square means tend to differ, $P = 0.06$).

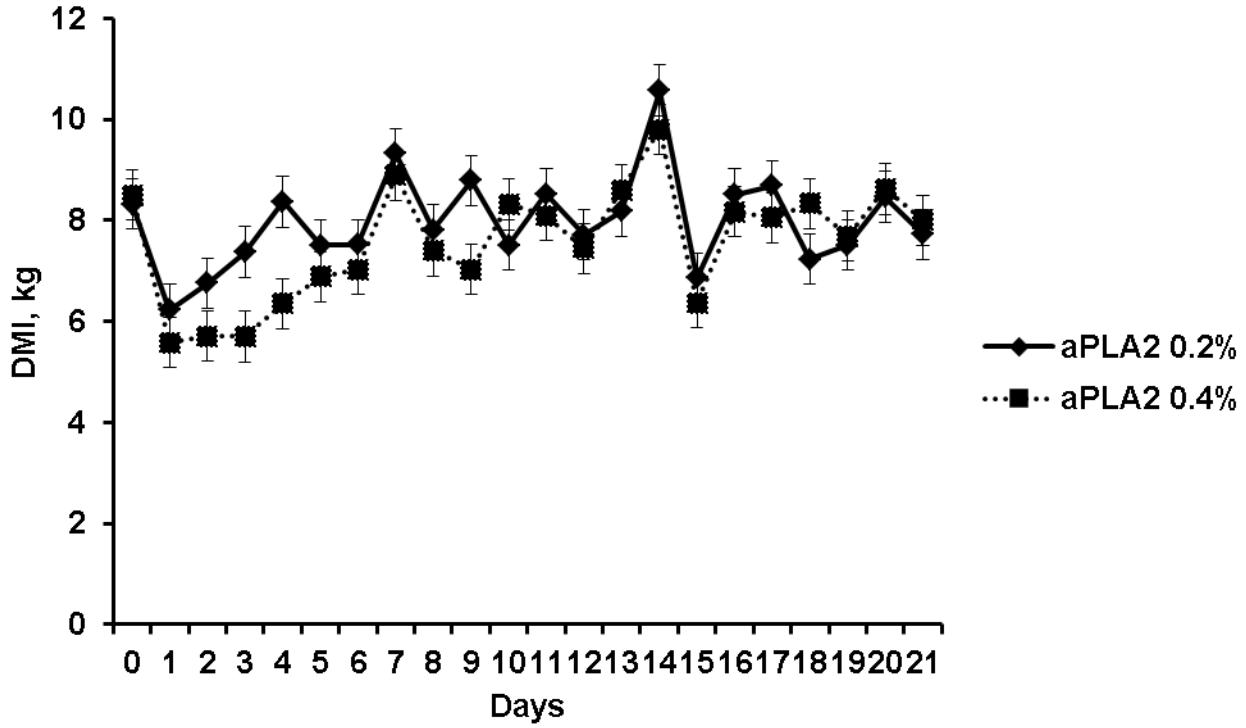

Figure 4-3. Average DMI by day of steers transitioned from a forage-based to a grain-based diet over a 21 d “step-up” adaptation period during Phase II. Contrast between aPLA2 0.2% and aPLA2 0.4% (*P* = 0.18).

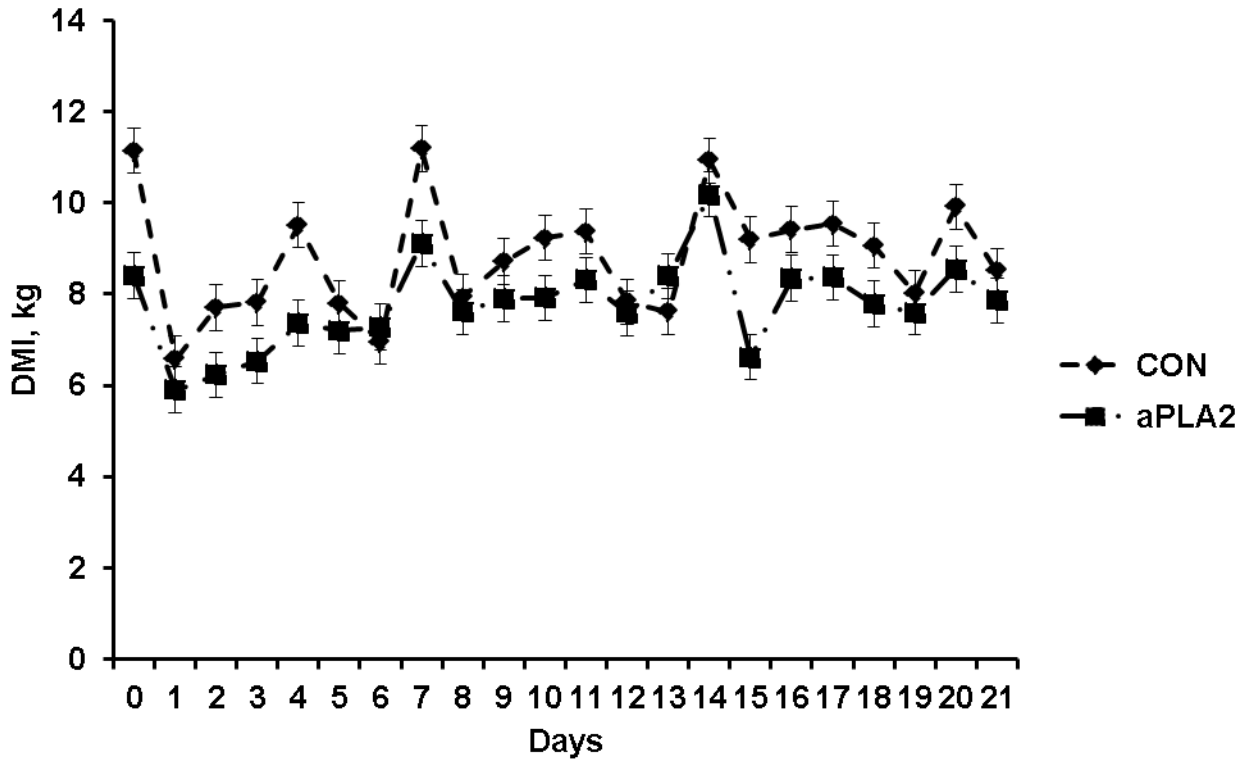

Figure 4-4. Average DMI by day of steers transitioned from a forage-based to a grain-based diet over a 21 d “step-up” adaptation period during Phase II. Contrast between both aPLA2 treatments combined and CON ($P < 0.0001$).

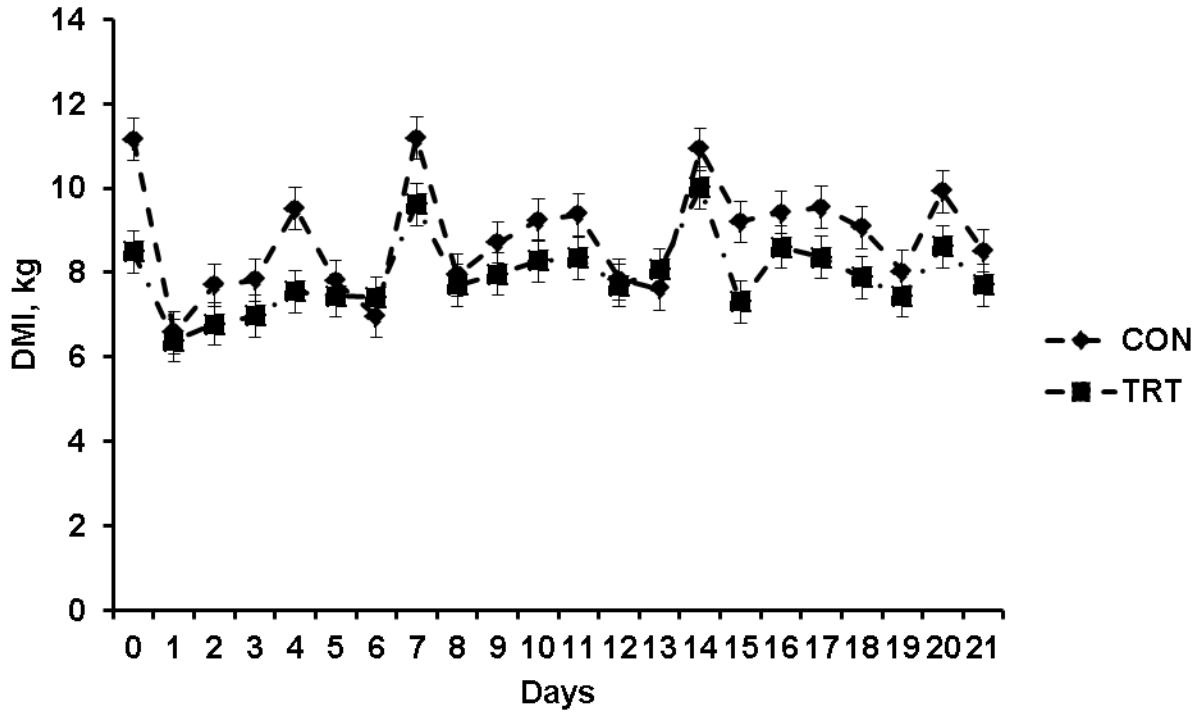

Figure 4-5. Average DMI by day of steers transitioned from a forage-based to a grain-based diet over a 21 d “step-up” adaptation period during Phase II. Contrast between both aPLA2 and MT treatments combined and CON ($P < 0.0001$).

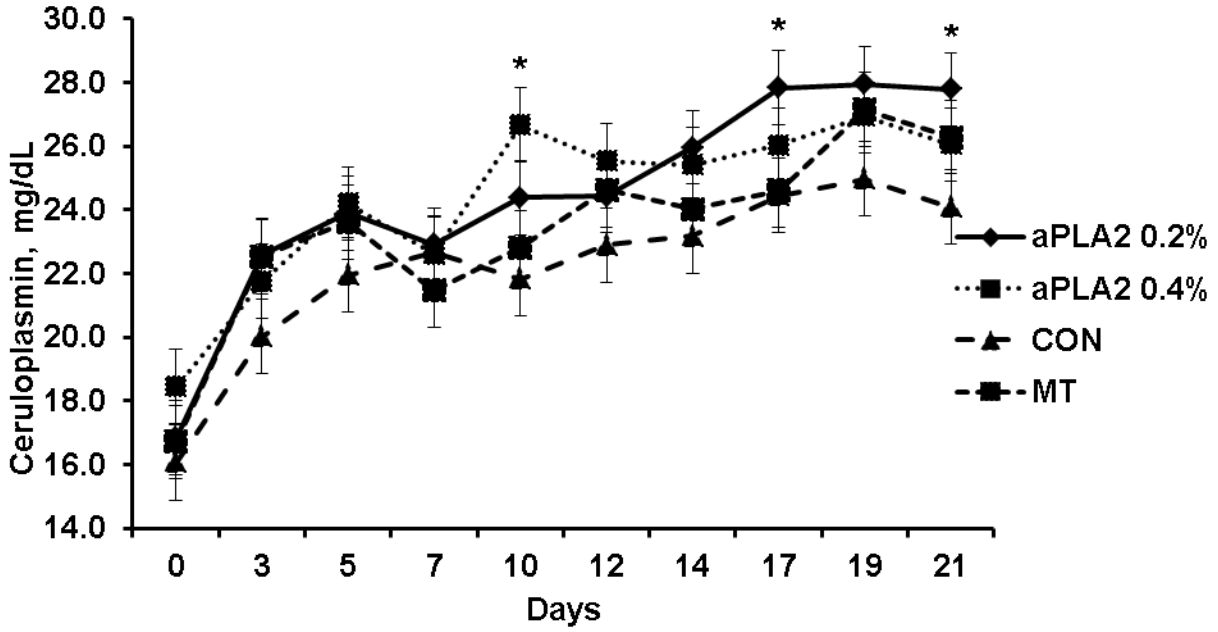

Figure 4-6. Concentrations of plasma ceruloplasmin by day of steers transitioned from a forage-based to a grain-based diet over a 21 d “step-up” adaptation period during Phase II. * Means differ within day, P = 0.01.

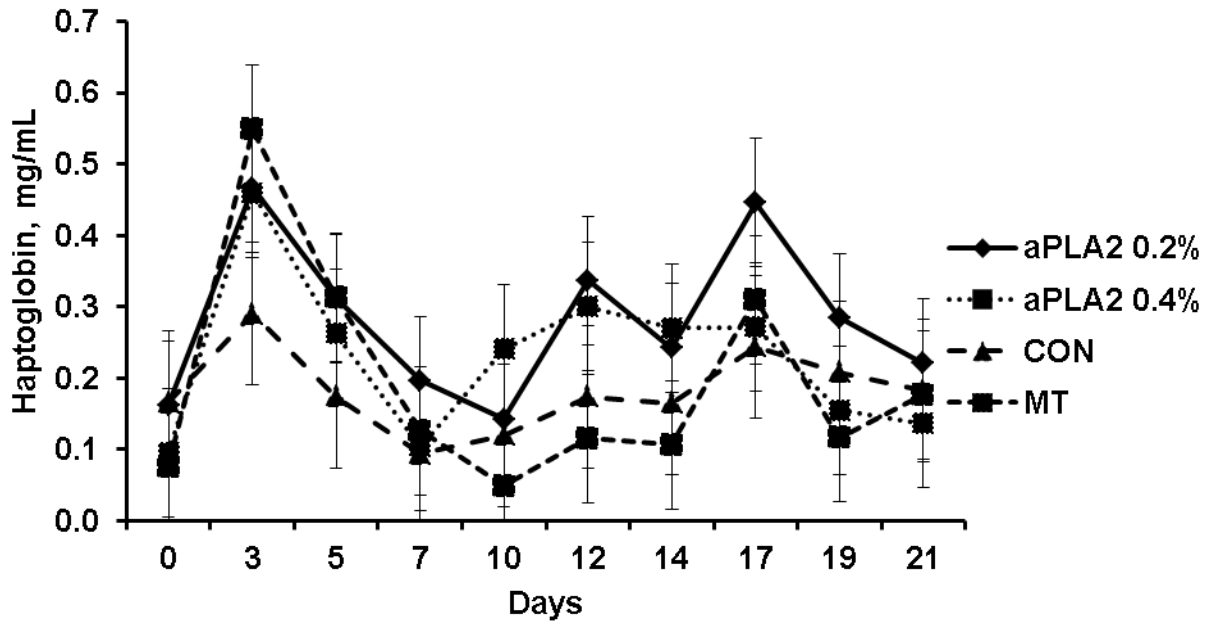

Figure 4-7. Concentrations of plasma haptoglobin by day of steers transitioned from a forage-based to a grain-based diet over a 21 d “step-up” adaptation period during Phase II.

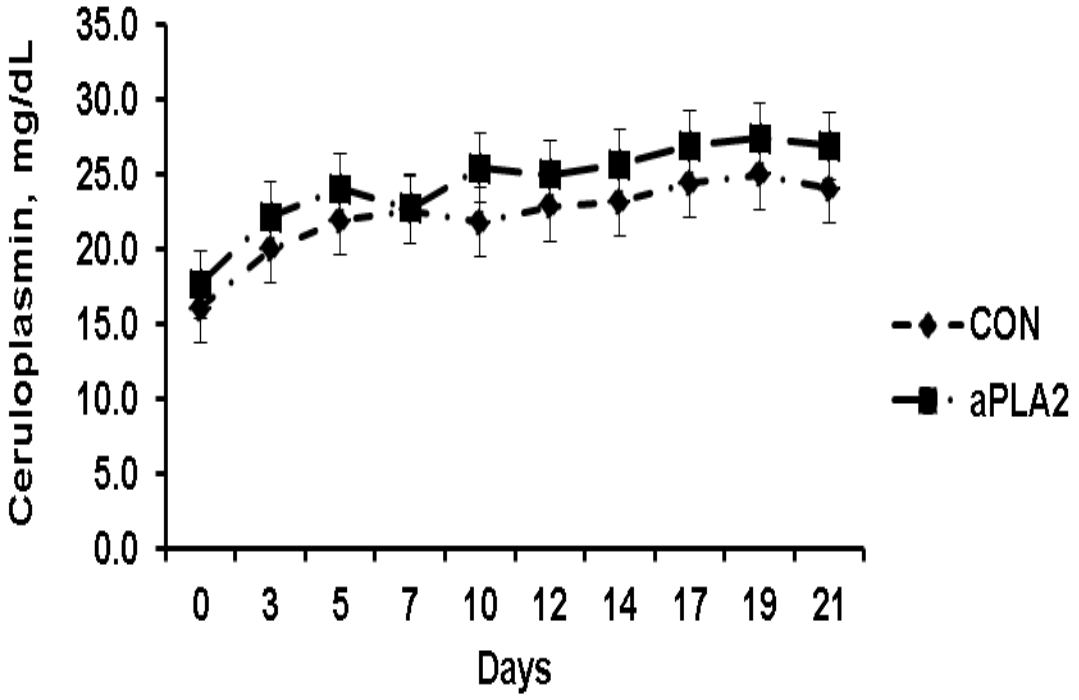

Figure 4-8. Concentrations of plasma ceruloplasmin by day of steers transitioned from a forage-based to a grain-based diet over a 21 d “step-up” adaptation period during Phase II. Contrast between both aPLA2 treatments combined against CON ($P < 0.0001$).

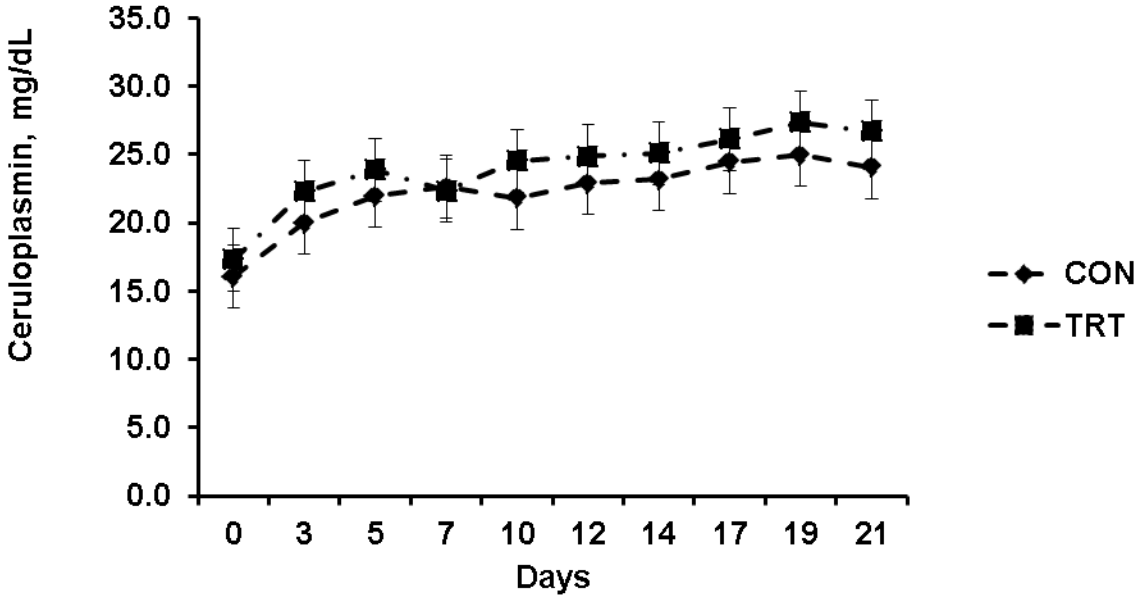

Figure 4-9. Concentrations of plasma ceruloplasmin by day of steers transitioned from a forage-based to a grain-based diet over a 21 d “step-up” adaptation period during Phase II. Contrast between both aPLA2 and MT treatments combined against CON ($P < 0.0001$).

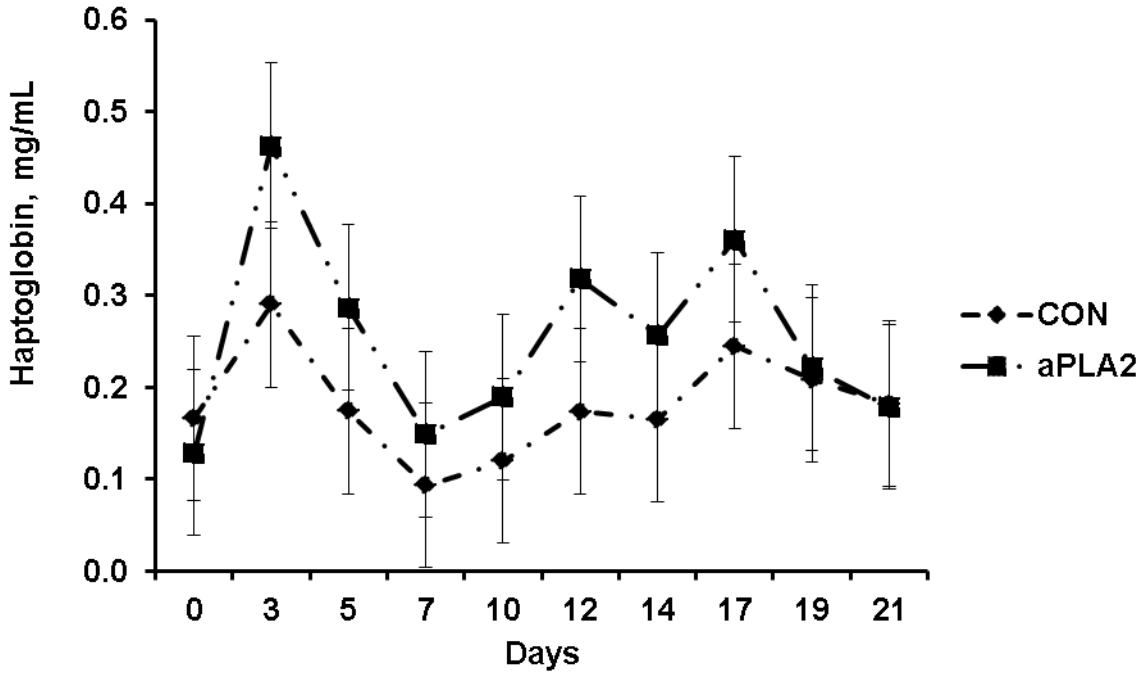

Figure 4-10. Concentrations of plasma haptoglobin by day of steers transitioned from a forage-based to a grain-based diet over a 21 d “step-up” adaptation period during Phase II. Contrast between both aPLA2 treatments combined against CON ($P < 0.05$).

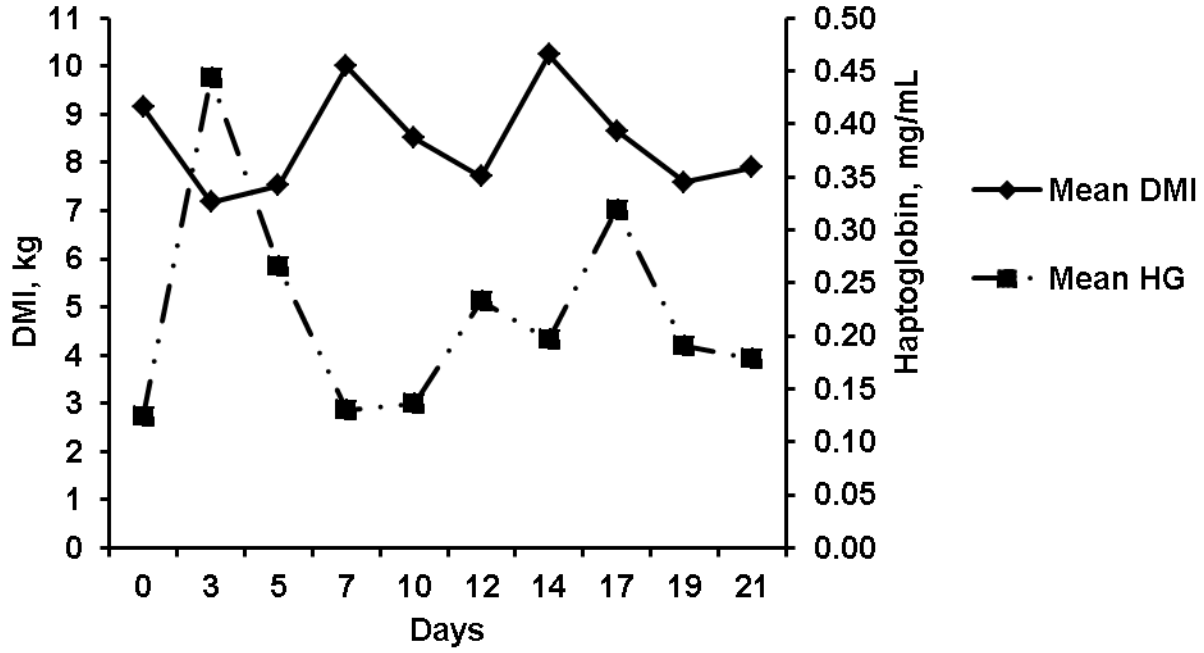

Figure 4-11. Correlation between plasma concentration of haptoglobin and average DMI by day of steers transitioned from a forage-based to a grain-based diet over a 21 d “step-up” adaptation period during Phase II, when combining all treatments ($P < 0.001$, $R2 = 0.02$).

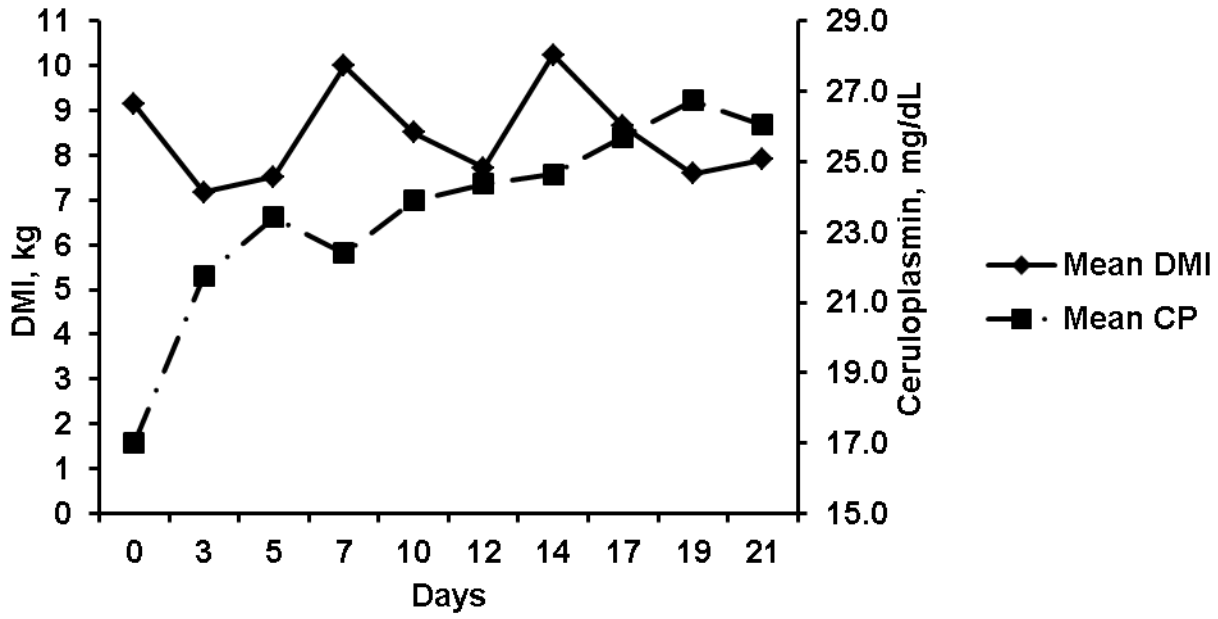

Figure 4-12. Correlation between plasma concentration of ceruloplasmin and average DMI by day of steers transitioned from a forage-based to a grain-based diet over a 21 d “step-up” adaptation period during Phase II, when combining all treatments ($P < 0.0001$, $R2 = 0.03$).

LIST OF REFERENCES

Ametaj, B.N., K.M. Koenig, S.M. Dunn, W.Z. Yang, Q. Zebeli, and K. a Beauchemin. 2009. Backgrounding and finishing diets are associated with inflammatory responses in feedlot steers. J. Anim. Sci. 87(4): 1314–20.

Andersen, P.H., B. Bergelin, and K. a Christensen. 1994. Effect of Feeding Regimen on Concentration of Free Endotoxin i n Ruminal Fluid of Cattle. J. Anim. Sci. 72(2): 487–91.

Araujo, D.B., R.F. Cooke, G.R. Hansen, C.R. Staples, and J.D. Arthington. 2010. Effects of rumen-protected polyunsaturated fatty acid supplementation on performance and physiological responses of growing cattle following transportation and feedlot entry. J. Anim. Sci. 88: 4120–4132.

Archer, J.A., E.C. Richardson, R.M. Herd, and P.F. Arthur. 1999. Potential for selection to improve efficiency of feed use in beef cattle: a review. Aust. J. Agric. Res. 50: 147–161.

Arthington, J.D., L.R. Corah, and F. Blecha. 1996. The Effect of Molybdenum-Induced Copper Deficiency on Acute-Phase Protein Concentrations, Superoxide Dismutase Activity, Leukocyte Numbers, and Lymphocyte Proliferation in Beef Heifers Inoculated with Bovine Herpesvirus-1. J. Anim. Sci. (74): 211–217.

Arthington, J.D., S.D. Eicher, W.E. Kunkle, and F.G. Martin. 2003. Effect of transportation and commingling on the acute-phase protein response , growth , and feed intake of newly weaned beef calves. 81: 1120–1125.

Arthington, J.D., X. Qiu, R.F. Cooke, J.M.B. Vendramini, D.B. Araujo, C.C. Chase, and S.W. Coleman. 2008. Effects of preshipping management on measures of stress and performance of beef steers during feedlot receiving. J. Anim. Sci. 86(8): 2016–23.

Arthur, P.F., and R.M. Herd. 2005. Efficiency of feed utilisation by livestock — Implications and benefits of genetic improvement. Can. J. Anim. Sci. 85: 281–290.

Barry, T.P., and M. Yang. 2008. Effects of Anti-phospholipase A 2 on the Growth of Rainbow Trout. North American Journal of Aquaculture 70(2): 236–239.

Beckett, S., I. Lean, R. Dyson, W. Tranter, and L. Wade. 1998. Effects of monensin on the reproduction, health, and milk production of dairy cows. J. Dairy Sci. 81(6): 1563–73.

Bergen, W.G., and D.B. Bates. 1984. Ionophores : Their Effect on Production Efficiency and Mode of Action. J. Anim. Sci. 58: 1465–1483.

Berry, B.A., A.W. Confer, C.R. Krehbiel, D.R. Gill, R.A. Smith, and M. Montelongo. 2004a. Effects of dietary energy and starch concentrations for newly received feedlot calves : II . Acute-phase protein response. 82: 845–850.

Berry, B.A., C.R. Krehbiel, A.W. Confer, D.R. Gill, R.A. Smith, and M. Montelongo. 2004b. Effects of dietary energy and starch concentrations for newly received feedlot calves : I . Growth performance and health. J. Anim. Sci. 82: 837–844.

Brody, S. 1945. Bioenergetics and growth: with special reference to the efficiency complex in domestic animals.

Burrin, D.G., and R.A. Britton. 1986. Response to Monensin in Cattle during Subacute Acidosis. J. Anim. Sci. 63: 888–893.

Carroll, J.A. 2008. Bidirectional communication: growth and immunity in domestic livestock. J. Anim. Sci. 86(14 Suppl): E126–37.

Carroll, J.A., J.D. Arthington, and C.C. Chase. 2009a. Early weaning alters the acute-phase reaction to an endotoxin challenge in beef calves. J. Anim. Sci. 87(12): 4167–72.

Carroll, J.A., N.C. Burdick, R.R. Reuter, C.C. Chase, D.E. Spiers, J.D. Arthington, and S.W. Coleman. 2011. Differential acute phase immune responses by Angus and Romosinuano steers following an endotoxin challenge. Domestic animal endocrinology 41(4): 163–73.

Carroll, J.A., and N.E. Forsberg. 2007. Influence of stress and nutrition on cattle immunity. The Veterinary clinics of North America. Food animal practice 23(1): 105–49.

Carroll, J.A., R.R. Reuter, C.C.C. Jr, S.W. Coleman, D.G. Riley, D.E. Spiers, J.D. Arthington, and M.L. Galyean. 2009b. Profile of the bovine acute-phase response following an intravenous bolus-dose lipopolysaccharide challenge. Innate Immun 15: 81–89.

Ceciliani, F., J.J. Ceron, P.D. Eckersall, and H. Sauerwein. 2012. Acute phase proteins in ruminants. Journal of proteomics 75(14): 4207–31.

Cook, M.E. 2004. Antibodies : Alternatives to Antibiotics in Improving Growth and Feed Efficiency. J. Appl. Poult. Res. 13: 106–119.

Cook, M.E. 2011. A review of science leading to host-targeted antibody strategies for preventing growth depression due to microbial colonization. J. Anim. Sci. 89: 1981–1990.

Cook, M.E., and D.L. Trott. 2010. IgY – Immune component of eggs as a source of passive immunity for animals and humans. World's Poultry Science Journal 66(02): 215–226.

Cooke, R.F., J.D. Arthington, D.B. Araujo, and G.C. Lamb. 2009a. Effects of acclimation to human interaction on performance, temperament, physiological responses, and pregnancy rates of Brahman-crossbred cows. J. Anim. Sci. 87(12): 4125–32.

Cooke, R.F., J.D. Arthington, B.R. Austin, and J.V. Yelich. 2009b. Effects of acclimation to handling on performance, reproductive, and physiological responses of Brahman-crossbred heifers. J. Anim. Sci. 87(10): 3403–12.

Cooke, R.F., and D.W. Bohnert. 2011. Technical note: Bovine acute-phase response after corticotrophin-release hormone challenge. J. Anim. Sci. 89(1): 252–7.

Cooke, R.F., D.W. Bohnert, B.I. Cappellozza, C.J. Mueller, and T. Delcurto. 2012a. Effects of temperament and acclimation to handling on reproductive performance of Bos taurus beef females. J. Anim. Sci.Available at http://jas.fass.org/content/early/2012/05/02/jas.2011-4768 (verified 11 July 2012).

Cooke, R.F., J.A. Carroll, J. Dailey, B.I. Cappellozza, and D.W. Bohnert. 2012b. Bovine acute-phase response after different doses of corticotropin-releasing hormone challenge. J. Anim. Sci. (90): 2337–2344.

Curley, K.O., D. a Neuendorff, A.W. Lewis, J.J. Cleere, T.H. Welsh, and R.D. Randel. 2008. Functional characteristics of the bovine hypothalamic-pituitary-adrenal axis vary with temperament. Hormones and behavior 53(1): 20–7.

Demetriou, J.A., P.A. Drewes, and J.B. Gin. 1974. Ceruloplasmin in Clinical Chemistry: Principles and Techniques. 2nd ed. Harper and Row, Hagerstown, MD.

DiLorenzo, N., C.R. Dahlen, F. Diez-Gonzalez, G.C. Lamb, J.E. Larson, and A. DiCostanzo. 2008. Effects of feeding polyclonal antibody preparations on rumen fermentation patterns, performance, and carcass characteristics of feedlot steers. J. Anim. Sci. 86(11): 3023–3032.

DiLorenzo, N., F. Diez-Gonzalez, and A. DiCostanzo. 2006. Effects of feeding polyclonal antibody preparations on ruminal bacterial populations and ruminal pH of steers fed high-grain diets. J. Anim. Sci. 84(8): 2178–85.

Dong, G., S. Liu, Y. Wu, C. Lei, J. Zhou, and S. Zhang. 2011. Diet-induced bacterial immunogens in the gastrointestinal tract of dairy cows: impacts on immunity and metabolism. Acta veterinaria Scandinavica 53(1): 48.

Drew, M.D., a G. Van Kessel, and D.D. Maenz. 2003. Absorption of methionine and 2-hydroxy-4-methylthiobutoanic acid in conventional and germ-free chickens. Poultry Sci. 82(7): 1149–53.

Duff, G.C., and M.L. Galyean. 2007. Board-invited review: recent advances in management of highly stressed, newly received feedlot cattle. J. Anim. Sci. 85(3): 823–40.

Duffield, T.F., J.K. Merrill, and R.N. Bagg. 2012. A Meta-analysis of the Effects of Monensin in Beef Cattle on Feed Efficiency, Gain and Dry Matter Intake. J. Anim. Sci.Available at http://jas.fass.org/content/early/2012/08/01/jas.2011-5018 (verified 16 August 2012).

Eckersall, P.D., and R. Bell. 2010. Acute phase proteins: Biomarkers of infection and inflammation in veterinary medicine. Veterinary journal 185(1): 23–7.

Elsasser, T.H., T.J. Caperna, C.-J. Li, S. Kahl, and J.L. Sartin. 2008. Critical control points in the impact of the proinflammatory immune response on growth and metabolism. J. Anim. Sci. 86(14 Suppl): E105–25.

Emmanuel, D.G.V., S.M. Dunn, and B.N. Ametaj. 2008. Feeding high proportions of barley grain stimulates an inflammatory response in dairy cows. J. Dairy Sci. 91(2): 606–14.

Emmanuel, D.G.V., K.L. Madsen, T. a Churchill, S.M. Dunn, and B.N. Ametaj. 2007. Acidosis and lipopolysaccharide from Escherichia coli B:055 cause hyperpermeability of rumen and colon tissues. J. Dairy Sci. 90(12): 5552–7.

FAO. 2009. How to Feed the World: Global Agriculture towards 2050.Available at http://www.fao.org/fileadmin/templates/wsfs/docs/Issues_papers/HLEF2050_Global_Agriculture.pdf.

Fike, K., and M.F. Spire. 2006. Transportation of cattle. The Veterinary clinics of North America. Food animal practice 22(2): 305–20.

Fitzhugh Jr., H.A., and C.S.S. Taylor. 1971. Genetic Analysis of Degree of Maturity. J. Anim. Sci. 33(4): 717–725.

Friend, T.H. 1991. Behavioral aspects of stress. J. Dairy Sci. 74(1): 292–303.

Galyean, M.L., K.J. Malcolm, and G.C. Duff. 1992. Performance of feedlot steers fed diets containing laidlomycin propionate or monensin plus tylosin, and effects of laidlomycin propionate concentration on intake patterns and ruminal fermentation in beef steers during adaptation to a high-concentrate diet. J. Anim. Sci. 70(10): 2950–8.

Galyean, M.L., L.J. Perino, and G.C. Duff. 1999. Interaction of cattle health / immunity and nutrition. J. Anim. Sci. 77: 1120–1134.

Galyean, M.L., C. Ponce, and J. Schutz. 2011. The future of beef production in North America. Animal Frontiers 1(2): 29–36.

Ganheim, C., S. Alenius, and K. Persson Waller. 2007. Acute phase proteins as indicators of calf herd health. Veterinary journal 173(3): 645–51.

Gifford, C.A., B.P. Holland, R.L. Mills, C.L. Maxwell, J.K. Farney, S.J. Terrill, D.L. Step, C.J. Richards, L.O. Burciaga Robles, and C.R. Krehbiel. 2012. Impacts of inflammation on cattle growth and carcass merit. J. Anim. Sci. 90: 1438–1451.

Gozho, G.N., D.O. Krause, and J.C. Plaizier. 2007. Ruminal lipopolysaccharide concentration and inflammatory response during grain-induced subacute ruminal acidosis in dairy cows. J. Dairy Sci. 90(2): 856–66.

Grandin, T. 1997. Assessment of Stress During Handling and Transport. J. Anim. Sci. 75: 249–257.

Haney, M E, J., and M.M. Hoehn. 1967. Monensin, a new biologically active compound I: Discovery and isolation. Antimicrobial Agents and Chemotherapy: 349.

Hegarty, R.S., J.P. Goopy, R.M. Herd, and B. McCorkell. 2007. Cattle selected for lower residual feed intake have reduced daily methane production. J. Anim. Sci. 85(6): 1479–86.

Herd, R.M., J.A. Archer, and P.F. Arthur. 2003. Reducing the cost of beef production through genetic improvement in residual feed intake : Opportunity and challenges to application. J. Anim. Sci. 81(E. Suppl. 1): 9–17.

Hulbert, L.E., J. a Carroll, N.C. Burdick, R.D. Randel, M.S. Brown, and M. a Ballou. 2011. Innate immune responses of temperamental and calm cattle after transportation. Veterinary immunology and immunopathology 143(1-2): 66–74.

Ishizaki, H., and Y. Kariya. 2010. Road transportation stress promptly increases bovine peripheral blood absolute NK cell counts and cortisol levels. The Journal of veterinary medical science / the Japanese Society of Veterinary Science 72(6): 747–53.

Johnson, R.W. 1997. Inhibition of Growth by Pro-Inflammatory Cytokines : An Integrated View. J. Anim. Sci. 75: 1244–1255.

Kellner, O. 1909. The Scientific Feeding of Animals. McMillan Co., New York, NY.

Khafipour, E., D.O. Krause, and J.C. Plaizier. 2009. A grain-based subacute ruminal acidosis challenge causes translocation of lipopolysaccharide and triggers inflammation. J. Dairy Sci. 92(3): 1060–70.

Kindt, T.J. 2007. Kuby Immunology (Richard A. Goldsby; Barbara A. Osborne, Ed.). 6th ed. W. H. Freeman and Company, New York, NY.

King, P. 1965. Infection in General Practice. Appl. Theri. 7: 490–495.

Klasing, K.C., D.E. Laurin, R.K. Peng, and D.M. Fry. 1987. Nutrition and Immunology Immunologically Mediated Growth Depression in Chicks : Influence of Feed Intake , Corticosterone and Interleukin-1. Nut. Immun. 117(85): 1629–1637.

Kleiber, M. 1947. Body size and metabolic rate. Physiol. Rev. 27: 511–541.

Koch, R.M.., L.A. Swiger, D. Chambers, and K.E. Gregory. 1963. Efficiency of Feed Use in Beef Cattle. J. Anim. Sci. 22: 486–494.

Kudo, I., and M. Murakami. 2002. Phospholipase A2 enzymes. Prostaglandins & other lipid mediators 68-69: 3–58.

Lev, M., and M. Forbes. 1959. Growth response to dietary penicillin of germ-free chicks with a defined intestinal flora. BR. J. Nutr. 13: 78–84.

Lippolis, J.D. 2008. Immunological signaling networks: integrating the body's immune response. J. Anim. Sci. 86(14 Suppl): E53–63.

Loerch, S.C., and F.L. Fluharty. 1999. Physiological Changes and Digestive Capabilities of Newly Received Feedlot Cattle. J. Anim. Sci. (77): 1113–1119.

Makimura, S., and N. Suzuki. 1982. Quantitative determinationof bovine serum haptoglobin and its elevation in some inflammatory disease. Jpn. J. Vet. Sci. 44: 15–21.

Merck Veterinary Manual. 2010. 10th ed. Merck and Co., Inc., Whitehouse, NJ.

Meyer, N.F., G.E. Erickson, T.J. Klopfenstein, M. a Greenquist, M.K. Luebbe, P. Williams, and M. a Engstrom. 2009. Effect of essential oils, tylosin, and monensin on finishing steer performance, carcass characteristics, liver abscesses, ruminal fermentation, and digestibility. J. Anim. Sci. 87(7): 2346–54.

Nagaraja, T.G., T.B. Avery, S.J. Galitzer, and D.L. Harmon. 1985. Effect of ionophore antibiotics on experimentally induced lactic acidosis in cattle. Am. J .Vet .Res. 46(12): 2444–2452.

Nagaraja, T.G., E.E. Bartley, L.R. Fina, and H.D. Anthony. 1978a. Relationship of Rumen Gram-Negative Bacteria and Free Endotoxin to Lactic Acidosis in Cattle. J. Anim. Sci. 47: 1329–1337.

Nagaraja, T.G., E.E. Bartley, L.R. Fina, H.D. Anthony, and R.M. Bechtle. 1978b. Quantitation of Endotoxin in Cell-Free Rumen Fluid of Cattle. J. Anim. Sci. 46: 1759–1767.

Nagaraja, T.G., and M.M. Chengappa. 1998. Liver Abscesses in Feedlot Cattle : A Review. J. Anim. Sci. 76: 287–298.

Nagaraja, T.G., and E.C. Titgemeyer. 2007. Ruminal acidosis in beef cattle: the current microbiological and nutritional outlook. J. Dairy Sci. 90 Suppl 1(07): E17–38.

Nevalainen, T.J., G.G. Graham, and K.F. Scott. 2008. Antibacterial actions of secreted phospholipases A2. Review. Biochimica et biophysica acta 1781(1-2): 1–9.

Nkrumah, J.D., J.A. Basarab, M.A. Price, E.K. Okine, A. Ammoura, S. Guercio, C. Li, B. Benkel, B. Murdoch, S.S. Moore, and C. Hansen. 2004. Different measures of energetic efficiency and their phenotypic relationships with growth, feed intake, and ultrasound and carcass merit in hybrid cattle. J. Anim. Sci. 82: 2451–2459.

Nkrumah, J.D., J.A. Basarab, Z. Wang, C. Li, M.A. Price, E.K. Okine, D.H. Crews, and S.S. Moore. 2007. Genetic and phenotypic relationships of feed intake and measures of efficiency with growth and carcass merit of beef cattle. J. Anim. Sci. 85(10): 2711–20.

Pacheco, R.D.L., D.D. Millen, N. Dilorenzo, C.L. Martins, C.T. Marino, M.V. Fossa, S.L. Beier, A. Dicostanzo, P.H.M. Rodrigues, and M.D.B. Arrigoni. 2012. Effects of feeding a multivalent polyclonal antibody preparation on feedlot performance , carcass characteristics , rumenitis , and blood gas profi le in Bos indicus biotype yearling bulls. J. Anim. Sci. 90: 1898–1909.

Pacák, K., and M. Palkovits. 2001. Stressor specificity of central neuroendocrine responses: implications for stress-related disorders. Endocrine reviews 22(4): 502–48.

Parker, L.C., L.R. Prince, and I. Sabroe. 2007. Translational mini-review series on Toll-like receptors: networks regulated by Toll-like receptors mediate innate and adaptive immunity. Clinical and experimental immunology 147(2): 199–207.

Petersen, H.H., J.P. Nielsen, and P.M.H. Heegaard. 2004. Application of acute phase protein measurements in veterinary clinical chemistry. Vet. Res. 35: 163–187.

Peterson, E.B., D.R. Strohbehn, G.W. Ladd, and R.L. Willham. 1989. Effects of Preconditioning on Performance of Beef Calves before and after Entering the Feedlot. J. Anim. Sci. (67): 1678–1686.

Qiu, X., J.D. Arthington, D.G. Riley, C.C. Chase, W. a Phillips, S.W. Coleman, and T. a Olson. 2007. Genetic effects on acute phase protein response to the stresses of weaning and transportation in beef calves. J. Anim. Sci. 85(10): 2367–74.

Richardson, L.F., A.P. Raun, E.L. Potter, and C.O. Cooley. 1976. Effect of Monensin on Rumen Fermentation in Vitro and in Vivo. J. Anim. Sci. 43: 657–664.

Rozenfeld, R.A., X. Liu, I. Deplaen, W. Hsueh, L. Khailova, K. Dvorak, K.M. Arganbright, M.D. Halpern, I.D.E. Plaen, W.E.I. Hsueh, and A. Ranna. 2001. Role of gut flora on intestinal group II phospholipase A2 activity and intestinal injury in shock. Am. J. Physiol. Gastrointest. Liver Physiol. 281: G957–G963.

Salyers, A. a. 2002. An overview of the genetic basis of antibiotic resistance in bacteria and its implications for agriculture. Animal biotechnology 13(1): 1–5.

Schwartz, M., M. Yang, and M. Cook. 2006. Effects of egg antibody to phospholipase A2 (aPLA2) on preventing coccidia-induced growth depression. Poult. Sci. 85 (Supp. : 38 (Abstr.).

Shimizu, M., R.C. Fitzsimmons, and S. Nakai. 1988. Anti-E. coli lmmunoglobulin Y Isolated from Egg Yolk of Immunized Chickens as a Potential Food Ingredient. J. Food Sci. 53: 1360–1366.

Spurlock, M.E. 1997. Regulation of Metabolism and Growth During Immune Challenge : An Overview of Cytokine Function. J. Anim. Sci. 75: 1773–1783.

Stock, R. a, S.B. Laudert, W.W. Stroup, E.M. Larson, J.C. Parrott, and R. a Britton. 1995. Effect of monensin and monensin and tylosin combination on feed intake variation of feedlot steers. J. Anim. Sci. 73(1): 39–44.

Voisinet, B.D., T. Grandin, J.D. Tatum, S.F.O. Connor, and J.J. Struthers. 1997. Feedlot cattle with calm temperaments have higher average daily gains than cattle with excitable temperaments. J. Anim. Sci. 75: 892–896.

Warnock, T.M., T.A. Thrift, M. Irsik, M.J. Hersom, J.V. Yelich, T.D. Maddock, G.C. Lamb, and J.D. Arthington. 2012. Effect of castration technique on beef calf performance, feed efficiency, and inflammatory response. J. Anim. Sci. 90(7): 2345–52.

Yang, M., M.E. Cook, and K. Roberson. 2003. The effect of feeding yolk antibody to phospholipase A2 (aPLA2) on growth and feed conversion in broiler chicks. Poultry Science Annual Meeting, Madison, Wisconsin A119.

Zayat, M., L.M. Lichtenberger, and E.J. Dial. 2008. Pathophysiology of LPS-induced gastrointestinal injury in the rat: role of secretory phospholipase A2. Shock 30(2): 206–211.

Zebeli, Q., S.M. Dunn, and B.N. Ametaj. 2010. Strong associations among rumen endotoxin and acute phase proteins with plasma minerals in lactating cows fed graded amounts of concentrate. J. Anim. Sci. 88(4): 1545–53.

BIOGRAPHICAL SKETCH

Vitor Rodrigues Gomes Mercadante was born in Piracicaba, São Paulo, Brazil in 1986. He was raised in Campo Grande in the state of Mato Grosso do Sul, where he had his first contact with beef cattle production. In 2005, Vitor entered the Veterinary Medicine School at the São Paulo State University in Botucatu, where he joined CONAPEC Jr., a student enterprise consulting on agricultural issues, as the director for beef projects. After graduation, Vitor moved to Gainesville, FL, US to pursue his Master's degree at the University of Florida Animal Sciences Department. He joined Dr. Lamb's research program, and focused his research on the effects of the activation of the immune system on performance and feed efficiency of beef cattle.